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Nutrient flow in improved upland aquaculture systems in Yen Chau, province Son La (Vietnam)

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Abbreviations

AChE	Acetyl-cholinesterase
ADF	Acid detergent fiber
ADP	Adenosindiphosphate
AFD	Average farm dose
AI	Active ingredient
ALC	Apparent lipid conversion
ANOVA	analysis of variance
ATP	Adenosintriphosphate
BChE	Butyl-cholinesterase
CyHV-3	Koi carp herpes virus
CA	Crude ash
ChE	Cholinesterase
CL	Crude lipid
CP	Crude protein
CPI	Consumer price index
DFG	Deutsche Forschungsgemeinschaft
DM	Dry matter
DO	Dissolved oxygen
DT_{50}	Degradation half-life time
EE	Ether extract
e.g.	exempli gratia
E_H	Redox potential
esp.	especially
FAO	Food and Agriculture Organization of the United Nations
FCR	Feed conversion ratio
FM	Fresh matter
FNU	Formazine Nephelometric Units
g	gravity acceleration
GCHV	Grass carp hemorrhagic virus
GE	Gross energy
His	Histidine
HK	Hexokinase
Ile	Isoleucine
K_{ow}	Log octanol-water partition coefficient
LC_{50}	Lethal concentration
LDS	Fisher´s least significant difference
Leu	Leucine
Lig	Lignin
Lys	Lysine
m	Mass
Met	Methionine
MJ	Mega joule
MLR	Maximum residue levels
n	number of sampled subjects

N	Nitrogen
NADPH	Nicotinamide adenine dinucleotide phosphate
n.d.	Not detected
NDF	Neutral detergent fiber
N_{diss}	Dissolved nitrogen
NO_2-N	Nitrite nitrogen
NO_3-N	Nitrate nitrogen
NOEC	No observed effects concentrations
OP	Organophosphate pesticides
Org.	Organisms
p	Significance level
P	Phosphorus
PCR	Polymerase chain reaction
PER	Protein efficiency ratio
Phe	Phenylalanine
PO_4-P	Ortho-phosphate phosphorus
PPV	Protein productive value
PVC	polyvinyl chloride
RIA 1	Research Institute for Aquaculture No. 1
PNEC	Predicted no effect concentration
rpm	Rounds per minute
RSD	Red spot disease
SD	Standard deviation
SDD	Secchi disc depth
SFB	Sonderforschungbereich
SGR	Specific growth rate
SRS	Self-recruiting species
SS_{com}	Combustible suspended solids
SS_{income}	Incombustible suspended solids
TAN	Total ammonia nitrogen
TC	Total carbon
Thr	Threonine
TI	Trypsin-Inhibitor
TN	Total nitrogen
Trp	Tryptophan
TNER	Total nitrogen efficiency ratio
TNPV	Total nitrogen productive value
TP	Total phosphorus
TPER	Total phosphorus efficiency ratio
TPPV	Total phosphorus productive value
TSS	Total suspended solids
TSS_{org}	Organic fraction of TSS
TSS_{inorg}	Inorganic fraction of TSS
TV	Television
UIA-N	Unionized ammonia nitrogen

USD	US dollar
v	Volume
VAC	Vietnamese acronym combining garden, fishpond and livestock pen
Val	Valine
VND	Vietnamese dong

1 General introduction

1.1 Contribution of aquaculture to global seafood supply

In human nutrition, fish and other aquatic products are an important source of high-value protein, essential amino acids, polyunsaturated fatty acids (e.g. omega-3 fatty acids), vitamins and minerals, and are fundamental to a well-balanced and healthy diet (FAO 2012). In 2009, the global consumption of fish and other aquatic products provided 16.6% of the animal protein intake of humans equivalent to 6.5% of their total protein intake (FAO 2012). Especially in developing countries in South-East Asia and Central Africa, fish is a food item of very high importance as it represents an affordable source of animal protein accounting for over 20% of animal-derived protein intake (FAO 2005, 2012). Unsurprisingly therefore, there is an increasing demand for aquatic foods due both to the increase in human population and the rising consumption per capita.

Over the past decade, the amount of fish and other aquatic products landed globally from marine and inland water bodies has been maintained at a relatively constant level of around 90 million tons per year through the utilisation of ever more effective fishing gear and landing technologies, and by the overexploitation of several natural stocks (Pauly 2009; FAO 2012). Marine capture fisheries are the main suppliers to world markets of piscivorous/carnivorous food fish species at higher trophic level (pollock, tuna, cod, hake, drums, croakers, snappers, groupers, flatfish, breams, basses, etc.), mollusc species (squid, cuttlefish, octopus) and crustaceans (shrimp, lobsters, crabs) (Tacon *et al.* 2010; Neori and Nobre 2012). The trophic level of an organism is describing its position in a food chain and ranges from 1 (primary producers) to 4-5 (top predators). However, the increasing demand for aquatic products cannot be satisfied by such fisheries, which has resulted in a steady proliferation of production by aquaculture over the last few decades.

The Food and Agriculture Organisation of the United Nations (FAO) defines aquaculture as "…farming of aquatic organisms including fish, molluscs, crustaceans and aquatic plants. Farming implies some sort of intervention in the rearing process to enhance production, such as regular stocking, feeding, protection from predators, etc…." (FAO 1997, p. 6). According to the FAO, global aquaculture production reached 83.7 million tons including aquatic plants, and 62.7 million tons excluding aquatic plants in 2011 (FAO 2011). Within the last three decades, global aquaculture production of food fish has increased steadily with an average annual rise of 8.8% and is therefore the fastest growing agricultural sector (FAO 2012). Since 2009, more than half of the fish being consumed worldwide has been produced by aquaculture (Naylor *et al.* 2009).

Aquaculture production techniques differ greatly with regard to the intensity of production. The intensity can be classified according to the yield per area or volume of water, the stocking density, level of management/technical requirements, capital and recurring costs, labour requirement, quantity and quality of external feed/fertiliser inputs, risk of diseases or technical failure, and degree of dependency on natural food resources (Edwards *et al.* 1988; Tacon 1988; Prein 2002). In general, aquaculture is classified into three intensities:

<u>Extensive aquaculture:</u> Fish mainly from lower trophic levels are grown solely on natural food resources (e.g. bacteria, phytoplankton, zooplankton, zoobenthos, detritus, prey fish) without

substantial external inputs. Typically, this aquaculture is characterised by low stocking densities and low input of money and labour. However, it also results in low yields.

<u>Semi-intensive aquaculture:</u> Fish are grown on a combination of natural food resources and external supplemental feed inputs. The farmer often encourages the production of natural food resources through fertilisation and proper water management. The quantity and quality of external feed resources are chosen so as to supplement the diet of fish with nutrients which are lacking in their natural food or which enable the most effective use of natural food-derived nutrients (De Silva 1993). Viola (1989) described digestible energy as the first factor limiting fish growth in semi-intensive aquaculture as natural food resources are generally rich in protein. The next limiting factors are protein, phosphorus and vitamins (Viola 1989). Semi-intensive aquaculture is characterised by medium stocking densities, minimal use of technical equipment (aeration, automatic feeders) and medium to high application of external sources of fertiliser and/or feeds.

<u>Intensive aquaculture:</u> Fish are grown solely on external feed inputs, which the farmer typically supplies in the form of pelleted feeds that are formulated to supply the cultured fish with all required nutrients and energy. This aquaculture makes use of high stocking density, high inputs of money and labour and uses technologically sophisticated equipment such as pumps, filters, disinfection units, heaters/coolers, feeders, water quality monitoring systems etc. Intensive aquaculture results in high yields but is also considered of being highly risky because of factors such as diseases, technical failure, price competition etc.

Nowadays, the FAO (2011) divides aquaculture production into 520 marine, brackish water and freshwater single species or groups of species (excluding plants and mammals). The cultured species differ greatly in their requirements and culture conditions that include factors such as water quality, stocking density and feeding behaviour. They range from those at low trophic levels that are planktivorous, herbivorous, detrivorous and omnivorous to those at high trophic levels that are piscivorous and carnivorous.

The species that form the bulk of the catch from capture fisheries come from the higher trophic levels while, with the exception of the Atlantic salmon, *Salmo salar*, and the rainbow trout, *Oncorhynchus mykiss*, the species with the global highest production by aquaculture are from lower trophic levels (Tacon *et al.* 2010; Neori and Nobre 2012). In aquaculture, lower trophic level species also dominate in terms of total monetary value (Neori and Nobre 2012). The cyprinid species silver carp (*Hypophthalmichthys molitrix*), grass carp (*Ctenopharyngodon idellus*), common carp (*Cyprinus carpio*), bighead carp (*Hypophthalmichthys nobilis*) as well as the cichlid species Nile tilapia (*Oreochromis niloticus*) are all at a trophic level below 2.5 and are the major species cultured extensively and semi-intensively in pond polycultures especially in South-East Asia (Milstein 1992; Kestemont 1995; Neori and Nobre 2012). In 2007, these fish were all among the ten species with the highest global aquaculture production volume (Tacon *et al.* 2010; Neori and Nobre 2012).

Generally, cultured species from lower trophic levels require lower quality and quantity of supplemental feeds and therefore their production is typically more sustainable and resource efficient than that of species from higher trophic levels (Tacon *et al.* 2010). However, the production of these low trophic level freshwater fish under extensive/semi-intensive aquaculture has a high importance for the food security and can only be further expanded by greater intensification as expansion in area is often limited due to high land pressure (Tacon *et al.* 2010).

Expanding aquaculture production, whether semi-intensive or intensive, requires an increase in aquafeed production and puts pressure on the supply of common aquafeed ingredients (Tacon and Metian 2008). Fishmeal and fish oil are the traditional source of animal protein and fatty acids (esp. omega-3 fatty acids) for aquafeeds and are finite in its production (Naylor *et al.*, 2009). Both fish meal and fish oil are mainly produced from pelagic forage/trash fish, but the amount of these fish landed world-wide fell from 30 million tons in 1994 to about 15 million tons in 2010 with large annual fluctuations in production caused by climatic circumstances, such El Niño (FAO 2012). The declining availability of pelagic forage/trash fisheries´ products and the increasing demand for these resources for aquafeed production is causing the price on international markets to rise as well as stimulating the search for alternatives. Other resources that have been studied and used in the manufacture of aquafeeds include soybean meal, various other plant resources and rendered animal products (Hardy 2010; Hernández *et al.* 2010). These feed resources are produced on a large scale and are traded internationally, but the extent of their use depends on their global market price and availability. This dependency makes it risky for small-scale farmers to invest in such resources for use in aquafeeds. To meet the needs of small-scale farmers and establish further alternative feed ingredients, researchers have investigated a number of highly nutritious alternative feed resources which can be produced and used also on a small-scale to make feeds based on farm wastes and by-products and which can be traded locally or regionally. These include insects, snails, termites and oligochaeta (Tacon *et al.* 1983; Nandeesha *et al.* 1988; Begum *et al.* 1994; Ogunji *et al.* 2008; Phonekhampheng *et al.* 2008; Sánchez-Muros *et al.* 2014).

1.2 Aquaculture in South-East Asia

In South-East Asia[1], total aquaculture production (excluding algae and mammals) grew about 2.8 fold from 3.1 to 8.6 million tons from 2001 to 2011. Marine and brackish water aquaculture production of crustaceans (e.g. shrimps, prawns), molluscs and fish also rose about 1.9 fold from 1.6 to 3.1 million tons (FAO 2011). With a 3.9 fold increase in production over the same period, the production of freshwater species grew even more from 1.4 to 5.5 million tons (FAO 2011). In freshwater aquaculture, the production of cyprinids (esp. carps and barbels) and cichlids (esp. tilapia) contributed the highest share with 3.0 million tons in 2011 (FAO 2011).

This tremendous growth in aquaculture production is driven by the increasing global demand for aquatic food products and is implemented by both an increasing of the area dedicated to aquaculture and by the application of more intensive aquaculture technologies. The intensive aquaculture practised in South-East Asia has been brought to public attention in Europe especially by the fast growing production of Pangasius catfish (*Pangasianodon hypophthalmus* and *Pangasius bocourti*) and shrimp, both are produced intensively in the major river deltas

[1] Includes Brunei, Cambodia, Indonesia, Laos, Malaysia, Myanmar, Philippines, Singapore, Thailand, Timor-Leste, and Vietnam.

and coastal regions, and are associated with a number of environmental concerns (Little *et al.* 2012; Bush *et al.* 2013). These intensive production systems combined with the corresponding processing industries merchandise medium- and high-value commodities to the more prosperous global or national markets. The products of intensive aquaculture are usually not affordable for poorer populations even though fish and other aquatic products are well-accepted and play an important role in the nutrition of such communities (Little *et al.* 1996; Edwards 2000; Prein and Ahmed 2000; Prein 2002; Hop 2003; Dey and Ahmed 2005). For these people, the consumption of fish provides a high proportion of their animal-derived protein intake and may even exceed 50% (Dey and Ahmed 2005; Mishra and Ray 2009). The aquatic products consumed by lower income groups are supplied by marine or freshwater fisheries (Islam and Wahab 2005; Kawarazuka 2010; FAO 2012), culture-based fisheries (Phan and De Silva 2000; Nguyen *et al.* 2001; De Silva 2003) and marine and freshwater aquaculture (Edwards 2000; Islam 2004; Dey and Ahmed 2005) or are produced locally in inland integrated agriculture-aquaculture (IAA) systems (Prein and Ahmed 2000; Prein 2002; Little and Edwards 2003).

In the lowlands and in sub-urban regions in many parts of South-East Asia, both agriculture and aquaculture are rapidly intensifying and the traditional integrated farming systems of small-scale farmers are changing. These processes have been catalysed by a number of factors including expanding markets for selling farm products and buying farm inputs (e.g. fertiliser, pesticides, seeds, feeds), and improvements in communications, infrastructure and resettlement of people from the lowlands. Examples of their impact on Northern Vietnam have been described by Minot *et al.* (2006) and Vien (2003). In integrated agriculture-aquaculture systems, small-scale aquaculture is often still performed by following traditional management schemes which coevolved with agriculture practice that are appropriate to local environmental, economic and social conditions (Vien 2003). These integrated agriculture-aquaculture systems differ regionally and are still in use especially in the more rural areas. One example is the traditional IAA system in Vietnam, which combines gardening, pond fish culture and animal husbandry at an intra-farm level (Luu 2001c). This practice is still often used in remote regions but differs between the flat areas (lowlands) and the mountainous areas (uplands) in the way in which resources are managed (Luu 2001c). The intensification of single farming activities has led to the development of several adapted integrated agriculture-aquaculture systems that combine single intensive, semi-intensive or extensive farming activities with pond aquaculture. Nowadays, aquaculture is managed in combination with intensive production systems that concentrate on livestock (Little and Edwards 2003; Nhan *et al.* 2007), fruit orchards (Nhan *et al.* 2007) and paddy rice (Little *et al.* 1996; Dey and Prein 2005; Ahmed *et al.* 2011). These steps towards more intensive farming activities create an imbalance with farming activities that are still traditionally managed and are causing concerns about the sustainability of such systems regarding water use, agrichemical pollution, erosion, desertification, eutrophication and human health. Land pressure in the lowlands has increased due to urbanisation and the need for higher food production. This situation has also increased the land pressure in the mountainous regions and led to migration towards the uplands along with infrastructural development, deforestation and agricultural intensification but also cultural conflicts (Vien 2003). However, little is known about the resulting challenges created within the uplands of South-East Asia. To investigate the potentials, threats and risks in the uplands, an interdisciplinary research program, the Upland Program (UHOH 2014), was carried out between 2000 and 2014 to investigate the changes in two mountainous regions in South-East Asia regarded as representative: Chiang Mai province in Northern Thailand and the Son La province in Northern Vietnam (Schreinemachers *et al.* 2013). In this thesis, the Yen Chau district in Son La province, Northern Vietnam, will be examined as a model for potential improvements of small-scale pond aquaculture in mountainous regions in South-East Asia. The area in which the presented research was performed is about four hours drive from the capital Hanoi and is situated near to the border of Lao People's Democratic Republic. It is a rural region which is populated by several ethnic minority groups and by people from the major ethnic group in Vietnam (Kinh ethnic group) who migrated in large numbers from the lowlands into the northern uplands (Vien 2003). In the study area, Kinh people settled in the main valleys while the ethnic minorities are more dominant in the villages in the mountains.

1.3 Farming system in Son La province, Northern Vietnam

The poorest people in Vietnam live mostly in remote and less developed areas such as the mountainous regions of Central and North Vietnam and often belong to ethnic minorities (Minot *et al.* 2006; Mishra and Ray 2009; IFAD 2012). One of the poorest provinces in the country is Son La province which is located in the north-west of the country and is populated by several ethnic minorities such as the Thai and Hmong. In the past, these people developed several land use and farming systems that were adapted to the topographic characteristics of karst mountains and a semitropical climate.

In Yen Chau district in central Son La province, there are several villages of Black Thai farmers established in the valleys. In one of the districts communes, Chieng Khoi, the agricultural activities of Black Thai farmers are partly supplied with water from a reservoir that irrigates the rice paddies in the valleys which allows the production of two rice crops per season. While paddy rice is mainly produced for subsistence, corn and cassava are mainly cultivated as cash crops on the hill slopes with very little attempt to apply any soil conservation methods (Saint-Macary *et al.* 2010). Corn culture in the region has been especially intensified by the use of chemical fertiliser and hybrid seeds, and by the ease of access to markets in the lowlands. The income of the Black Thai farmers comes mainly from selling corn via a commodity chain to buyers in the lowlands for animal feed production. To a smaller extent, vegetables and fruits are grown in gardens and secondary forests. Common farm animals are poultry, ruminants and occasionally pigs and goats. Pond aquaculture is a common farming practice in the region and has been practiced for over 50 year to provide a more varied household diet and to help the perpetual fight against hunger and poverty (Minh 2010). Even though the demand for fish is rising in the Northern Vietnam uplands and the access to external high quality feed inputs is improving (Anrooy 2001), pond aquaculture is still performed mainly for subsistence and to supply local markets (Luu 2001a, b, c; Minh 2010; Steinbronn 2009).

The traditional pond aquaculture management used by famers in Yen Chau district was described in detail by Steinbronn (2009) and is part of an integrated agriculture-aquaculture system. Traditionally, the ponds are stocked with a polyculture of the macroherbivorous grass carp (*Ctenopharyngodon idella*) as the main species (accounting for approx. 50% of stocked fish) together with 3-5 other non-herbivorous fish species such as common carp (*Cyprinus carpio*), silver carp (*Hypophthalmichthys molitrix*), bighead carp (*Aristichthys nobilis*), mrigal

(*Cirrhinus mrigala*), mud carp (*Cirrhinus molitorella*), silver barb (*Barbus gonionotus*) and Nile Tilapia (*Oreochromis niloticus*). With a rearing period of 21 months, the production of the aquaculture system amounts to 1.54 ± 0.33 t ha^{-1} a^{-1}. Since the growth of fish strongly depends in external feed resources, this pond management can be considered as being intensive. However, in terms of fish yield, this aquaculture can rather be categorised as extensive or semi-intensive aquaculture (Edwards *et al.* 1988).

In Chieng Khoi commune, nearly every household has at least one pond of, on average, 800 m^2 in size, which serves multiple purposes and is operated as a flow-through-system for most of the year depending on the availability of water (Steinbronn 2009; Anyusheva *et al.* 2012). The steady water flow is advantageous for the culture of grass carp whose natural habitat is in large rivers (Chilton and Mouneke 1992), but may cause a continuous loss of nutrients as it has been shown to exist in ponds in the Red River Delta, Northern Vietnam (Nhan *et al.* 2008). Further, the water flow-through results in high turbidity and sedimentation in the ponds caused by particles eroded from the upland fields (Nikolic *et al.* 2008; Schmitter *et al.* 2010a; 2010b). The high turbidity in the ponds limits the development of phytoplankton and zooplankton as natural food resources for non-herbivorous fish species in the polyculture as well as lowering the water quality and limiting oxygen production in the ponds. All these factors have been described as limiting the production of fish (Steinbronn 2009).

For external feed inputs, the farmers use mainly green leaves from banana, bamboo, cassava, maize and grass and crop residues such as rice bran, rice husk, cassava root peel and distillery residues (Luu 2001c; Steinbronn 2009). These items are cheap on-farm resources that are intermittently available in large quantities but are mostly useful only for the production of the macro-herbivorous grass carp. They are also of low quality due to their low crude protein content and high level of antinutrients and fibres (Dongmeza *et al.* 2009). The feeds used in the study area are reported to be of insufficient nutritional quality and limit local fish production (Dongmeza *et al.* 2009; Thai *et al.* 2006; Thai *et al.* 2007; Steinbronn 2009). Some of these feeds have even been shown to result in negative growth (Dongmeza *et al.* 2010). None of the non-herbivorous fish species of the polyculture are actively fed by the farmer and show a rather poor growth (Steinbronn 2009). Manures of ruminants and, to a lesser extent, of pigs are used as direct feed and fertiliser to increase natural food availability.

Since 2003, an undiagnosed disease has been threatening grass carp production. Little is known about its definition or aetiology but it causes high morbidity and mortality in grass carp in affected ponds especially in the periods between March and April as well as between September and October and thereby decreases the dietary protein supply and income of Black Thai farmers. A detailed investigation on the grass carp specific disease will be the scope of another PhD thesis at the University of Veterinary Medicine, Vienna, Austria. The disease is described by farmers as the main risk and limiting factor of pond aquaculture as it has had a major economic impact on the earnings from fish-farming in Yen Chau region (Steinbronn 2009) and occurs also in other regions of Vietnam (Edwards 2000). Other fish species in the same ponds are not affected. Besides parasites and bacterial or viral agents, pesticides were also considered as potential factors leading to the disease of grass carp in the region (Steinbronn 2009). In Vietnam in general and in the study area in particular, pesticides are known to be used at higher than recommended levels (Lamers *et al.* 2011; Normile 2013). In Yen Chau district, surface waters are reported to be contaminated with high levels of pesticide that are toxic to aquatic organisms

(Lamers *et al.* 2011). As shown by Anyusheva *et al.* (2012), pesticides applied to rice paddies enter the pond via the water inflow. Up to now, weeds or surplus rice plants from paddies contaminated with pesticides have not been considered as potential threats to grass carp, but their effects will be discussed in a later part of this thesis.

The occurrence of the aforementioned disease of grass carp and the linked reduction in food production and income from aquaculture as a farming activity emphasise the need to change the local pond management towards safer, more productive and beneficial practice while maintaining the use of locally available resources. In the course of the Uplands Program, three dissertations were produced: (1) investigating the current state and limitations of the traditional integrated agriculture-aquaculture farming system (Steinbronn 2009), (2) analysing the nutritional quality of currently used and potentially useful local feed resources (Dongmeza 2009) and (3) developing cost-effective supplemental feeds for common carp based on locally-available feed ingredients (Tuan 2010). Based on these works, a set of improvements to the traditional pond management were defined in collaboration with local farmers. In the following chapters, this set of improvements is referred to as "modified pond management" and is compared with the traditional pond management. It is tested in ponds in the study area under field conditions for its effects on water quality, production conditions, nutrient utilisation efficiency, for its suitability for implementation by local farmers, and for its potential for improving their fish production and increasing their potential income.

1.4 Objectives

The main goals of this thesis were to evaluate the nutrient flows in fishponds under the modified pond management and to compare the modified and the traditionally applied pond management under local field conditions. These investigations may both complement and extend the work of previous scientists and provide local farmers and extension workers with information and ideas that would be an aid to future rural development in mountainous regions in South-East Asia that are comparable to the study area.

Specific goals were:

1.) to investigate the effects of the pesticide contaminated grass that was being used as feed for grass carp,

2.) to examine the effects of pond management modifications on limnological parameters, nutrient utilisation, natural food webs, fish production and financial benefit,

3.) to assess the potential use of alternative feed resources as supplemental feeds for common carp, and

4.) to evaluate the willingness of local farmers to adopt the different modifications in pond aquaculture.

1.5 Organisation of the thesis

The thesis is written as a cumulative dissertation and combines six articles and a book chapter. Three of the six articles has been accepted for publication by a peer-reviewed journal while two manuscripts are currently under the review processes of peer-reviewed journals. The book chapter is published and was reviewed by two reviewers chosen by the book editors. All research activities described in the thesis were funded by the Deutsche Forschungsgemeinschaft (DFG) and were performed under the umbrella of the Uplands Program (SFB 564).

The first article, “Pesticide contaminated feeds in integrated grass carp aquaculture: Toxicology and bio-accumulation” (chapter 2), evaluates the effects of pesticide contaminated grass from rice paddy dikes on the health and feed intake of grass carp as well as the accumulation of active compounds in the flesh of this fish species cultured under field conditions in the uplands of Northern Vietnam. This paper is published in the journal *Diseases of Aquatic Organisms* and is a valuable resource for anyone attempting to improve feeding management in integrated aquaculture systems that use plant material from pesticide treated crops as feeds for grass carp.

The second article, “Effects of modified pond management on limnological parameters in small-scale aquaculture ponds in mountainous Northern Vietnam” (chapter 3), compares the traditional pond management of small-scale farmers in Northern Vietnam with a modified pond management system with regard to limnological factors and the abundance of natural food resources in the ponds. This paper is published in the journal *Aquaculture Research* and may help to better understand the biological processes and culture conditions in small aquaculture ponds under different management. Further, this work may help small-scale farmers and their advisors who want to adopt management strategies that improve fish production by changing pond conditions.

The third article, “Pond management strategies for small-scale aquaculture in Northern Vietnam: Fish production and economic performance” (chapter 4), compares the traditional and the modified pond management with regard to fish production, the efficiency of applied nutrients (feeds, fertilisers) in increasing fish biomass, and economic benefits. This paper is published in the journal *Aquaculture International* and may help to better understand the utilisation efficiencies of nutrients from fertilisers and feeds into fish biomass in polycultures in pond aquaculture. Further, it may help to increase fish production by small-scale farmers in subtropical upland areas through the change of water management and the use of supplemental pelleted feeds and higher quality fertilisers.

Whether the traditional and modified pond managements differ in the nitrogen dynamics within the pelagic and benthic web of natural food resources is evaluated in the fourth article entitled “^{15}N tracer application to evaluate nitrogen dynamics of food webs in two subtropical small-scale aquaculture ponds under different managements” (chapter 5). The paper is published in the journal *Isotopes in Environmental and Health Studies* and might contribute to a better understanding of fertilisation management as a means of improving nutrient utilisation in ponds.

In the fifth article, “Earthworm meal as fishmeal replacement in plant-based feeds for common carp in semi-intensive aquaculture in rural Northern Vietnam” (chapter 6), the potential for replacement of fishmeal in supplemental feeds by earthworm meal is evaluated under pond conditions. This article is published in the *Turkish Journal of Fisheries and Aquatic Sciences* and may support the utilisation of alternative feed ingredients in aquafeeds allowing fishmeal replacement in aquaculture at different scales.

The book chapter "Improved sustainable aquaculture systems for small-scale farmers in northern Vietnam" (chapter 7) is part of the book "Sustainable land use and rural development in South-East Asia: Innovations and policies for mountainous areas" published by Springer Verlag. This book chapter summarises the traditional aquaculture in the study area, its limitations and the potential for improvements in pond management in small-scale farms. The chapter also contains results from an interdisciplinary investigation that evaluated the willingness of farmers to change their traditional pond management and apply the tested modifications. This information is valuable for those who want to modify any proposed changes in pond management so that they are more acceptable to small-scale farmers or to design efficient training and dissemination strategies in the uplands of Northern Vietnam.

In chapter 8, the findings of the research presented in this thesis are discussed and conclusions drawn. Finally, summaries of the thesis are given in chapters 9 (English summary) and 10 (German summary).

1.6 References

Ahmed N, Zander KK, Garnett ST (2011) Socioeconomic aspects of rice-fish farming in Bangladesh: opportunities, challenges and production efficiency. The Australian Journal of Agricultural and Resource Economics 55:199-219

Anrooy R van (2001) Fish marketing in the northern mountains of Viet Nam. FAO Aquaculture Newsletter 28:8-10

Anyusheva M, Lamers M, La N, Nguyen VV, Streck T (2012) Fate of pesticides in combined paddy rice–fish pond farming systems in Northern Vietnam. Journal of Environmental Quality 41:515-525

Begum NN, Chakraborty SC, Zaher M, Abdul MM, Gupta MV (1994) Replacement of Fishmeal by Low-Cost Animal Protein as a Quality Fish Feed Ingredient for Indian Major Carp, *Labeo rohita*, Fingerlings. Journal of the Science of Food and Agriculture 64:191-197

Bush SR, Belton B, Hall D, Vandergeest P, Murray FJ, Ponte S, Oosterveer P, Islam MS, Mol APJ, Hatanaka M, Kruijssen F, Ha TTT, Little DC, Kusumawati R (2013) Certify Sustainable Aquaculture? Science 341:1067-1068

Chilton EW, Mouneke MI (1992) Biology and management of grass carp (*Ctenopharyngodon idella*, Cypinidae) for vegetation control: a North American perspective. Reviews in Fish Biology and Fisheries 2:283-320

De Silva SS (1993) Supplemental feeding in semi-intensive aquaculture systems. In: New MB, Tacon AGJ, Csavas I (eds.) Farm-made aquafeeds. Proceedings of the FAO/AADCP Regional Expert Consultation on Farm-Made Aquafeeds, 14-18 December 1992. FAO-RAPA/AADCP, Bangkok, Thailand, 434p., pp. 24-60

De Silva SS (2003) Culture-based fisheries: an underutilised opportunity in aquaculture development. Aquaculture 221:221-243

Dey MM, Ahmed M (2005) Aquaculture - Food and livelihood for the poor in Asia: A brief overview of this issues. Aquaculture Economics and Management 9:1-10

Dey MM, Prein M (2005) Increased income from seasonally flooded rice fields through community based fish culture in Bangladesh and Vietnam. Plant Production Science 8:349-353

Dongmeza E (2009) Studies on the nutritional quality of plant materials used as fish feed in Northern Vietnam (PhD thesis). Institute for Animal Productions in the Tropic and Subtropics, Hohenheim Stuttgart, Germany, University of Hohenheim, 55p.

Dongmeza E, Steinbronn S, Francis G, Focken U, Becker K (2009) Investigations on the nutrient and antinutrient content of typical plants used as fish feed in small scale aquaculture in the moutainous regions of Northern Vietnam. Animal Feed Science and Technology 149:162-178

Dongmeza E, Francis G, Steinbronn S, Focken U, Becker K (2010) Investigation on the digestibility and metabolizability of the major nutrients and energy of maize leaves and barnyard grass in grass carp (*Ctenopharyngodon idella*). Aquaculture Nutrition 16:313-326

Edwards P, Pullin RSV, Gartner JA (1988) Research and education for the development of integrated crop-livestock-fish farming systems in the tropics. ICLARM Studies and Reviews 16, International Center for Living Aquatic Resources Management, Manila, Philippines, 53p.

Edwards P (2000) Aquaculture, poverty impacts and livelihoods. ODI Natural Resource Perspectives 56:1-4

FAO (1997) Aquaculture Development. FAO Technical Guidelines for Responsible Fisheries No. 5, FAO, Rome, 40p.

FAO (2005) Increasing the Contribution of Small-scale Fisheries to Poverty Alleviation and Food Security. FAO Technical Guidelines for Responsible Fisheries No. 10, FAO, Rome, 79p.

FAO (2011) Fisheries and Aquaculture Department, Statistics and Information Service FishStatJ: Universal software for fishery statistical time series.

FAO (2012) The State of World Fisheries and Aquaculture. FAO Fisheries and Aquaculture Department, FAO, Rome, 209p.

Hardy RW (2010) Utilization of plant proteins in fish diets: Effects of global demand and supplies of fishmeal. Aquaculture Research 41:770-776

Hernández C, Olvera-Novoa MA, Hardy RW, Hermosillo A, Reyes C, González B (2010) Complete replacement of fish meal by porcine and poultry by-product meals in practical diets for fingerling Nile tilapia *Oreochromis niloticus*: Digestibility and growth performance. Aquaculture Nutrition 16:44-53

Hop LT (2003) Programs to improve production and consumption of animal source foods and malnutrition in Vietnam. Journal of Nutrition 133:4006-4009

IFAD (2012) Rural Poverty Portal: Rural poverty in Viet Nam. http://www.ruralpovertyportal.org/country/home/tags/viet_nam. Accessed on 15th Feb 2013

Islam MS (2004) Urban and peri-urban aquaculture as an immediate source of food fish: Perspectives of Dhaka City, Bangladesh. Urban Ecosystems 7:341-359

Islam MS, Wahab MA (2005) A review on the present status and management of mangrove wetland habitat resources in Bangladesh with emphasis on mangrove fisheries and aquaculture. Hydrobiologia 542:165-190

Kawarazuka N (2010) The contribution of fish intake, aquaculture, and small-scale fisheries to improving food and nutrition security: A literature review. The WorldFish Center Working Paper No. 2106. The WorldFish Center, Penang, Malaysia, 51p.

Kestemont P (1995) Different systems of carp production and their impacts on the environment. Aquaculture 129:347-372

Lamers M, Anyusheva M, La N, Nguyen VV, Streck T (2011) Pesticide pollution in surface- and groundwater by paddy rice cultivation: A case study from Northern Vietnam. Clean – Soil, Air, Water 39:356-361

Little DC, Surintaraseree P, Innes-Taylor N (1996) Fish culture in rainfed rice fields of Northeast Thailand. Aquaculture 140:295-321

Little DC, Edwards P (2003) Integrated livestock-fish farming systems. FAO, Rome, 177p.

Little DC, Bush SR, Belton B, Phuong NT, Young JA, Murray FJ (2012) Whitefish wars: Pangasius, politics and consumer confusion in Europe. Marine Policy 36:738–745

Luu LT (2001a) Sustainable aquaculture for poverty alleviation (SAPA): A new rural developement strategy for Viet Nam - Part 1. In: FAN, FAO Aquaculture Newsletter (27). FAO, Rome, pp. 12-17

Luu LT (2001b) Sustainable aquaculture for poverty alleviation (SAPA): A new rural developement strategy for Viet Nam - Part 2: Implementation of the SAPA strategy. In: FAN, FAO Aquaculture Newsletter (28). FAO, Rome, pp. 4-7

Luu LT (2001c) The VAC system in Northern Vietnam. In: Integrated agriculture-aquaculture: A primer. FAO/IIRR/WorldFish Center, Fisheries Technical Paper 407, Rome, pp. 29-32

Milstein A (1992) Ecological aspects of fish species interactions in polyculture ponds. Hydrobiologia 231:177-186

Minh T (2010) Agriculture innovation system in Vietnam Northern mountainous region - Six decade shift from a supply driven to a diversification oriented system. Kommunikation und Beratung 95, Margraf Publishers, Weikersheim, 271p.

Minot N, Epprecht M, Anh TTT, Trung LQ (2006) Income diversification and poverty in the Northern Uplands of Vietnam. International Food Policy Research Institute (IFPRI), Research Report 45, Washington, D.C., 137p.

Mishra V, Ray R (2009) Dietary diversity, food security and undernourishment: The Vietnamese evidence. Asian Economic Journal 23:225-247

Nandeesha MC, Srikanth GK, Basavaraja N, Keshavanath P, Varghese TJ, Bano K, Ray AK, Kale RD (1988) Influence of earthworm meal on the growth and flesh quality of common carp. Biological Wastes 26:189-198

Naylor RL, Hardy RW, Bureau DP, Chiu A, Elliott M, Farrell AP, Forster I, Gatlin DM, Goldburg RJ, Hua K, Nichols PD (2009) Feeding aquaculture in an era of finite resources. PNAS, 106:15103-15110

Neori A, Nobre AM (2012) Relationship between trophic level and economics in aquaculture. Aquaculture Economics and Management 16:40-67

Nguyen SH, Bui AT, Le LT, Nguyen TTT, De Silva SS (2001) The culture-based fisheries in small, farmer-managed reservoirs in two provinces of Northern Vietnam: an evaluation based on three production cycles. Aquaculture Research 32:975-990

Nhan DK, Phong LT, Verdegem MCJ, Duong LT, Bosma RH, Little DC (2007) Integrated freshwater aquaculture, crop and livestock production in the Mekong delta, Vietnam: Determinants and the role of the pond. Agricultural Systems 94:445-458

Nhan DK, Verdegem MCJ, Milstein A, Verreth JAV (2008) Water and nutrient budgets of ponds in integrated agriculture–aquaculture systems in the Mekong Delta, Vietnam. Aquaculture Research 39:1216 -1228

Nikolic N, Schultze-Kraft R, Nikolic M, Boecker R, Holz I (2008) Land degradation on barren hills: A case study in northeast Vietnam. Environmental Management 42:19-36

Normile D (2013) Vietnam Turns Back A `Tsunami of Pesticides`. Science 341:737-738

Ogunji JO, Kloas W, Wirth M, Neumann N, Pietsch C (2008) Effect of housefly maggot meal (magmeal) diets on the performance, concentration of plasma glucose, cortisol and blood characteristics of *Oreochromis niloticus* fingerlings. Journal of Animal Physiology and Animal Nutrition 92:511-518

Pauly D (2009) Beyond duplicity and ignorance in global fisheries. Scientia Marina 73:215-224

Phan PD, De Silva SS (2000) The fishery of the Ea Kao reservoir, southern Vietnam: a fishery based on a combination of stock and recapture, and self recruiting populations. Fisheries Management and Ecology 7:251-264

Phonekhampheng O, Hung LT, Lindberg JE (2008) Nutritive value of potential feed resources used in Laos for African catfish (*Clarias gariepinus*) production. Livestock Research for Rural Development 20: Article No. 207. Available at: http://www.lrrd.org/lrrd20/12/phon20207.htm. Accessed on November 9, 2013.

Prein M, Ahmed M (2000) Integration of aquaculture into smallholder farming systems for improved food security and household nutrition. Food and Nutrition Bulletin 21:466-471

Prein M (2002) Integration of aquaculture into crop-animal systems in Asia. Agricultural Systems 71:127-146

Saint-Macary C, Keil A, Zeller M, Heidhues F, Dung PTM (2010) Land titling policy and soil conservation in the Northern uplands of Vietnam. Land Use Policy 27:617-627

Sánchez-Muros M-J, Barroso FG, Manzano-Agugliaro F (2014) Insect meal as renewable source of food for animal feeding: a review. Journal of Cleaner Production 65:16-27

Schmitter P, Dercon G, Hilger T, Ha TTL, Thanh NH, Lam N, Vien TD, Cadisch G (2010a) Sediment induced soil spatial variation in paddy fields of Northwest Vietnam. Geoderma 155:298-307

Schmitter P, Dercon G, Hilger T, Hertel M, Treffner J, Lam N, Vien TD, Cadisch G (2010b) Linking spatio-temporal variation of crop response with sediment deposition along paddy rice terraces. Agriculture, Ecosystems and Environment 140:34-45

Schreinemachers P, Fröhlich HL, Clemens G, Stahr K (2013) From Challenges to Sustainable Solutions for Upland Agriculture in Southeast Asia (Chapter 1). In: Fröhlich HL, Schreinemachers P, Clemens G, Stahr K (eds.) Sustainable Land Use and Rural Development in Southeast Asia: Innovations and Policies for Mountainous Areas. Springer Verlag, Heidelberg, New York, Dordrecht, London, pp. 3-27

Steinbronn S (2009) A case study: Fish production in the integrated farming system of the Black Thai in Yen Chau district (Son La province) in mountainous North-Western Vietnam - Current state and potential (PhD thesis). Institute for Animal Productions in the Tropic and Subtropics, Hohenheim Stuttgart, Germany, University of Hohenheim, 207p.

Tacon AGJ, Stafford EA, Edwards CA (1983) A preliminary investigation of the nutritive value of three terrestrial lumbricid worms for rainbow trout. Aquaculture 35:187-199

Tacon AGJ (1988) The nutrition and feeding of farmed fish and shrimp - a training manual. In: 3. Feeding methods. FAO Field Document, Project GCP/RLA/075/ITA. Field Document 7/E, FAO, Brasilia, Brazil, 208 pp.

Tacon AGJ, Metian M (2008) Global overview on the use of fish meal and fish oil in industrially compounded aquafeeds: Trends and future prospects. Aquaculture 285:146-158

Tacon AGJ, Metian M, Turchini GM, de Silva SS (2010) Responsible aquaculture and trophic level implications to global fish supply. Reviews in Fisheries Science 18:94-105

Thai BT, Pham TA, Austin CM (2006) Genetic diversity of common carp in Vietnam using direct sequencing and SSCP analysis of the mitochondrial DNA control region. Aquaculture 258:228–240

Thai BT, Burridge CP, Austin CM (2007) Genetic diversity of common carp (*Cyprinus carpio* L.) in Vietnam using four microsatellite loci. Aquaculture 269:174-186

Tuan NN (2010) Development of supplemental diets for carp in Vietnamese upland ponds based on locally available resources (PhD thesis). Institute for Animal Productions in the Tropic and Subtropics, Hohenheim Stuttgart, Germany, University of Hohenheim, 159p.

Vien TD (2003) Culture, environment, and farming systems in Vietnam's Northern mountain region. Southeast Asian Studies 41:180-205

Viola S (1989) Production of commercial feeds for warm water fish. In: Shilo M, Sarig S (eds.) Fish Culture in Warm Water Systems: Problems and Trends. CRC Press Inc., Boca Raton, Florida, pp. 143-162

2 Pesticide contaminated feeds in integrated grass carp aquaculture: Toxicology and bio-accumulation

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Abstract

Effects of dissolved pesticides on fish are widely described, but little is known about effects of pesticides contaminated feeds orally been taken up by fish. In integrated farms, pesticides used on crops may affect grass carp which has fed on plants from these fields. In Northern Vietnam, grass carp suffer seasonal mass mortalities which may be caused by pesticide contaminated plant feeds. To test effects of pesticide contaminated feeds on health and bioaccumulation in grass carp, a net-cage trail was conducted, feeding five differently contaminated grasses. Grass was spiked with two levels of trichlorfon/fenitrothion and fenobucarb. Unspiked grass was used as a control. Fish were fed at a daily rate of 20% of body mass for 10 days. The concentrations of fenitrothion and fenobucarb in pond water increased over time. Effects on fish mortality were not found. Fenobucarb in feed showed strongest effects on fish by lowering feed uptake, deforming hepatopancreas, increasing blood glucose and reducing cholineesterase activity in blood serum depending on feed uptake. Fenobucarb showed increased levels in flesh in all treatments suggesting bio-concentration. Trichlorfon and fenitrothion did not significantly affect feed uptake but showed concentration dependant reduction of cholineesterase activity and hepatopancreatic changes. Fenitrothion showed bioaccumulation in flesh which was dependant on feed uptake whereas trichlorfon was only detected in very low concentrations in all treatments. Pesticide levels were all detected below the maximum residue levels in food. Neither tested pesticide contaminated feeds caused mortality in grass carp but were associated with negative physiological responses and may thereby raise susceptibility towards diseases.

2.1 Indroduction

Mountainous areas of northwest Vietnam have a long tradition of rice and fish culture. Typically, rice paddies and fish ponds are located side by side, with irrigation water flowing from rice paddies to fish ponds and vice versa. To increase yields, rice paddy management in this region includes the use of highquality seeds and chemical fertilizers as well as herbicides, insecticides, rodenticides and fungicides. Pesticides are typically applied 2 or 3 times per rice cultivation period. Most farmers apply pesticides at the same time, following the advice of the governmental extension service, which is communicated viara dio and local loud speakers. While rice paddy culture has changed significantly and has become an intensive production system over the past decade, aquaculture management is still performed traditionally. In this study, we focussed on 3 pesticides (trichlorfon, fenitrothion, fenobucarb; Table 2.1) that have often been applied in northern Vietnam. Trichlorfon and fenitrothion are organophosphate (OP) pesticides, whereas fenobucarb is a carbamate.

Table 2.1: Properties of applied pesticides (Hertfordshire 2011).

Pesticide	Water solubility [mg L^{-1}]	Log K_{ow}	Acute 96h LC_{50} to fish [mg L^{-1}]	Chronic 21 day NOEC [mg L^{-1}]
Trichlorfon	120000	0.43	0.7[1]	-
Fenitrothion	19	3.32	1.3[1]	0.088[1]
Fenobucarb	420	2.78	1.7[2]	0.2[a]

[1] to *Onchorhynchus mykiss* (rainbow trout)

[2] to *Cyprinus carpio* (common carp)

[a] 14 d-NOEC to *Cyprinus carpio* (MEJ, 2013)

All 3 substances are non-specific insecticides. Their mode of action is non-systemic with contact and stomach action. Their toxic effect is based on inhibition of cholinesterase (ChE) by binding to the esteratic site of the enzyme. ChE has a crucial function in all vertebrates; it hydrolyses acetylcholine, a neurotransmitter in the central and peripheral nervous system that transmits nerve signals across cholinergic synapses and is afterwards hydrolysed by acetyl- or butyryl-ChE (AChE or BChE). If this process is interrupted by inhibiting the ChE, the result is an accumulation of acetylcholine in the synapses and thus overstimulation of postsynaptic cholinergic receptors (Pope 1999), leading to rapid twitching of voluntary muscles followed by paralysis (Fulton & Key 2001). The inhibition of ChE by binding OP pesticides is irreversible, and recovery depends on new enzyme synthesis, whereas carbamated enzymes can slowly recover (Sturm *et al.* 2000). This mechanism is so specific that it has been widely accepted as a bioindicator of exposure to OP and carbamate insecticides. More specifically, numerous studies have inves tigated ChE inhibition in fish species related to exposure to dissolved pesticides in surrounding water (Weiss 1961, Gruber & Munn 1998, Chuiko 2000, Kirby *et al.* 2000, Sturm *et al.* 2000, Fulton & Key 2001, Aker *et al.* 2008, Halappa & David 2009, Kumar *et al.* 2011). While pesticide contamination via water inflow to ponds has been studied elsewhere (e.g. Lamers *et al.* 2011, Anyusheva *et al.* 2012), the import pathway of pesticides to fish ponds via contaminated feeding material and their bioaccumulation in fish has not yet been considered in environmental studies, although it may also constitute a significant potential threat to integrated aquaculture. The grass carp *Ctenopharyngodon idella*, a member of the family Cyprinidae, plays an important role in carp polyculture systems in Asia. Above a body length of about 25 mm (De Silva & Weerakoon 1981), grass carp are capable of efficiently shredding plantderived material such as filamentous algae, emerged plants, grasses and leaves of trees and crops (Prowse 1971, Opuszynski 1972). Due to the low quality and digestibility of these feed resources, the amount ingested by grass carp may reach more than 100% of their body mass per day at water temperatures of 22 to 33°C (Opuszynski 1972). However, the actual amount varies greatly depending on water temperature (Opuszynski 1972, Cai & Curtis 1990, Osborne & Riddle 1999) and fish live mass (Osborne & Riddle 1999). This macro-herbivore feeding habit allows farmers to grow grass carp with low financial input and to run a cash-generating pond aquaculture by feeding agricultural by-products. For more than a decade, the populations of grass carp in the uplands and lowlands of northern Vietnam have been suffering from high mortality rates, usually in the rainy season from March to November (Van *et al.* 2002, Steinbronn 2009, Pucher *et al.* 2013). Diseased fish show typical symptoms such as haemorrhagic changes and ulcers on the skin, darkening or bleaching of the skin, loss of scales, haemorrhagic intestines, necrosis of the gills, exophthalmia or enoph thalmia and erratic swimming behaviour (Van *et al.* 2002, Steinbronn 2009, Pucher *et al.* 2013). Mass mortalities of grass carp are attributed to a multi-factorial disease primarily caused by bacterial agents and may be triggered by unsuitable environmental factors, such as poor water quality, limited oxygen supply, poor feed bases and chronic or acute exposure to pesticides dissolved in water or included in feeds (Van *et al.* 2002, Steinbronn 2009, Anyusheva *et al.* 2012, Pucher *et al.* 2013). In mountainous northern Vietnam, leaves of maize, banana, cassava and bamboo as well as weeds, grasses, human wastes and animal manure are commonly applied to ponds (Luu 2001, Steinbronn 2009). On a dry matter basis, 17 ± 6% (mean ± SD) of all applied feed and fertilizer inputs were reported to originate from rice paddies and dykes, and the fresh matter amounted to 22.3 ± 10.7 t ha^{-1} $pond^{-1}$ yr^{-1} (Steinbronn 2009). These feed resources originating from rice paddies are especially available in the hot rainy season when rice is produced and include mixed weeds, water hyacinths and rice plants uprooted while thinning. Farmers have stated that they use feed resources from their own paddies and dikes or from collective feed resources (e.g. channels, rivers), which are potentially contaminated by pesticides (Steinbronn 2009). Pesticides in food are differently degradable by cooking at degradation temperatures above (e.g. fenitrothion), below or around 100°C (e.g. fenobucarb, trichlorfon; http://sitem.herts.ac.uk/aeru/footprint/index2.htm). In northern Vietnam, fish is often consumed raw (Kino *et al.* 1998, Steinbronn 2009, Phan *et al.* 2011). In Nam Dinh province, for example, 78% of persons interviewed stated that they eat raw fish (Nguyen & Thanh 2011). In the research area, raw or slightly acid marinated filets of silver carp and grass carp are combined with cut leaves to make a traditional salad. Consumers of raw fish run the risk of infection by parasites such as Chlonorchis sinensis or fishborne zoonotic trematodes (Kino *et al.* 1998, Phan *et al.* 2010, 2011) or ingestion of pesticides which have accumulated in vegetables and fish (Hoai *et al.* 2011). The aims of the present study were to investigate (1) whether pesticide-contaminated feed applied for a limited period of time (10 d, resembling the pesticide application practices of local farmers) leads to increased mortality or morbidity that has a measurable negative health effects in fish, and (2) whether pesticides taken up by fish via contaminated feed during a pesticide application campaign accumulate in fish flesh and thereby pose a potential threat to human health.

2.2 Materials and Methods

Experimental setup

In summer 2010, a survey of 145 small-scale farmers was performed in Chieng Khoi commune, Yen Chau district, Son La province, northern Vietnam. The sizes of paddy fields and the kind as well as the amount of pesticides applied were recorded and used to calculate the amounts of individual pesticides per application and per unit of paddy area. The 2 commonly used pesticide products Ofatox (trichlorfon and fenitrothion) and NIBAS (fenobucarb) were selected for this study. In 10 replicates, the biomass of grass per unit area of paddy dike was determined by cutting all biomass above the ground into defined areas of 5 rice paddy dikes. Under the assumption that the same amounts of pesticides are applied to the rice paddy and the paddy dike, we estimated the amounts of active ingredients (AIs) per kg fresh weight of grass grown on the paddy dike. These amounts were most likely to be found in grass collected by farmers to feed their carp and were called 'average farm dose' (AFD), equalling 65 mg each of trichlorfon and fenitrothion kg^{-1} fresh weight of grass in paddy areas sprayed with Ofatox and 113 mg fenobucarb kg^{-1} fresh weight of grass in paddy areas sprayed with NIBAS. A total of 30 kg of uncontaminated grass was collected for the fish trial. Four aliquots of fresh grass were treated with Ofatox or NIBAS at AFD concentrations of trichlorfon + fenitrothion (Treatment A) and fenobucarb (Treatment D) and at doubled AFD concentrations (Treatments B and E, respectively). A control aliquot was treated only with water (Treatment C). The levels of pesticide in the grass in each of the 5 treatments are given in Table 2.2.

Table 2.2: Concentrations of active ingredients in fresh matter (FM) of grass fed to grass carp.

Treatment	Active ingredients	Concentration [mg kg^{-1} FM grass]	Contamination level
A	Trichlorfon & Fenitrothion	65	Farmer practice
B	Trichlorfon & Fenitrothion	130	Doubled farmer practice
C	None	0	Negative control
D	Fenobucarb	113	Farmer practice
E	Fenobucarb	226	Doubled farmer practice

The pesticides were applied to the grass aliquots using aerosol cans. For each pesticide product and the control, a separate aerosol can was used. Low pesticide concentrations were sprayed first. For spraying, the total amount of each grass aliquot was spread on a plastic sheet (2 × 3 m) lying on the ground. The respective amount of Ofatox or NIBAS was dissolved in 500 ml of water and sprayed onto the grass aliquot under temporary mixing of the grass to ensure a homogenous inoculation. After inoculation, the pesticide solution was left to soak in for about 30 min. The inoculated grass was then weighed out in daily feeding portions equivalent to 20% of the total body mass of all grass carp in each net cage. These feed portions were stored at -18°C and defrosted 1 h before feeding. Twenty net cages (1.5 × 1.5 × 2 m) were installed in a pond (depth 1.2 m, in Sap Vat village, Yen Chau district) to assure the same environmental conditions in all replicates. Each cage rested at the bottom of the pond and was covered with a net over the top to prevent the fish from jumping out. Each cage was stocked with 3 grass carp (1 yr old; 171 ± 33 g body mass, 25.4 ± 1.8 cm total body length, n = 60, mean ± SD) taken from a neighbouring pond, which had been stocked with a single batch of grass carp fingerlings from the hatchery in Son La city. The treatments were distributed randomly among the cages. In 4 replicates, the groups of grass carp were offered the 5 trial feeds at a level of 20% of body mass per day for a period of 10 d from 5 to 14 November 2011. The grass used as feed was confined to the net cages and could not contaminate other replicates. We checked daily to determine whether any fish had died. After the final harvest of fish on Day 10, the uneaten grass in the cages was weighed to estimate the percentage of feed uptake.

Sampling and analysis

Mixed-grab water samples were taken on Days 0, 5 and 10 at 3 positions in the pond and were analysed for water-soluble pesticide exposure and nitrogen compounds. Temperature, oxygen concentration and pH were constantly monitored using a multi-electrode data logger in situ (Troll 9500). On Day 0, 4 groups of 3 grass carp, and on Day 10, all grass carp from each net cage were killed, scaled, filleted and freeze dried to determine pesticide concentrations in the flesh. The percentage of intestinal filling was estimated as a second measure to evaluate feed uptake. Macroscopic liver alterations were evaluated for individual fish modified after Adams *et al.* (1993) and were were rated on a scale of 1 to 5 (1: no deformation; 2: slight deformation; 3: medium deformation; 4: strong deformation; 5: very strong deformation). Blood samples were taken from all grass carp by heart puncture after they had been anaesthesised by percussion of the head and before they were killed by exsanguination. The blood was stored at 4°C for 10 h to allow clotting, before being centrifuged (700 × g for 10 min) to separate blood clots and serum. Blood serum was stored at -18°C and analysed for the concentration of BChE and glucose. Blood analysis of fish serum was performed on a fully selective auto-analyser for clinical chemistry (Cobas 6000/501c) and by the use of the ChE 2 Kit (Roche®), according to the manufacturers' recommendations. The principle of this method is that ChE hydrolyses butyrylthiocholine to thiocholine and butyrate. Thiocholine reduces the yellow pigment hexacyanoferrate III to the nearly colourless hexacyanoferrate II. This decrease in colour intensity is measured at 700/415 nm wavelengths at 37°C and is directly proportional to the ChE activity. BChE was used as a biomarker because it has been reported to be more active compared to AChE if BChE is present in the serum of cyprinids (Chuiko 2000). For measurement of the glucose concentration in serum, the auto-analyser Cobas 6000/501c and a glucose HK Kit (Roche®) were used according to the manufacturers' recommendations. In this method, hexokinase (HK) cata lyses the phosphorylation of glucose by ATP to form glucose-6-phosphate and ADP. To measure the extent of this reaction, a second enzyme, glucose-6-phosphate dehydrogenase is used to catalyse oxidation of glucose-6-phosphate by $NADP^+$ to form NADPH. The concentration of the NADPH formed is directly proportional to the glucose concentration, which is determined by measuring the increase in absorbance due to NADPH at 340 nm. For preparation of fish samples for pesticide analysis, fish flesh samples were ground with a grinder after the fish had been freeze-dried. Two grams of sample were weighed in a glass bottle. Surrogate diazinon-d10 and acetonitrile solvent were added to the bottle. The mixture was homogenized for 5 min. The extract was filtered through a layer of anhydrous sodium sulphate and concentrated to 1 mL by a rotary vacuum evaporator. The extracts were 'cleaned up' on 3 extraction columns (C18 [5 mg/3 ml, RP-18-erck K91203423], NH_2 [2 g/12 mL, OROCHEM – SY NH_2] and carbon [0.5 g/glass column, 20 cm length × 0.5 cm diameter]). Pesticides for analysis were eluted through the clean-up columns using 8, 25 and 60 ml of a solvent mixture of acetonitrile and toluene at a ratio of 3:1 (v/v). The eluate was concentrated to 1 mL. After adding 10 mL of a solvent mixture of hexane and acetone (1:1 v/v) and the internal standard chrysene-d12, the extract was again concentrated to 1 mL using a stream of nitrogen gas. For preparation of grass samples for pesticide analysis, dried grass samples were ground with a grinder. One gram of sample was weighed into a polypropylene tube. Surrogate diazinon-d10 and acetonitrile solvent were added to the tube, and the sample was homogenized for 1 min. After adding 4 g $MgSO_4$ and 1 g NaCl, the sample tube was shaken well for 1 min and centrifuged (400 × g) for 5 min. Half of the sample extract was concentrated by a rotary vacuum evaporator. The extract was cleaned up on the following columns: a carbon column (0.5 g/column, 20 cm length × 0.5 cm diameter), a Florisil cartridge (1 g/6 mL, Merck K93101027) and an NH_2 cartridge (2 g / 12 mL, OROCHEM - SY NH_2). Pesticides for analysis were eluted through the clean-up columns in 60, 10 and 25 mL volumes of a solvent mixture of acetonitrile and toluene at a ratio of 3:1 (v/v), respectively. The eluate was concentrated to 1 mL. After adding 10 mL of a solvent mixture of hexane and acetone (1:1 v/v) and an internal standard (chrysene-d12), the extract was concentrated to 1 mL again with a nitrogen gas stream. Two µL of pond water and the final sample extracts of fish and grass were analysed by capillary gas chromatography–mass spectrometry using GCMSQP2010 (Shimadzu) equipped with a capillary column 'OV-5MS' (30 m × 0.25 mm i.d. × 0.25 µm film thickness). The analytical conditions were as follows: injector temperature 250°C, injection mode splitless: split ratio 1:20, ion source temperature 230°C and detector temperature 290°C. The oven temperature was initially set to 100°C for 3 min, raised to 300°C at a rate of 10°C min^{-1}, and held for 10 min. Calibration was done with an internal standard (chrysen-d12). Diazinon-d10 was used as a surrogate standard. The specific ion mass (m/z) for quantification was 110, 121 and 277 for trichlorfon, fenobucarb and fenitrothion, respectively. For confirmation, the specific ion mass was 109, 145 and 79 for trichlorfon, 150 for fenobucarb and 260 and 125 for fenitrothion. Analytical methods for fish and grass samples were applied according to Takatori *et al.* (2008). For fish and grass samples, the detection limits of pesticides were 1.28, 1.21 and 0.74 ng g^{-1} of sample dry matter (DM) for trichlorfon, fenobucarb and fenitrothion, respectively. Detection limits of water-dissolved pesticides were 0.01, 0.005 and 0.01 ng L^{-1} for trichlorfon, fenobucarb and fenitrothion, respectively.

Statistical analysis

Data sets were analysed by 1-way ANOVAs with time 0 (T0) and the 5 feeding treatments as factors. Data sets were tested for homogeneity by Levene's test. Data distributions were checked visually for normality. To meet the assumptions required before ANOVA could be rigorously applied, data sets were log or square root transformed if needed. Fisher's least significant difference (LDS) tests were performed as post hoc tests. Data sets (trichlorfon flesh accumulation, % intestinal fill, grass eaten, liver alterations) which did not meet the assumptions necessary for ANOVAs or which were ordinal were analysed by using the non-parametric Kruskal-Wallis test followed by multiple comparison of mean ranks for all groups. All statistical analyses were performed using STATISTICA 8 (StatSoft®).

2.3 Results

Water parameters

The water quality parameters over the 10 d of the feeding trial were typical for an aquaculture pond. The pH of the pond water was 7.7, the redox potential was 782 ± 9 mV, and the average temperature was 24.9°C, with minimum and maximum water temperatures of 24.2 and 25.8°C. The dissolved oxygen was low with a mean (±SD) of 2.7 ± 1.7 mg L^{-1} and averaged daily minima of 0.9 ± 0.8 mg L^{-1}, which typically occurred at dawn. The turbidity was on average 35.8 ± 10.2 formazine nephelometric units (FNU). Concentrations of total ammonia nitrogen, nitrite nitrogen, nitrate nitrogen, orthophosphate phosphorus, total nitrogen and total phosphorus were 0.03 ± 0.00, 0.04 ± 0.00, 0.3 ± 0.1, 0.00 ± 0.00, 1.3 ± 0.2 and 0.1 ± 0.0 mg L^{-1}, respectively. The concentrations of fenitrothion and Fenobucarb in water increased from Day 0 to Day 10, whereas for trichlorfon a concentration of 0.31 µg L^{-1} was detected on Day 0, but on Days 5 and 10, the concentration was below the detection limit (Table 2.3).

Table 2.3: Means concentration of dissolved active ingredients (±SD) in pond water over the period of the feeding trial.

Day	Trichlorfon [µg L^{-1}]	Fenitrothion [µg L^{-1}]	Fenobucarb [µg L^{-1}]
0	0.031±0.010	0.021±0.003	0.12±0.03
5	n.d.	0.058±0.006	0.34±0.042
10	n.d.	0.075±0.001	0.465±0.021

n.d.: not detected

The concentrations of fenitrothion and fenobucarb increased by a factor of 3.5 and 3.9 from Day 0 to Day 10, respectively. Maximum concentrations reached 0.075 and 0.465 µg L^{-1} for fenitrothion and fenobucarb, respectively. The grass fed to grass carp within the trial had a mean (±SD) dry mass of 28.1 ± 1.8% of fresh mass (n = 6; crude ash 15.6% of DM, crude lipid of 1.7% of DM). Table 2.4 summarizes the amounts of AIs fed daily per g fresh mass of fish in the 5 treatment groups. No contami nation of pesticide AIs was detected on grass which was fed to the control group of fish (Treatment C) (Table 2.4). Control fish consumed all offered grass, which was confirmed by the feed uptake estimates shown in Table 2.5. When sprayed on the feed, trichlorfon and fenitrothion (Treatments A and B) led to a reduction in feed uptake compared to the control and accounted for about 70% of offered feed. Fenobucarb in concentrations found in AFD (Treatment D) reduced the feed uptake to about 40% of offered feed and to about 20% under the doubled concentration.

Table 2.4: Means (±SD) of feed uptake estimates and macroscopic alterations of liver on the 10th day of the trial and concentration of blood serum glucose and Butyl-Cholinesterase (BChE) activity in blood serum of grass carp before (T0) and after 10 days of feeding grass treated with pesticide products.

Treatment	**Intestine filling [%]**	**Grass eaten [% of a daily grass offered]**	**Macroscopic liver deformation scale**	**Glucose [mg dL^{-1} serum]**	**BChE [U L^{-1} serum]**
T0	-	-	-	115±13 z	93±33 zx
A	70±44 zx	75±6 zx	1.0±0.0 z	101±19 zx	84±50 zxt
B	72±42 zx	65±13 zx	2.3±0.9 zx	96±19 yxw	48±17 ywv
C	100±0 z	100±0 z	1.0±0.0 z	91±11 y	83±22 zxut
D	36±40 yx	40±8 yx	4.1±0.2 yx	107±22 zw	55±20 yvt
E	10±0 yx	23±15 yx	5.0±0.0 y	117±9 z	63±25 zxv

Mean values in the column that do not share the same superscript(s) differ significantly at $P \leq 0.05$.
Macroscopic alterations of liver are transformed into a scale from 1 to 5 (1: no deformation; 2: slight deformation; 3: medium deformation; 4: strong deformation; 5: very strong deformation)

Table 2.5: Means (±SD) of the amounts of active ingredients (AI) daily offered on spiked grass per g fresh mass of grass carp fresh matter (FM) over the 10 day period of the feeding trial and AI accumulated in the flesh (DM) of grass carp fed before (T0) and after 10 days of feeding the contaminated grass. Cells with gray shading refer to grass carp groups been fed with the respective AI.

Treatment	**Trichlorfon**		**Fenitrothion**		**Fenobucarb**	
	AI fed [ng [AI] g^{-1} fish FM]	**AI in flesh [ng [AI] g^{-1} flesh DM]**	**AI fed [ng [AI] g^{-1} fish FM]**	**AI in flesh [ng [AI] g^{-1} flesh DM]**	**AI fed [ng [AI] g^{-1} fish FM]**	**AI in flesh [ng [AI] g^{-1} flesh DM]**
T0		0.0±0.0		3.9±3.5 z		3.4±0.9 z
A	2417±157	0.0±0.0	2205±144	103.7±29.0 y	18±1	12.5±6.6 y
B	3667±239	1.1±2.2	5463±356	156.3±23.6 y	31±2	9.0±3.7 y
C	0±0	6.0±7.0	0±0	8.7±5.8 z	0±0	10.1±2.8 y
D	9±1	0.0±0.0	7±0	10.9±2.9 z	16764±1092	13.9±4.6 y
E	7±0	1.2±2.2	26±2	10.9±8.9 z	22566±1469	11.5±1.0 y

Mean values in the columns of AI accumulated in fish flesh that do not share the same superscript(s) differ significantly at $P \leq 0.05$. Mean values in column without superscripts did not differ significantly.

Health effects

Although over the 10 d of the feeding experiment none of the fish died, various other health effects were observed. BChE was detected in blood serum of all grass carp. BChE activity in the blood serum of grass carp was lowered when the fish were offered feed treated with pesticides (Table 2.5), especially high concentrations of trichlorfon and fenitrothion (Treatment B). Compared to the control group, BChE activity decreased by 43% in Treatment B, by 34% in Treatment D and by 24% in Treatment E. In Treatment A, activity increased slightly by 1%. The blood serum glucose concentration was significantly lower in fish fed unspiked grass (Treatment C) than in fish that were sampled before the trial started (Table 2.5). Within the trial, fish in all treatments had higher blood serum glucose levels than the controls, but fish that were treated with fenobucarb (Treatments D and E) had higher levels than those dosed with tri chlorfon + fenitrothion (Treatments A and B). Macroscopic symptoms of disease, such as haemorrhagic changes and ulcers on the skin, darkening or bleaching of the skin, loss of scales, necrosis of the gills, exophthalmia, enophthalmia and erratic swimming behaviour, were not apparent in any of the grass carp in this trial. Fish were examined for internal macroscopic changes, whereupon we found that the treatments had profound and different effects on the colour of the liver (Table 2.5). Fish fed grass with no AIs (Treatment C) had red livers. Feeding of trichlorfon and fenitrothion at the concentration of AFD (Treatment A) did not affect the colour of the liver, but double the AFD amount resulted in a yellow colouration. The application of fenobucarb had a profound effect on liver colour, which changed from intense yellow to brown with increasing concentration in the grass offered (Treatments D and E).

Pesticide concentration in flesh

The fresh fish flesh sampled for the pesticide accumulation measurements accounted for 34.9 ± 2.1% (mean ± SD) of the sampled grass carp. These samples had a mean dry mass of 20.9 ± 0.8% of the fresh mass. The flesh of experimental fish sampled at T0 showed low accumulation of all 3 tested AIs. Trichlorfon offered on grass did not accumulate in the flesh (Table 2.4), even though most of the offered grass was consumed by the fish (Table 2.5). Trichlorfon was only detected in 1 fish in 2 other treatments each. Fenitrothion fed to grass carp accumulated significantly in the flesh over the 10 d of feeding and showed significantly higher accumulation compared to that in fish that were not fed grass spiked with Fenitrothion (Table 2.4). Fenobucarb showed an increased accumulation in all fish, but the increase was statistically significant only in the flesh of fish which were offered grass spiked with the lower concentration of Fenobucarb (Table 2.4). Offering grass spiked with higher concentrations of this AI did not increase the accumulation in flesh, as the grass was not accepted by grass carp (Table 2.4).

2.4 Discussion

Water parameters

The water parameters of the experimental pond met the requirements for grass carp culture. The water temperature was in the optimal temperature range for intensive feeding of grass carp (Opuszynski 1972). In the morning, dissolved oxygen levels were recorded to be near the minimal tolerable level of 0.5 mg L^{-1} for grass carp (Froese & Pauly 2013) which are typical in this region (Steinbronn 2009, Pucher *et al.* 2013) but did not visibly affect the health of the experimental grass carp. In pond water, concentrations of fenitrothion and fenobucarb increased significantly during the study, whereas concentrations of trichlorfon could not be detected on Days 5 and 10. This finding is surprising, given that trichlorfon has by far the highest water solubility of the 3 tested substances. On the other hand, trichlorfon also quickly degrades to dichlorvos, which was not analysed. The increasing concentrations of fenitrothion peaked at 0.075 µg L^{-1} and of fenobucarb at 0.465 µg L^{-1}. These results clearly indicate that a fraction of the pesticides spiked on the grass fed to the fish also dissolved in the water of the pond. The more soluble Fenobucarb reached higher concentrations than the less soluble fenitrothion. In all cases, the concentration levels remained below the lethal concentration (LC_{50}) given in Table 2.1. However, fish in the control group (Treatment C) showed some alterations of the measured parameters in comparison to fish before the treatments (T0), thus indicating a contribution of waterborne pesticide exposure to the observed effects. Very limited information is available on pesticide effects on grass carp. The highest measured concentrations of fenitrothion and feno bucarb (0.075 and 0.465 µg L^{-1}, respectively) were far below the no observed effects concentrations (NOECs) to Cyprinus carpio reported at 0.088 and 0.2 mg L^{-1}, respectively (see Table 2.1). For trichlorfon, no NOEC was found in the literature. However, given the fact that trichlorfon was not found at concentrations above the detection limit (0.01 ng L^{-1}) in the course of the experiment, no effect of trichlor fon is expected, but effects of its metabolite dichlorvos (not measured) cannot be excluded. However, NOECs are usually determined under controlled laboratory conditions without other stressors for the fish. Under pond conditions, health effects of pesticide concentrations could occur below the NOEC, due to coupled effects of a variety of stress factors. This is indicated by the predicted no effect concentration (PNEC), below which absolutely no effects would be expected. PNEC values are often considerably lower than the NOEC values. In case of fenitrothion, for instance, the PNEC is 0.00021 µg L^{-1} (vs. the NOEC of 0.088 mg L^{-1}) and hence was exceeded by the observed concentration. However, in this trial, feedborne pesticide exposure effects were evaluated, not the potential waterborne pesticide exposure effects from dissolved pesticide concentrations carried into the pond via the treated feeding material. Therefore, all measured effects of all treatment groups were evaluated versus the control group and not versus T0.

Health effects

The type and amount of grass was fully accepted by grass carp, as the amount offered (20% of live weight per day) was consumed completely. Spiking the feeds with the 2 pesticide products had significant effects on the feed acceptance of grass carp. Trichlorfon and fenitrothion on the grass resulted, on average, in a slight reduction of feed uptake which was not concentration dependent. Fenobucarb on the grass resulted in a significantly reduced feed uptake of spiked grass which was concentration dependent. It is not known whether the refusal of pesticide spiked grass was caused by a decrease in palatability or by reduced appetite caused by negative effects of the AIs on the health of the fish. However, pesticides are known to reduce feed intake in fish (Kestemont & Baras 2001). The spiked feeds had feed-dependent effects on macroscopic liver colouration. In crucian carp, trichlorfon dissolved in ambient water had strong effects on hepatic pathways of lipid metabolism and increased lipid accumulation in the liver (Xu *et al.* 2012). Little information has been published on macroscopic changes in the colour of fish livers. However, laboratory tests and field studies have demonstrated that fish exposed to pesticides show pathologic changes in the liver (Kumar & Ansari 1986, Gill *et al.* 1988) and that OPs are reported to have negative effects on the antioxidant system of carp and therefore lead to 'oxidative stress' (Hai *et al.* 1997). This 'oxidative stress' has been found to be the cause of jaundice, which causes yellowish decolouration in the liver of yellowtails Seriola quinqueradiata (Sakai *et al.* 1998). Wolf & Wolfe (2005) stated that a general macroscopic response of the fish liver to toxins is a darkening of the liver. This liver colouration is a strong indication of pathological damage to the organ. Liver changes affect fitness of fish (Rodrigues & Fanta 1998). BChE was detected in the blood serum of all grass carp. This makes grass carp a member of the family Cyprinidae in which BChE can be used as marker for pesticide treatment (Chuiko 2000). All treatments, including the control group Treatment C, showed decreased BChE activity compared to fish sacrificed at T0. Fish in Treatment C, which had been fed uncontaminated feed, showed a BChE activity decrease of 11%. This effect is likely to be derived from pesticides dissolved in the water. However, the changes in BChE activity of treatment groups compared to the control group can be attributed to effects caused by the pesticide-spiked feeding material and therefore indicate that pesticide intake via feed plays an important role in addition to any effects due to dissolved compounds. The feeding of trichlorfon and fenitrothion at AFD levels (Treatment A) did not lead to any significant change in BChE activity compared to the control group and therefore seems to be tolerable for grass carp. However, the feeding of the same AI at double the AFD concentrations led to the highest BChE decrease of all treatments; the BChE activity was decreased by 43% compared to Treatment C. The feed acceptance of fish in Treatments A and B was comparable. Therefore, the level of BChE activity decrease seems to be concentration dependent. Both AIs of Treatments A and B are OP pesticides which lead to irreversible BChE inhibition (Sturm *et al.* 2000). The decrease in BChE activity in fish from Treatments D and E was less distinct. Feeding of the AI fenobucarb at AFD levels (Treatment D) resulted in 34% reduced BChE activity compared to Treatment C (control). Feeding of the same AI at double the AFD concentrations led to a smaller reduction in BChE activity of 24% compared to Treatment C. This effect might be explained by the fact that Fenobucarb seemed to have a strong effect on feed acceptance. Grass in Treatment E was less well accepted than that in Treatment D. Furthermore, macroscopic health effects indicated that fish from Treatment E were suffering stronger impacts on their vitality than fish from Treatment D. Thus, the fish from Treatment E incorporated less feeding material and therefore less fenobucarb than fish from Treatment D. Furthermore, fenobucarb is a carbamate insecticide, and its ChE inhibiting effects are to a certain degree reversible (Sturm *et al.* 2000). The recovery from ChE inhibition could have been expressed to different degrees in individual fish. Overall, our findings are comparable to data in the current scientific literature. Gruber & Munn (1998) reported that the mean whole-brain ChE activity of carp exposed to OP and carbamate insecticides was 34% less than that of carp from a lake that was not influenced by agricultural irrigation waters. A review on AChE inhibition in estuarine fish as an indicator of OP insecticide exposure (Fulton & Key 2001) indicates that AChE inhibition levels of 50% can lead to sublethal effects on stamina and that AChE inhibition levels of >70% are associated with mortality in most tested species. Concentration dependency of AChE inhibition was shown by Kumar *et al.* (2011), who reported that increasing levels of endosulphan in the water reduced the AChE activity in Nile tilapia. Serum glucose levels are described as a general stress-indicating parameter in fish (Wedemeyer 1972). In this study, the pesticide-contaminated feeds affected the serum glucose significantly, with higher levels of glucose in the serum of fish fed pesticide treated grass. No comparative data are available in the literature for the haematological response of fish to pesticide-contaminated feed. However, sub-lethal concentrations of pesticides in water increased blood glucose levels in fish showing an increased stress level (Chandrasekar & Jayabalan 1993, Sweilum 2006, Kumar *et al.* 2011). The elevated glucose level in grass carp at T0 is most likely caused by the stress of handing and by the limited acclimatization time in net cages prior to the trial.

Pesticide concentration in flesh

Among the 3 tested AIs, only fenitrothion showed a clearly feed-dependent accumulation in the fresh flesh with a bioaccumulation rate of about 1% and 0.6% (Treatments A and B) of the offered amount of AI over the feeding period. Fenitrothion concentrations increased in all fish compared to concentrations at T0. However, the fenitrothion concentration in fish of Treatment groups C, D and E increased only by a factor of 2 to 3× compared to T0, whereas in the 2 groups which were fed fenitrothion (Treatments A and B), the increase was by a factor of 26× (Treatment A) and 40× (Treatment B). This very high increase in fish fed with fenitrothion-spiked grass compared to other groups must be caused by intake via feeding materials. Trichlorfon and fenobucarb levels in fish flesh did not show treatment-dependent differences but increased significantly during the course of the feeding trial, which suggests that the concentration of these 2 pesticides dissolved in pond water is of more significance than the contamination of feed. These findings are in line with the physical properties of the 3 AIs and also with the results of pesticide concentrations in the water and feed acceptance by the experimental fish. The AI showing the highest food-dependent accumulation, fenitrothion, has the lowest water solubility and the highest log octanol-water partition coefficient (K_{ow}) and hence the highest potential for bio-accumulation. Therefore, we expected to find comparatively low concentrations of fenitrothion in the water but high bioaccumulation once it had been ingested by the fish via the feed. Fenobucarb on the other hand shows higher water solubility and a comparatively long aqueous degradation half-life time (DT_{50}). Therefore, higher concentrations of fenobucarb in water could be expected. Furthermore, feno bucarb-treated feed was much less acceptable to the fish than fenitrothion- and trichlorfon-treated feed. However, accumulation of trichlorfon did not follow the same pattern. Trichlorfon did not accumulate in the flesh of any of the treated groups. Only in fish of the control group (Treatment C) could any increase in trichlorfon concentration in fish flesh be observed. However, this high trichlorfon level in the control group was most likely an artefact as can be seen from the high standard deviation and the fact that in all other treatment groups, trichlorfon was below the detection level. Furthermore, although trichlorfon has the highest water solubility of all 3 tested AIs, it also has a very short aqueous DT_{50} half-life and is rapidly degraded to dichlorvos. Trichlorfon was not detected dissolved in the water on Days 5 and 10 and has a very low K_{ow} value of 0.43. Considering these facts, significant bioaccumulation in fish flesh was not to be expected. However, measuring dichlorvos would be beneficial in future studies. Trichlorfon and fenobucarb were detected in fish flesh prior to the feeding trial in comparable amounts to those reported by Hoai *et al.* (2011), who performed a survey on pesticide contamination of food fish in northern Vietnam. We can conclude that contamination of feeds with the AIs used in this study directly reduces fish production by reducing feed intake in the case of fenobucarb and by affecting fish health status in the case of all tested AIs, confirming that the intensification of rice farming is a threat to integrated aquaculture. Future studies on the effects of orally applied pesticides on fish health and production should focus on realistic scenarios in several ponds by including seasonal and spatial differences over the complete production cycle. For consumers of such fish, orally applied pesticides after a short period of 10 d pose little risk, as the levels of fenitrothion and trichlorfon in the flesh of grass carp before and after the trial were below the maximum residue levels (fenitrothion at 0.01 mg kg^{-1} fresh fish and trichlorfon at 0.1 mg kg^{-1} fresh fish) given by the European Commission for terrestrial animal products (EC 2005, aquatic animal products not listed). For example, taking the highest accumulation of fenitrothion under AFD conditions (Treatment A) after 10 d of feeding, a person weighing 50 kg would have to consume about 13.6 kg of grass carp filet in a day to reach even the minimum intake deemed to be harmful (acceptable daily intake) of 0.006 mg kg^{-1} body weight (WHO 2009). However, to evaluate the potential risk of consumers from accumulated pesticides in fish, a steady state pesticide accumulation assessment would be needed, taking into account the entire production cycle of fish for consumption.

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2.5 References

Adams SM, Brown AM, Goede RW (1993) A quantitative health assessment index for rapid evaluation of fish condition in the field. Transactions of the American Fisheries Society 122:63−73

Aker WG, Hu X, Wang P, Hwang HM (2008) Comparing the relative toxicity of malathion and malaoxon in blue cat-fish Ictalurus furcatus. Environmental Toxicology 23:548−554

Anyusheva M, LamersM, La N, Nguyen VV, Streck T (2012) Fate of pesticides in combined paddy rice−fish pond farming systems in Northern Vietnam. Journal of Environmental Quality 41:515−525

Cai Z, Curtis LR (1990) Effects of diet and temperature on food consumption, growth rate and tissue fatty-acid composition of triploid grass carp. Aquaculture 88:313−327

Chandrasekar S, Jayabalan N (1993) Hematological responses of the common carp, *Cyprinus carpio* L. exposed to the pesticide endosulfan. Asian Fisheries Science Journal 6:331−340

Chuiko GM (2000) Comparative study of acetylcholin-esterase and butyrylcholinesterase in brain and serum of several freshwater fish: specific activities and in vitro inhibition by DDVP, an organophosphorus pesticide. Comparative Biochemistry and Physiology - Part C: Toxicology & Pharmacology 127:233−242

De Silva SS, Weerakoon DEM (1981) Growth, food intake and evacuation rates of grass carp, *Ctenopharyngodon idella* fry. Aquaculture 25:67−76

EC (European Commission) (2005) EU pesticides database: pesticide EU- MRLs (maximum residue levels) based on the Regulation (EC) No 396/2005. European Parliament and of the

Council. Available at http://ec.europa.eu/sanco_pesticides/public/index.cfm (accessed on 20 February 2013)

Froese R, Pauly D (eds) (2013) FishBase. Species summary: *Ctenopharyngodon idella* (Valenciennes, 1844), Grass carp. www.fishbase.org/summary/Ctenopharyn godonidella.html (accessed on 8 January 2013)

Fulton MH, Key PB (2001) Acetylcholinesterase inhibition in estuarine fish and invertebrates as an indicator of organophosphorus insecticide exposure and effects. Environmental Toxicology and Chemistry 20:37−45

Gill TS, Pant JC, Pant J (1988) Gill, liver and kidney lesions associated with experimental exposures to carbaryl and dimethoate in the fish (*Puntius conchonius* Ham.). Bulletin of Environmental Contamination and Toxicology 41:71−78

Gruber SJ, Munn MD (1998) Organophosphate and carbamate insecticides in agricultural waters and choline-esterase (ChE) inhibition in common carp (*Cyprinus carpio*). Archives of Environmental Contamination and Toxicology 35:391−396

Hai DQ, Varga SI, Matkovics B (1997) Organophosphate effects on antioxidant system of carp (*Cyprinus carpio*) and catfish (*Ictalurus nebulosus*). Comparative Biochemistry and Physiology Part C: Pharmacology, Toxicology and Endocrinology 117:83−88

Halappa R, David M (2009) Behavioural responses of the freshwater fish, *Cyprinus carpio* (Linnaeus) following sub lethal exposure to chlorpyrifos. Turkish Journal of Fisheries and Aquatic Sciences 9:233−238

Hoai PM, Sebesvari Z, Minh TB, Viet PH, Renaud FG (2011) Pesticide pollution in agricultural areas of northern Vietnam: case study in Hoang Liet and Minh Dai communes. Environmental Pollution 159:3344−3350

Kestemont P, Baras E (2001) Environmental factors and feed intake: mechanisms and interaction. In: Houlihan D (ed) Food intake in fish. Blackwell Science, London, p 131–146

Kino H, Inaba H, Van De N, Van Chau L and others (1998) Epidemiology of clonorchiasis in Ninh Binh Province, Vietnam. The Southeast Asian Journal of Tropical Medicine and Public Health 29:250−254

KirbyMF, Morris S, Hurst M, Kirby SJ, Neall P, Tylor T, Fagg A (2000) The use of cholinesterase activity in flounder (*Platichthys flesus*) muscle tissue as a biomarker of neurotoxic contamination in UK estuaries. Marine Pollution Bulletin 40:780−791

Kumar K, Ansari BA (1986) Malathion toxicity: effect on the liver of the fish *Brachydanio rerio* (Cyprinidae). Ecotoxicology and Environmental Safety 12:199−205

Kumar N, Prabhu PAJ, Pal AK, Remya S and others (2011) Anti-oxidative and immuno-hematological status of tilapia (*Oreochromis mossambicus*) during acute toxicity test of endosulfan. Pesticide Biochemistry and Physiology 99:45−52

LamersM, Anyusheva M, La N, Nguyen VV, Streck T (2011) Pesticide pollution in surface- and groundwater by paddy rice cultivation: a case study from Northern Vietnam (short communication). CLEAN Soil Air Water 39:356−361

Luu LT (2001) The VAC system in northern Viet Nam. In: Integrated agriculture-aquaculture. Fisheries Tech Pap 407. FAO, Rome, p 29–32

MEJ (Ministry of the Environment in Japan) (2013) Results of eco-toxicity tests of chemicals. Available at www.env.go.jp/chemi/sesaku/02e.pdf (accessed on 1 December 2013)

Nguyen VD, Thanh HL (2011) Human infections of fishborne trematodes in Vietnam: prevalence and molecular specific identification at an endemic commune in Nam Dinh province. Experimental Parasitology 129:355−361

Opuszynski K (1972) Use of phytophagous fish to control aquatic plants. Aquaculture 1:61−74

Osborne JA, Riddle RD (1999) Feeding and growth rates for triploid grass carp as influenced by size and water temperature. Journal of Freshwater Ecology 14:41−45

Phan VT, Ersbøll AK, Nguyen KV, Madsen H, Dalsgaard A (2010) Farm-level risk factors for fish-borne zoonotic trematode infection in integrated small-scale fish farms in northern Vietnam. PLoS Neglected Tropical Diseases 4:e742

Phan VT, Ersbøll AK, Do DT, Dalsgaard A (2011) Raw- fisheating behavior and fishborne zoonotic trematode infection in people of northern Vietnam. Foodborne Pathogens and Disease 8:255−260

Pope CN (1999) Organophosphorus pesticides: Do they all have the same mechanism of toxicity? Journal of Toxicology and Environmental Health, Part B 2:161−181

Prowse GA (1971) Experimental criteria for studying grass carp feeding in relation to weed control. Progressive Fish-Culturist 33:128−131

Pucher J, Steinbronn S, Mayrhofer R, Schad I, El-Matbouli M, Focken U (2013) Improved sustainable aquaculture systems for small-scale farmers in northern Vietnam. In: Fröhlich HL, Schreinemachers P, Cle mens G, Stahr K (eds) Sustainable land use and rural development in Southeast Asia: innovations and policies for mountainous areas. Springer Verlag, Berlin, p 281–317

Rodrigues EL, Fanta E (1998) Liver histopathology of the fish *Brachydanio rerio* Hamilton Buchmann after acute exposure to sublethal levels of the organophosphate Di-methoate 500. Revista Brasileira de Zoologia 15:441−450

Sakai T, Murata H, Endo M, Shimomura T and others (1998) Severe oxidative stress is thought to be a principal cause of jaundice of yellowtail (*Seriola quinqueradiata*). Aquaculture 160:205−214

Steinbronn S (2009) A case study: fish production in the integrated farming system of the Black Thai in Yen Chau district (Son La province) in mountainous North-Western Vietnam—current state and potential. PhD thesis, University of Hohenheim, Stuttgart

Sturm A, Wogram J, Segner H, Liess M (2000) Different sensitivity of organophosphates of acetylcholinesterase and butyrylcholinesterase from three-spined stickleback (*Gasterosteus aculeatus*): application in biomonitoring. Environmental Toxicology and Chemistry 19:1607−1615

Sweilum MA (2006) Effect of sublethal toxicity of some pesticides on growth parameters, haematological properties and total production of Nile tilapia (*Oreochromis niloticus* L.) and water quality of ponds. Aquaculture Research 37:1079−1089

Takatori S, Okihashi M, Okamoto Y, Kitagawa Y and others (2008) A rapid and easy multiresidue method for the determination of pesticide residues in vegetables, fruits, and cereals using liquid chromatography/tandem mass spectrometry. Journal of AOAC INTERNATIONAL 91:871−883

Van PT, Khoa LV, Lua DT, Van KV, Ha NT (2002) The impacts of red spot disease on small-scale aquaculture in Northern Vietnam. In: Arthur JR, Phillips MJ, Subasinghe RP, Reantaso MB, MacRae IH (eds) Primary aquatic animal health care in rural, small-scale aquaculture development. FAO, Rome, p 165–176

Wedemeyer GA (1972) Some physiological consequences of handling stress in the juvenile coho salmon (*Oncorhynchus kisutch*) and steelhead trout (*Salmo gairdneri*). Journal of the Fisheries Research Board of Canada 29:1780−1783

Weiss CM (1961) Physiological effect of organic phosphorus insecticides on several species of fish. Transactions of the American Fisheries Society 90:143−152

WHO (World Health Organization) (2009) Inventory of IPCS and other WHO pesticide evaluations and summary of toxicological evaluations performed by the Joint Meeting on Pesticide Residues (JMPR) through 2009. Available at www.who.int/ipcs/publications/jmpr/pesticide_inventory_edition10.pdf (accessed on 20 February 2013)

Wolf JC, Wolfe MJ (2005) A brief overview of nonneoplastic hepatic toxicity in fish. Toxicologic Pathology 33:75−85

Xu W, Liu W, Shao X, Jiang G, Li X (2012) Effect of Trichlorfon on hepatic lipid accumulation in crucian carp Carassius auratus gibelio. Journal of Aquatic Animal Health 24:185−194

3 Effects of modified pond management on limnological parameters in small-scale aquaculture ponds in mountainous Northern Vietnam

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Abstract

In mountainous Northern Vietnam, traditional pond aquaculture is part of the integrated farming activity contributing to food safety and to income generation for small-scale farmers of ethnic minorities. Traditional pond management consists of a polyculture of macro-herbivorous grass carp with 3-5 other fish species that are cultured in small ponds with constant water flow-through. The main limitations to production are species-specific mass mortalities of grass carp, a poor feed base especially for all species but grass carp, and poor water quality. In this study, we compared the traditional pond management to a semi-intensive pond management that was based on the traditional management system but included changes designed by researchers to increase fish production. The modifications consisted of water inflow control, supplemental fertilization and feeding, and a polyculture dominated by common carp. The changes in management significantly reduced the turbidity and increased oxygen supply, as well as the natural food base within the pond. These changes in pond management provide farmers with the possibility to improve their pond aquaculture scheme and overcome previous limitations.

3.1 Introduction

In mountainous regions of Northern Vietnam, rapid agricultural intensification of cropping systems has changed the traditional integrated farming systems of small-scale farmers (Minot *et al.* 2006) and negatively affected single farming activities such as pond aquaculture which is important for food security and income generation (Steinbronn 2009). Exemplarily, Pucher *et al.* (2014) evaluated that pesticide contaminated leave material collected from rice paddy fields and used as feed for fish might negatively affect feed uptake and health parameters of fish. Even though demand for fish and accessibility for external high quality inputs is rising in the Northern Vietnam uplands (Anrooy 2001), pond aquaculture is still performed mainly for subsistence and supply to local markets (Luu 2001; Minh 2010; Steinbronn 2009). In this region, pond aquaculture is still managed traditionally by using on-farm resources that are of poor quality fed to a carp / tilapia polyculture with macro-herbivorous grass carp (*Ctenopharyngodon idella*) as a primary species, together with 3-5 secondary fish species such as common carp (*Cyprinus carpio*), silver carp (*Hypophthalmichthys molitrix*), bighead carp (*Aristichthys nobilis*), mrigal (*Cirrhinus mrigala*), mud carp (*Cirrhinus molitorella*), and Nile tilapia (*Oreochromis niloticus*) (Steinbronn 2009).

Traditionally applied feeds and fertilizers consist of cheap, low quality on-farm resources which are temporally available in large quantities such as leaves of banana, maize, bamboo and cassava, grass, rice bran and other by-products such as manure of ruminants (Dongmeza *et al.* 2009; Steinbronn 2009; Dongmeza *et al.* 2010). Use of these resources results in low aquaculture production of about 1.54±0.33 t ha^{-1} a^{-1} (Steinbronn 2009). Farmers claim grass carp specific mass mortalities, water shortage, high labour requirement, bad water quality and infiltration of ponds by pesticides as the most severe problems for aquaculture production. Steinbronn (2009) and Thai and colleagues (2006; 2007) additionally suggested that low quality of external feed and fertilizer resources, as well as high turbidity of pond water, which reduce the natural food base and oxygen supply, as major limitations for productive aquaculture. Aquaculture is strongly integrated into the water and organic matter flows of the traditional integrated farming systems (Luu 2001; Steinbronn 2009). It is performed with constant water flow through the ponds (Steinbronn 2009; Anyusheva *et al.* 2012) in which eroded particles from highly sloped maize and cassava fields (Wezel *et al.* 2002; Nikolic *et al.* 2008) are transported by the irrigation system into paddy rice fields (Schmitter *et al.* 2012) and fish ponds (Steinbronn 2009) which act as sediment traps. The suspended particles impair the penetration of sunlight and thereby inhibit primary and secondary production and may change the nutritional quality of natural food resources (Cashman *et al.* 2013). The low primary productivity limits oxygen production as well as the entire natural food base especially for the planktivorous and benthivorous fish species of the carp / tilapia polyculture.

In the present study, a semi-intensive pond management that included the application of locally available, higher quality inputs was compared to the traditional system for its ability to improve water quality and enhance natural food availability which are both essential for a productive small-scale aquaculture practice (De Silva 1995; Knud-Hansen *et al.* 2003; Rahman *et al.* 2008). The following modifications were developed to improve the water quality and natural food base for fish in the research area:

1.) Application of basic pond hygiene measures

2.) Control of water inflow towards a static pond to reduce turbidity as well as outflow of nutrients and thereby increase primary production

3.) Supplemental inorganic fertilization

4.) Replacement of the disease-prone grass carp with the omnivorous common carp as the main species

5.) Supplemental feeding of the common carp with pelleted feed mainly based on locally available resources

3.2 Material and methods

Study area and site selection:

The study was carried out in six pilot ponds belonging to Black Thai farmers in Chieng Khoi commune, Yen Chau district, Son La province, Northern Vietnam. The trial was performed as an action-research trial in the ponds of the farmers and managed by the farmers themselves. This implies the active involvement of the farmers in the daily feeding, fertilization, water management and general pond management in both, traditional and modified pond systems. The trial was conducted for a period of 7 months between April and October 2009. Three pairs of ponds were selected. The members of each pair were contiguous but the pairs themselves differed in their locations, type of water supply and surroundings. The first pair of ponds (T1, S1; pond T1 [X: 429938; Y: 2323462; altitude: 465], size 916 m^2, depth 1.2 m, round shape; pond S1 [X: 429963; Y: 2323530; altitude: 466], size 943 m^2, depth 1.1 m, rectangular shape) was located in the upper hill slopes cropped with maize and cassava. The pond water was mainly supplied by rain collected in hills with sandy red soils (Clemens *et al.* 2010) and occasionally by reservoir water. The second pair of ponds (T2, S2; pond T2 [X: 429831; Y: 2324914; altitude: 435], size 634 m^2, depth 0.9 m, rectangular shape; pond S2 [X: 429978; Y: 2324817; altitude: 421], size 598 m^2, depth 1.0 m, rectangular shape) was located in a valley supplied only by rain water. This watershed was characterized by secondary forest and cropping maize, cassava and banana on small, steep fields (< 2000 m^2) on yellow soil and good black soil (Clemens *et al.* 2010). The third pair of ponds (T3, S3; pond T3 [X: 428865; Y: 2325346; altitude: 435], size 545 m^2, depth 1.3 m, rectangular shape; pond S3 [X: 428940; Y: 2325328; altitude: 438], size 982 m^2, depth 1.3 m, rectangular shape) was connected to the irrigation channel system and was thus constantly supplied with reservoir water. These ponds were located on a plane of irrigated rice paddies on good black soil (Clemens *et al.* 2010). All GPS data are shown in the WGS84 format and are in the zone North 48.

Comparison of pond managements

Before starting the trial, all ponds were completely drained and harvested. To start the trial, all ponds were filled, fertilized and, ten days later, stocked at a stocking density of 1.5 fingerlings m^{-2}. During the study, two types of management (traditional and semi-intensive) were applied to the ponds in each pair by the farmers under advice of researchers.

Traditional pond management

One pond of each pair (T1, T2, and T3) was managed by farmers applying their traditional pond management practice. The ponds were drained, filled with water and fertilized with cow or buffalo manure from farmers' dung heaps (2.3 t ha^{-1}). Mud of ponds was not removed as this practice is rarely performed by farmers since chemical fertilizers are cheaply available. This reduces the benefit of farmers´ labour and time effort for transferring pond mud to fields and gardens for fertilization. Ponds were stocked with grass carp as main species (60% of individuals) and common carp, silver carp, bighead carp and Nile tilapia (each 10% of individuals) as by-species of the polyculture. The water inflow was not controlled and depended on farmers' practice as well as on the availability of water from rain or reservoirs. As common in the research area (Steinbronn 2009, Pucher *et al.* 2013), the farmers used leaves from banana, maize, cassava, weeds and rice plants as well as farm by-products (rice bran, cassava peel) as feed once or twice a day. As fertilizers, the farmers used manure from cows, buffaloes and pigs which was applied fresh or dried to the pond according to its availability on farm. Amounts of feeds and fertilizer applied to the ponds were recorded daily by farmers.

Semi-intensive pond management

The second pond of each pair (S1, S2, and S3) was managed semi-intensively. The ponds were drained, limed with locally available agricultural lime at 900 kg ha^{-1} $CaCO_3$ and left to dry for 10 days before they were filled and fertilized with urea (12 kg ha^{-1} total nitrogen (TN)), single superphosphate (6 kg ha^{-1} total phosphorus (TP)), and cow or buffalo manure from farmers' dung heaps (2.3 t ha^{-1}). The ponds were stocked with common carp as the main species (60% of individuals) and grass carp, silver carp, bighead carp and Nile tilapia (each 10% of individuals) as by-species. The water inflow and outflow was controlled by polyvinylchlorid (PVC) pipes. Water inflow was minimized so that it just maintained the normal level in the ponds with clear water and was stopped completely during rain events or when pesticides had been applied to areas upstream less than a week previously. To decrease the inflow of runoff water from surrounding slopes, channels were installed to direct runoff water around the ponds. Every day, the farmers used leaves from banana, maize, cassava, weeds and rice plants as well as and by-products (rice bran, cassava peel) to feed the grass carp and applied supplemental feeding with pellets for common carp. The fertilization was adjusted to a TN/TP ratio of 3:1 by chemical fertilizers (urea (producers specification: 46% TN) and phosphorus fertilizer (producers specification: 8.7% TP)) and ruminant manure. The amount used of the latter was calculated using the values for the nitrogen and phosphorus contents of ruminant manure in the region reported by Kumar *et al.* (2004; 2005). Chemical fertilizers were applied in a fine mesh bag which was suspended in the pond and then lifted every day to slowly release the nutrients. The amounts of fertilizers were adjusted according to the application rate of on-farm available organic fertilizers (farmer decision) and the current concentrations of total ammonia, total nitrogen and total phosphorus in the pond. The amounts of chemical fertilizer applied were calculated to raise dissolved nitrogen and phosphorus to 1.0 ppm and 0.5 ppm, respectively (Kumar *et al.* 2004). The inorganic phosphorus fertilizer, which was locally traded as single superphosphate fertilizer, showed significantly lower content of TP in the chemical analysis than given in specifications of the producer and did not match the specifications given by FAO (2012). This resulted in a lower application of TP than targeted.

Water parameters and limnological monitoring

Monthly, seven days after applications of chemical fertilizer, three water samples of 1.5 L were taken at three different sites in each pond at 25 cm water depth by means of a water sampler which allowed total displacement of air in the bottle by pond water (water inflow tube diameter 1.8 cm, air outflow tube diameter 0.5 cm). The water depth of 25 cm was chosen to sample the horizontal variation in the trophogenic zone under both managements without distorting the water sample by sediments been stirred up in the process of sampling. Water samples were transported to the field laboratory (10 minutes' drive). For determination of suspended particle concentration, sample water was sucked under weak vacuum through pre-dried (60°C) and pre-weighed glass fibre filters (pore size 1.2 μm; GF/C; Whatman, Piscataway, NJ, USA). Dry mass (105°C) and mass after combustion were used to determine the concentrations of total suspended solids (TSS), incombustible suspended solids (SS_{incom}) and combustible suspended solids (SS_{com}).

Filtered water samples from the suspended solids determinations were analysed in duplicates for dissolved total ammonia nitrogen (TAN), nitrite nitrogen (NO_2-N), nitrate nitrogen (NO_3-N) and ortho-phosphate phosphorus (PO_4-P) using Merck Spectroquant test kits (catalogue numbers 14752, 14776, 09713 and 14848, respectively, Merck Chemicals, Darmstadt, Germany). The concentrations of unionized ammonia nitrogen (UIA-N) were calculated according to Emerson *et al.* (1975).

To evaluate TN and TP in the entire pond depth, water samples of three 0.5 L aliquots taken in each pond at 25, 50 and 75 cm water depth were mixed and analysed. Duplicate 20 mL samples of mixed pond water were completely oxidized by adding two microspoons of Oxisolv® ((Merck, Darmstadt, Germany) modified after Köthe & Bitsch (1992)) and autoclaving for 10 minutes. The digested water samples were analysed for dissolved NO_3-N and PO_4-P (described above) being equivalent to TN and TP. Photometrical analyses of dissolved TAN, NO_2-N, NO_3-N, PO_4-P, TN and TP were performed using a field photometer (Photolab S12; WTW, Weilheim, Germany).

Once a month, profiles of dissolved oxygen, pH and temperature were measured from platforms at sunrise, midday and sunset in water depths of 5, 25, 50, 75 and 100 cm. Net oxygen production was calculated for each pond between sunrise, sun zenith and sunset using the profiles of dissolved oxygen concentrations. Sediment pH and redox potential were measured in 2 and 5 cm sediment depth of sediment cores taken monthly by a PVC pipe (Hussenot & Martin 1995; Somsiri *et al.* 2006; Ø 4.5 cm). Measurements of dissolved oxygen, water temperature, water pH, sediment pH and sediment redox potential were measured using a handheld pH/Oxi 340i Meter (WTW) equipped with a CellOx 325 sensor (WTW), SenTix 20 pH probe (WTW), SenTix SP pH probe (WTW), and SenTix ORP redox probe (WTW), respectively. Alkalinity and total hardness of the pond water sources were tested before filling the ponds using test kits (Aquamerck; catalogue number 111109 and 111104, respectively). Alkalinity is expressed as acid capacity (mmol L^{-1} H^{+}) until pH 8.2 (K_S 8.2) and pH 4.3 (K_S 4.3) is reached.

For determination of abundance and species composition of phytoplankton and zooplankton, three 1.5 L water samples were taken at a depth of 25 cm at three different sites in each pond once a month. The water samples were filtered through 60 µm and 30 µm gauze and the fractions of plankton were stored in 5% formalin solution. Zooplankton and phytoplankton were counted, identified down to the level of classes and subclasses (Balcer *et al.* 1984; Sládecek 1983; Van 2003) and divided into size classes. Based on zooplankton and phytoplankton counts, estimates of the available natural food stocks in DM were made for zooplankton using DM-length regressions from literature (Dumont *et al.* 1975; Culver *et al.* 1985; Hellung-Larsen & Andersen 1989; Ejsmont-Karabin *et al.* 2004) and for phytoplankton using DM estimates based on the method described by Schwoerbel (1994) by using genus specific volumina (Nauwerck 1963) and the assumption of a fresh matter (FM) density of 1.02 g cm^{-3} and DM of 10% of FM (Ruttner 1938).

Monthly, three sediment cores per pond were taken at different sites of each pond using a PVC pipe (Ø 4.5 cm) (Somsiri *et al.* 2006). The pond sediment cores were washed through a 500 µm mesh and macro-zoobenthos were segregated and stored in 5% formalin solution. Macro-zoobenthos were identified to family level, divided into body length classes and the DM biomass estimated per pond area by using DM-length regressions from literature (Miserendino 2001; Miyasaka *et al.* 2008). The nutritional quality of phytoplankton, zooplankton and zoobenthos were estimated based on the chemical compositions given by Hepher (1988 modified by De Silva 1995).

To determine the chemical composition of sedimented particles on the pond bottom within the trial, the top layer (2 cm) of the pond bottom was sampled at five sites in each pond using a corer. The samples were sieved through a mesh (0.5 mm) before analysis.

Chemical analysis

Samples of fertilizers, feeds and sediments were analysed for FM, DM and crude ash according the AOAC (1990). TN and TC were determined using a C/N analyser (Vario MAX CN, Elementar Analysensysteme GmbH, Hanau, Germany). TP was analysed with a spectrophotometer (U-2000, Hitachi, Japan) using the colorimetric method of Gericke & Kurmies (1952). Pond bottom samples were dried and analysed for crude ash and content of TC, TN and TP.

Statistical analysis

The pond input data are shown in kg 1000 m^{-2} as this area is close to the average pond area owned and managed by a Black Thai farmer household in the research area (Steinbronn 2009). Data were processed and depicted using SigmPlot 11.0 and statistically analysed using STATISTICA 8 (StatSoft®, Tulsa, OK, USA). The two types of pond management were compared with regard to pond inputs, water parameters and natural food abundances by means of one-way ANOVAs and repeated measures ANOVAs. Data sets were tested for homogeneity by the Levene´s test and checked visually for normality. Data sets for repeated measures ANOVAs were tested for sphericity by means of the Mauchley sphericity test. To meet the assumptions, data sets were log or square root transformed and “Greenhouse-Geisser" corrected (for repeated measures ANOVAs), if needed. Data sets which strongly violated the assumptions of one-way ANOVA and repeated measures ANOVA, were analysed by non-parametric equivalent tests for pond management effects (Mann-Whitney U Test) and time effects (Friedman´s ANOVA by ranks), respectively. Repeated measures data sets are additionally shown as the differences between the pairs of ponds ($\Delta\ S_{ij}-T_{ij}$, with “i” being the number of the pond pair and “j” being the repeated measure).

3.3 Results

The utilization of higher quality fertilizer and feed resources under semi-intensive pond management resulted in a reduced total DM and TC input compared to traditional pond management while inputs of TN and TP were similar under both managements (Table 3.1). The nutrient inputs added by fertilizers were similar for organic fertilization under both managements but under semi-intensive management they were raised 1.5 fold and 4.9 fold for TN and TP, respectively by chemical fertilization. With the higher stocking density of grass carp under traditional pond management, the input of plant material and farm by-products as feed for the macro-herbivorous fishes were significantly higher under traditional pond management resulting in higher DM, TC, TN and TP inputs compared to nutrients originating from feed (including pellets) under semi-intensive management.

Most of the limnological factors showed a higher variability under traditional than under semi-intensive management due to uncontrolled water inflow (including runoff) which fluctuated in volume and quality depending on rainfall, work load of the farmer, surrounding land use and the water source. In rain fed ponds, water inflow ranged from zero on days without rain up to 154 L min^{-1} equalling 0.24 L m^{-2} pond area (without taking lateral inflows such as runoff and leakage through the dikes into account). In ponds fed by irrigation channels, the water flow was more constant with inflow rates between 7.6 and 61.6 L min^{-1} (equivalent to 0.01 and 0.11 L m^{-2} pond area) as water was constantly available in the channels. Depending on the season and weather, the inflowing water had a total suspended solid content of up to 0.49 mg L^{-1} with an average 0.24±0.19 mg L^{-1}.

In the pond water, the concentrations of dissolved nutrient compounds as well as TN and TP did not differ between the two pond managements and over time (Table 3.2). Dissolved nitrogen compounds, such as TAN, NO_2-N and NO_3-N, were detected in low concentrations. Fish toxic UIA-N were regularly recorded at 4.1±3.1 µg L^{-1} under traditional and 6.2±6.2 µg L^{-1} under semi-intensive pond management. However, the TAN concentration and the sum of dissolved nitrogen compounds in ponds under traditional pond management showed a high variability depending on the weather and land use surrounding the ponds with high TAN of up to 0.41 mg L^{-1}. The concentration of PO_4-P was near the lower detection limit of the method used (0.01 mg L^{-1} PO_4-P). On average, no significant differences in TN and TP were measured in the ponds over the experimental period. The ratios N_{diss}/PO_4-P and TN/TP did not show significant management dependant differences, but TN/TP showed a time effect with high ratios in June and July under both types of pond managements.

In ponds under semi-intensive management, the minimized water inflow resulted in an increased water transparency, reduction of TSS content, reduction of SS_{incom} content and an increased percentage of SS_{com} of TSS compared to ponds under traditional management (Table 3.3). SS_{com} showed a significant time effect with higher percentage of SS_{com} of TSS in July and August. This effect was specifically instinct under semi-intensive pond management.

The turbidity in traditionally managed ponds was affected by the source of the constantly inflowing water. Ponds with an uncontrolled inflow of runoff water from the surrounding upland fields showed a higher load of TSS than ponds situated in the paddy rice terraces which had a constant inflow of reservoir water with lower TSS via the channel system.

The differences in the water management and fertilizing scheme under semi-intensive management had effects on limnological parameters within the ponds. Under both managements, the ponds underwent a complete turnover of the water body in the early morning, which is illustrated in the profiles of water temperature, dissolved oxygen and water pH (Figure 3.1). During the day, stratifications occurred in all ponds, but the thermocline under traditional pond management was established at a lower depth than in the semi-intensively managed ponds. Also the trophogenic zones in ponds under traditional management only reached water depths between 25 and 50 cm, while semi-intensively managed ponds had trophogenic zones down to 75 cm water depth.

Table 3.1: Mean inputs of dry matter (DM), total carbon (TC), total nitrogen (TN) and total phosphorus (TP) in organic fertilizers inorganic fertilizers and feeds (± standard deviations of the pond means) in traditional and semi-intensive pond management systems during the experimental phase (based on 39 fertilizer and 48 feed samples)

		Traditional management				Semi-intensive management			
	[kg 1000 m^{-2}]	**DM**	**TC**	**TN**	**TP**	**DM**	**TC**	**TN**	**TP**
Fertilizer	Organic	341±92	122±33	12.2±2.9	0.7±0.3	390±110	140±40	10.7±4.9	1.1±0.5
	Inorganic	-	-	-	-	58±2	0±0	7.4±0.7	2.2±0.1
	Total	**340±92**	**122±33**	**12.2±2.9**	**0.7±0.3[b]**	**448±108**	**140±40**	**18.1±5.2**	**3.4±0.5[a]**
Feed	Plant material	908±280[a]	411±123[a]	23.3±5.8[a]	2.2±0.7[a]	320±193[b]	143±89[b]	7.5±4.9[b]	0.7±0.4[b]
	By-products	637±556	291±255	5.7±4.3	3.0±2.3	175±16	81±7	1.0±0.5	0.5±0.3
	Pellets	-	-	-	-	322±102	152±48	14.0±4.4	1.6±0.5
	Total	**1545±787**	**702±356**	**28.9±9.7**	**5.3±2.8**	**818±205**	**376±97**	**22.5±6.6**	**2.9±0.9**
	Total (Fertilizer & Feeds)	**1886±878**	**824±389**	**41.1±12.2**	**6.0±3.1**	**1266±141**	**516±74**	**40.6±1.4**	**6.2±1.0**

Mean values in the rows that do not share the same superscript(s) differ significantly in the pond management effect at $p \leq 0.05$. Mean values in the rows without superscripts did not differ significantly.

Table 3.2: Mean dissolved nutrients, total nitrogen and total phosphorus (± standard deviations of the pond means) under two types of pond management during the experimental phase (based on 61 duplicated measurements)

Sample preparation	Nutrient	Inflowing Water	Traditional management	Semi-intensive management	$\Delta(T_{ij}-S_{ij})$
Filtered	TAN [mg L^{-1}]	0.34±0.23	0.13±0.15	0.09±0.05	-0.01±0.11
	NO_3-N [mg L^{-1}]	0.54±0.38	0.19±0.18	0.23±0.18	-0.06±0.08
	NO_2-N [mg L^{-1}]	0.05±0.03	0.02±0.00	0.02±0.00	0.00±0.01
	Sum N_{diss} [mg L^{-1}]	0.92±0.44	0.34±0.33	0.33±0.23	-0.07±0.17
	PO_4-P [mg L^{-1}]	0.06±0.11	0.02±0.01	0.01±0.00	0.01±0.02
	N_{diss}/PO_4-P	81±60	27±22	24±12	-6±13
Raw	TN [mg L^{-1}]	3.91±1.01	3.39±0.29	3.31±0.1	-0.37±0.48
	TP [mg L^{-1}]	0.23±0.14	0.12±0.05	0.15±0.02	0.02±0.03
	TN/TP	23±11	34±17	28±7	3±12

Mean values in the rows without superscripts did not differ significantly.

N_{diss}: Sum of nitrogen from dissolved nitrogen compounds; TP: Total phosphorus; TN: Total nitrogen; TAN: Total ammonia nitrogen; UIA-N: Unionized ammonia nitrogen

Table 3.3: Mean concentrations of suspended solids (± standard deviations of the pond means) under two pond management systems during the experimental phase (based on 42 duplicated samples)

Parameter	Traditional management	Semi-intensive management	$\Delta(S_{ij}-T_{ij})$
TSS [mg L^{-1}]	133.2±157.1*	36.5±11.8	-97.7±151.8
SS_{incom} [mg L^{-1}]	120.3±148.8*	28.8±10.1	-92.5±145.3
SS_{com} [% of TSS]	15.7±6.4	20.7±3.1	5.0±6.3

Mean values in the rows did not differ significantly.

TSS: Total suspended solids; SS_{incom}: Incombustible suspended solids; SS_{com}: Combustible suspended solids

*: extremely high variation under traditional pond management due to lateral inflow of water transporting eroded particles

Table 3.4: Mean net and gross production and respiration of oxygen between sunrise, zenith and sundown (± standard deviations of the pond means) under two pond management system during the experimental phase based on the profiles shown in Figure 3.1.

	Traditional management	Semi-intensive management	$\Delta(S_{ij}-T_{ij})$
Net production [mg O_2 m^{-2} 12 h $light^{-1}$]	$1525±310^{b}$	$3971±638^{a}$	2446±467
Gross production [mg O_2 m^{-2} 12 h $light^{-1}$]	$3051±621^{b}$	$7975±1275^{a}$	4892±934
Respiration [mg O_2 m^{-2} 12 h^{-1}]	$-1525±310^{a}$	$-3971±638^{b}$	-2446±467

Mean values in the rows that do not share the same superscript(s) differ significantly in the pond management effect at p≤0.05.

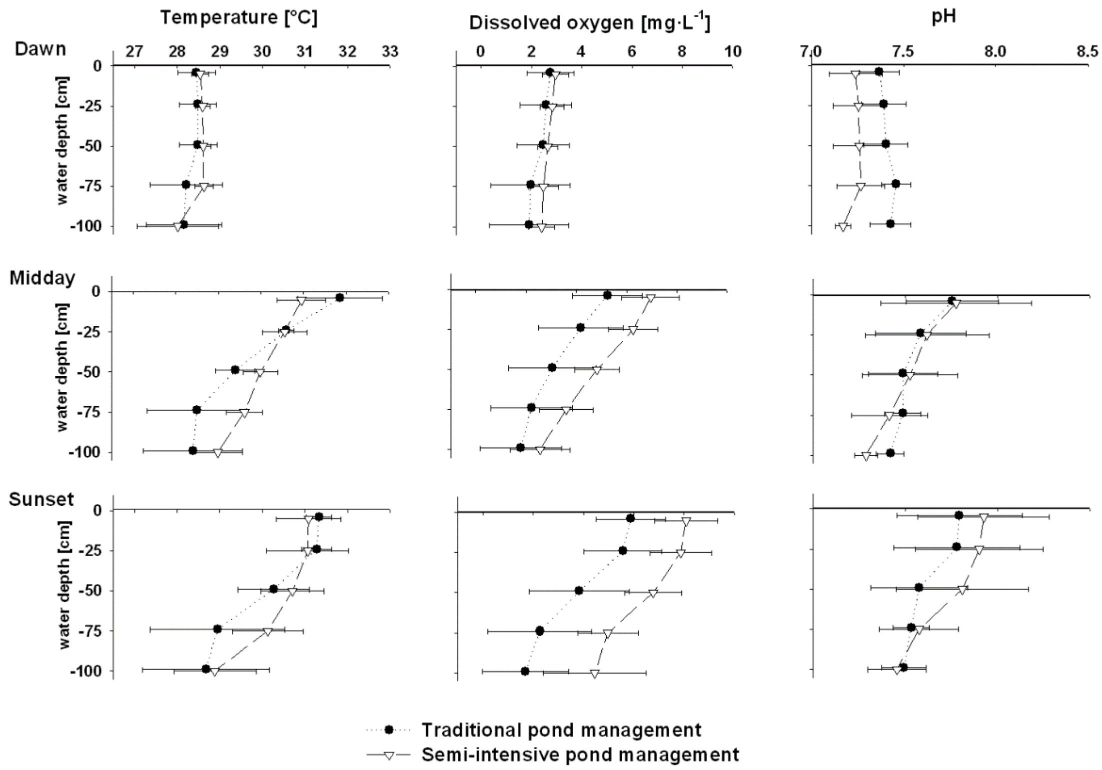

Figure 3.1: Depth profiles of water temperature, dissolved oxygen concentration and pH shown as pond means (± standard deviations) under two pond management systems during the experimental phase at dawn, zenith and sunset (based on 698 in-situ measurements of dissolved oxygen, pH and temperature). Secchi disc depths (SDD) were 28±22 cm under traditional and 97±106 cm under semi-intensive pond management.

The reduced turbidity and the deeper trophogenic zones in ponds under semi-intensive pond management resulted in a significantly higher net and gross oxygen production (Table 3.4). In addition to the management dependant differences, significant time and time*management differences of the oxygen production were detected with higher oxygen production in July and August especially under semi-intensive pond management and resulted in pH values of occasionally higher than 8.5 under both pond managements. Respiration was significantly higher in July and August under both managements (Figure 3.1 and Table 3.4). The total hardness and alkalinity of the water sources did not differ in the six ponds and gave average values of 10±1°d, K_S 8.2 of 0.2±0.1 mmol L^{-1} H^+ and K_S 4.3 of 3.4±0.2 mmol L^{-1} H^+, respectively.

Sediment parameters

The pond bottoms under traditional pond management were highly loaded with organic matter which accumulated over the years under traditional pond management. The organic matter originated from uneaten leaves and branches used as feed, fish faeces and organic fractions of sediments. After the trial, the top layer of pond sediments did not show any management-dependant differences in crude ash, TC, TN, or TP (in % of DM) content accounting for 95.4±1.2, 2.3±2.0, 0.1±0.0, and 0.05±0.1 under traditional pond management, and 95.8±0.7, 2.4±1.3, 0.1±0.0, and 0.05±0.1 and semi-intensive pond management, respectively.

The characteristics of the pond sediments were found to vary depending on the surrounding land use and pond history as the sediments had different colouration, thickness and particle fraction above 0.5 mm size. Ponds surrounded with steeply sloped fields showed reddish, sandy-loamy characteristics with higher fractions of larger particles, while ponds lower down in the paddy rice terraces showed dark, loamy sediments with hardly any particles above 0.5 mm in size. At pond sediment depths of 2 and 5 cm, redox potential was 1±20 (-13±13) and 17±19 (7±35) mV under traditional and semi-intensive pond management, respectively, with significantly higher redox potential in July and August under both types of pond managements. Down to a sediment depth of 5 cm, the redox potential never reached conditions suitable for methanogenesis. Sediment pH at 2, 5 and 10 cm sediment depth was the same (6.7±0.2) under both types of pond management.

Natural food availability

The two types of pond management affected the abundance of natural pond products differently. Figure 3.2 shows the abundance of different taxa of phytoplankton and zooplankton under both types of management over time. Euglenophyta were the most abundant group of phytoplankton followed by chlorophyta and pyrrophyta (Figure 3.2). Under semi-intensive pond management, euglenophyta, chlorophyta and total count of phytoplankton showed significantly higher abundances, especially at the end of the experiment. Even though the total number of phytoplankton increased, the total DM estimate of phytoplankton declined over the course of the experiment under both types of pond management as the phytoplankton composition changed towards smaller cells. In August, pond productivity was occasionally adversely affected by temporal blooms of coccal chlorophyceae. In several ponds in the study area as well as in one of the trial ponds, a thick, red- brown, light-impermeable layer of algae spores of Haematococcus sp. established itself on the pond surface during the day which interfered the photosynthesis in the body of the pond. The estimates of DM biomass, CP, CL and GE of phytoplankton were higher under semi-intensive pond management but did not differ significantly from the traditional pond management (Table 3.5).

The communities of zooplankton were dominated by rotatoria and copepods at juvenile stages (Figure 3.2). All types of zooplankton taxa, especially rotatoria under semi-intensive pond management, showed significant variations in their abundances over time with higher abundances in the first months of the experiment. Under semi-intensive pond management, zooplankton abundances as well as estimates of DM, CP, CL and GE were slightly higher than the corresponding values under traditional pond management (Table 3.5).

The abundance and species composition as well as the estimates of DM, CP, CL and GE of zoobenthos in the ponds did not differ between the two pond managements (Table 3.6). In the sediments, the zoobenthos community consisted mainly of oligochaeta, chironomidae larvae, and gastropoda. Occasionally, larvae of odonata were found. Neither composition, abundance of size classes nor sum of zoobenthic living organisms were affected by the type of pond management, and accounted for 104±84 individuals m^{-2}. Oligochaeta were mainly found found from May until September while chironomidae larvae predominated in April and May.

Table 3.5: Mean estimates of the masses of dry matter (DM), crude protein (CP), crude lipid (CL) and gross energy (GE) of phytoplankton and zooplankton of size > 30 µm (± standard deviations of the pond means) under two pond management systems during the experimental phase (based on 29 triplicate samples)

	Traditional management	**Semi-intensive management**	**Δ(S_{ij} -T_{ij})**
Phytoplankton			
DM estimate [µg L^{-1}]	104±31	266±135	162±145
CP estimate [µg L^{-1}]	31.5±10.3	79.3±39.7	47.8±43.3
CL estimate [µg L^{-1}]	10.0±4.3^{b}	27.1±7.1^{a}	17.1±5.7
GE estimate [J L^{-1}]	16.7±6.0	40.4±21.8	23.7±21.4
Zooplankton			
DM estimate [µg L^{-1}]	912±539	1102±827	189±1304
CP estimate [µg L^{-1}]	473±282	523±376	50±628
CL estimate [µg L^{-1}]	219±127	264±215	104±333
GE estimate [J L^{-1}]	10.7±6.4	12.6±10.0	5.4±16.7

Mean values in the rows that do not share the same superscript(s) differ significantly in the pond management effect at p≤0.05. Mean values in the rows without superscripts did not differ significantly.

Table 3.6: Mean estimates of masses of dry matter (DM), crude protein (CP), crude lipid (CL) and gross energy (GE) of zoobenthos per m^{-2} (± standard deviations of the pond means) under two pond management systems during the experimental phase (based on 40 triplicate measurements)

	Traditional management	**Semi-intensive management**	**Δ(S_{ij} -T_{ij})**
DM estimate [mg m^{-2}]*	533±672	603±591	70±1131
CP estimate [mg m^{-2}]*	301±387	349±350	48±662
CL estimate [mg m^{-2}]*	45±46	40±27	5±63
GE estimate [J m^{-2}]*	11519±14366	12860±12420	1340±23949

Mean values in the rows did not differ significantly.
*excluding Gastropoda

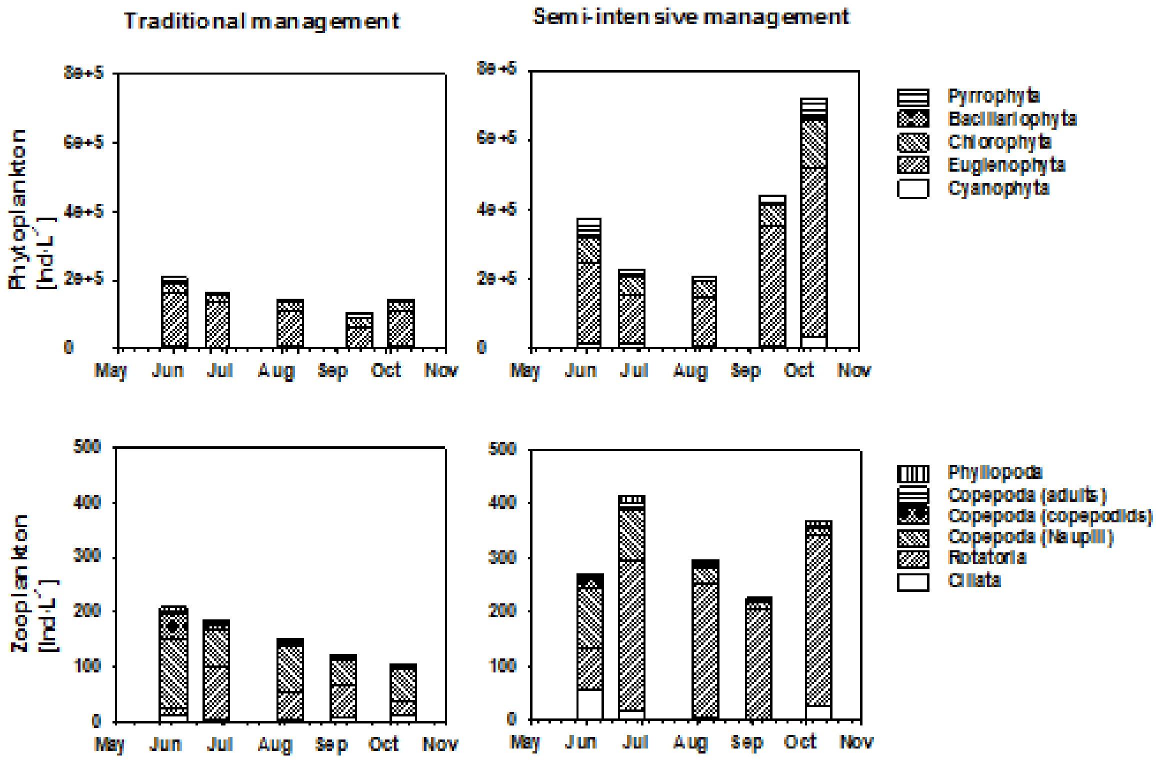

Figure 3.2: Average abundances of phyto- and zooplankton in taxonomic classes under both pond management systems during the experimental phase (based on 29 triplicate samples)

3.4 Discussion

The organic fertilizer inputs applied by farmers in this trial were similar to those reported for traditional aquaculture systems in Northern Vietnam (Luu 2001; Steinbronn 2009). Under traditional pond management, a larger nutrient pool in the ponds could have been reactivated within the experiment, as, following the traditional pond management, the mud was not remove from the pond beforehand. In spite of this potentially higher contribution of nutrients from the sediments, TN and TP phototrophic zone were lower under traditional pond management. This indicates the nutrient release from pond bottom is a minor contribution to total nutrient supply compared to the nutrients applied in the semi-intensive system. Even with additional use of inorganic fertilization under semi-intensive pond management, the amount of TN applied by organic and inorganic fertilization did not lift the nitrogen input up to the level recommended by Edwards *et al.* (1994; 1996) of 4 kg ha^{-1} day^{-1} TN. The application of additional fertilizers may be increased in future application because the concentrations of N_{diss} in pond water were low. Under semi-intensive pond management, nitrogen and phosphorus were applied at approximately similar rates via feeds and fertilizers, as opposed to the traditional pond management. Under traditional pond management the main inputs of nitrogen and phosphorus were applied in the form of leaves from banana, maize, cassava and weeds as well as farm by-products (especially rice bran) as feeds for the macro-herbivorous grass carp dominated polyculture. The applied leaf material and by-products are reported to be of lower feeding quality (Dongmeza *et al.* 2009) with a food conversion ratio (FCR) of about 7.7 ± 2.3 on a DM basis (Steinbronn 2009). Although the inputs of nutrients differed in quality between the two types of management, the composition of the pond sediment did not differ after the trial, which may have been caused by benthic fish, such as the common carp stirring up the sediment during their scavenging activities. This feeding process is known to increase the bio-availability of nitrogen and phosphorus. It aerates sediments, re-suspends deposited nutrients (Jana & Sahu 1993; Milstein *et al.* 2002; Frei & Becker 2005; Rahman *et al.* 2008) and increases turbidity (Chumchal *et al.* 2005) as well as stimulates primary production (Chumchal & Drenner 2004) and accumulation of nutrients in plankton (Rahman *et al.* 2008). Even with the high inputs of TC via organic fertilizers and low quality leaf material, sediment pH was in the optimal range but sediment redox potential was below the optimal range of 100-200 mV and above the critical value of -200 mV at which sulphides start to form (Hussenot & Martin 1995).

In all ponds, the concentrations of dissolved nitrogen compounds and TN in water were similar to those reported for reservoir water and channel water in the research area (Fröhlich *et al.* 2013), which suggests a high removal rate of the nutrients added via feeds and fertilizers in the ponds. The concentrations of fish toxic ammonia (TAN and UIA) and other dissolved nitrogen compounds under both types of pond management were below sub-lethal concentrations (Dabrowska & Sikora 1986; Dabrowska & Wlasow 1986; Abbas 2006). The low and comparable concentration of N_{diss} and PO_4-P and the comparable N_{diss}/ PO_4-P ration under both types of pond management may be explained by different factors such as (1) biological assimilation and/or (2) loss by deposition of nutrient rich particles in sediments and/or (3) leakage by horizontal water flow through the dikes or vertical flow into ground water. The ratios N_{diss}/ PO_4-P and TN/TP of pond water were higher than the TN/TP ratio of the fertilizer inputs, which implies lower removal rate of nitrogen than phosphorus although phosphorus is known to be the major limiting nutrient in aquatic environments (Elser *et al.* 1990), especially due to the fast deposition in bottom sediments (Correll 1998).

Under semi-intensive pond management, the inhibition of water inflow and the channels dug round the ponds to prevent ingress of runoff water from the surrounding fields reduced the introduction of eroded particles and nutrients. However, seepage of water was not inhibited by this pond management strategy which could have led to nutrient leakage by horizontal water flow through the dikes or vertical flow into ground water (as shown in rice paddies by Reinhardt *et al.* 2012). Proper sealing of dikes or dikes made of concrete or bricks may help to control these losses. Some farmers in the study area practice this already with the primary goal to increase the stability of dikes during flood events which are affecting the area at increasing frequencies (Schad *et al.* 2012). However, the effect of lining the dike have so far neither been studied with respect to limnology nor to economy.

Under traditional pond management, the flow rates were similar to those reported by two previous studies in the research area with flow rates of 120±40 L m^{-2} d^{-1} (Anyusheva *et al.* 2012) and 34-202 L m^{-2} d^{-1} (Steinbronn 2009), with TSS concentrations and nutrient loads being highly dependent on the season and weather conditions, such as intensity of rain (Schmitter *et al.* 2010a; 2012).

Although the introduction of eroded particles and suspended nutrients have a fertilizing effect in rice paddies (Schmitter *et al.* 2010a; 2010b), the high TSS concentration of inflowing water in traditionally managed ponds caused an averaged SDD of about 30 cm, which is considered to be turbid (Sevilleja *et al.* 2001). The turbid pond water had strong effects on physico-chemical water parameters by reducing light penetration during traditional pond management.

Turbid ponds absorbed the solar radiation in the top water layers and developed a strong stratification over the day, as was shown for shallow turbid water bodies by Condie & Webster (2002). The strong stratifications were especially observed under traditional pond management and were not inhibited by turbulences caused by constantly inflowing water.

Low oxygen levels are described as being the major limiting factor in aquatic environments (Black 1998; Ross 2000). At high water temperatures, dissolved oxygen content of the water decreases due to lower oxygen solubility while the oxygen requirements of poikilothermal organisms increase due to their higher levels of activity, thus limiting the oxygen supply for fish. In the present study, as in Steinbronn (2009), oxygen supply of ponds under traditional pond management was a serious problem for fish culture in the study area. Even the turnover effect of constant inflowing water was not enough to counteract the oxygen depletion under traditional management. Semi-intensively managed ponds had a higher net oxygen production along with lower turbidity, better light penetration, a deeper phototrophic water layer and higher abundances of phytoplankton.

Levels of respiration under semi-intensive management were similar to those in ponds intensively fertilized with chicken litter (Teichert-Coddington & Green 1993). However, ponds under both types of management had low oxygen levels at dawn which were caused by respiration and decomposition of organic matter in the sediments, as shown by Barik *et al.* (2000). The decomposition processes at the pond bottom may hinder macrobenthic communities by limiting oxygen concentrations (Kolar & Rahel 1993), thereby reducing the natural food resources of bottom feeders such as common carp. The higher oxygen supply under semi-intensive pond management did not increase the zoobenthic food availability, because of higher predatory pressure from common carp stocked as the main species. These fish are known to prefer zoobenthos (Kolar & Rahel 1993; Milstein *et al.* 2002; Rahman *et al.* 2008; Rahman & Meyer 2009), but also feed on zooplankton as soon as preferred feed resources become limited (Rahman *et al.* 2008; Pucher & Focken 2013).

Even though the higher phytoplankton biomass under semi-intensive pond management serves as a potential feed base that could produce higher levels of zooplankton, the biomass estimates of zooplankton were statistically similar under both pond managements. The low zooplankton abundance, especially of the larger plankton, under both pond managements shows a high predatory pressure on the part of stocked fish species such as bighead carp that prefer to filter feed on zooplankton (Burke *et al.* 1986; Milstein 1992; Cooke *et al.* 2009). In a bottom-up and top-down control mechanism as described by Burke *et al.* (1986), the high predatory pressure and the mesosaprobity of the habitat resulted in a change of zooplankton composition towards smaller plankton dominated by rotatoria (Sládecek 1983). Also the phytoplankton community, that had adapted to water loaded with organic compounds, was dominated by euglenophyceae under both types of pond management (Seenayya *et al.* 1971). Under semi-intensive pond management, the phytoplankton abundance as well as the estimates of algal DM, CP, CL and GE were higher than under traditional pond management and served as an increased natural food resource for the stocked silver carp which is described in the literature as a filter feeder of phytoplankton (Spataru 1977; Schroeder *et al.* 1990). The increased total natural food base under semi-intensive pond management was caused by the higher qualitative fertilization, lower turbidity, higher light penetration and by water flow control, which inhibited continuous loss of nutrients and increased the water retention times (Demir & Kirkagac 2005; Nhan *et al.* 2008).

Generally, the natural food base is improved by semi-intensive management including higher quality fertilization as reported by Spataru (1977) and Turker *et al.* (2003) for filter feeders such as silver carp, bighead carp and tilapia.

The management changes of the grass carp-dominated traditional pond aquaculture towards a common carp-dominated semi-intensive pond aquaculture by using locally available higher qualitative fertilizer and feed resources and a restricting water inflow scheme did improve the water quality, oxygen supply as well as the natural food availability and improved conditions for profitable aquaculture production. The modifications in pond management have the potential to raise aquaculture production especially in mountainous watersheds that experience strong erosion from uplands as is the case in Northern Vietnam. In this experiment, net fish production over the seven months of the trial under the traditional pond management system was 87.4 ± 43.6 kg 1,000 m^{-2} and was increased to 227.5 ± 41.6 kg 1,000 m^{-2} under the semi-intensive management system (Pucher *et al.* 2013), showing the efficiency of the modifications. A change of flow-through systems towards static water systems reduces the impact of surrounding land uses on ponds, and may turn aquaculture under semi-intensive pond management into a more predictable farming activity with reduced risks of high fluctuations of water quality and natural food availability.

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3.5 References

Abbas HH (2006) Acute toxicity of ammonia to common carp fingerlings (*Cyprinus carpio*) at different pH levels. Pakistan Journal of Biological Sciences 9:2215–2221

Anrooy R van (2001) Fish marketing in the northern mountains of Viet Nam. FAO Aquaculture Newsletter 28:8–10

Anyusheva M, Lamers M, La N, Nguyen VN, Streck T (2012) Fate of pesticides in combined paddy rice–fish pond farming systems in Northern Vietnam. Journal of Environmental Quality 4:515-525

AOAC (1990) Official Methods of Analysis, 15th ed. Association of Official Analytical Chemists, Arlington, VA

Balcer MD, Korda NL, Dodson SI (1984) Zooplankton of the Great Lakes - A guide to the identification and ecology of the common crustacean species, 1st edition. The University of Wisconsin Press, London

Barik SK, Mishra S, Ayyappan S (2000) Decomposition patterns of unprocessed and processed lignocellulosics in a freshwater fish pond. Aquatic Ecology 34:185–204

Black KD (1998) The environmental interactions associated with fish culture. In: Biology of farmed fish (ed. by Black K.D. & Pickering A.D.), pp 284–325. Sheffield Academic Press, Sheffield

Burke JS, Bayne DR, Rea H (1986) Impact of silver and bighead carp on plankton communities of channel catfish ponds. Aquaculture 55:59–68

Cashman MJ, Wehr JD, Truhn KAM (2013) Elevated light and nutrients alter the nutritional quality of stream periphyton. Freshwater Biology 58:1447-1457

Chumchal MM, Drenner RW (2004) Interrelationships between phosphorus loading and common carp in the regulation of phytoplankton biomass. Archiv für Hydrobiologie 161:147–158

Chumchal MM, Nowlin WH, Drenner RW (2005) Biomass-dependent effects of common carp on water quality in shallow ponds. Hydrobiologia 545:271–277

Clemens G, Fiedler S, Cong ND, Van DN, Schuler U, Stahr K (2010) Soil fertility affected by land use history, relief position, and parent material under a tropical climate in NW-Vietnam. Catena 81:87–96

Condie SA, Webster IT (2002) Stratification and circulation in a shallow turbid waterbody. Environmental Fluid Mechanics 2:177–196

Cooke SE, Hill WR, Meyer KP (2009) Feeding at different plankton densities alters invasive bighead carp (*Hypophthalmichthys nobilis*) growth and zooplankton species composition. Hydrobiologia 625:185–193

Correll DL (1998) The role of phosphorus in the eutrophication of receiving waters: A review. Journal of Environmental Quality 27:261–266

Culver DA, Boucherle MM, Bean DJ, Fletcher JW (1985) Biomass of Freshwater Crustacean Zooplankton from Length-Weight Regressions. Canadian Journal of Fisheries and Aquatic Sciences 42:1380–1390

Dabrowska H, Sikora H (1986) Acute toxicity of ammonia to common carp (*Cyprinus carpio* L.). Polskie Archiwum Hydrobiologii 33:121–128

Dabrowska H, Wlasow T (1986) Sublethal effect of ammonia on certain biochemical and haematological indicators in common carp (*Cyprinus carpio* L.). Comparative Biochemistry and Physiology - C Pharmacology Toxicology and Endocrinology 83:179–184

De Silva SS (1995) Supplemental feeding in semi-intensive aquaculture systems. In: Farm-made aquafeed (ed. by New MB, Tacon AGJ, Csavas I), pp 24–60. FAO, Rome

Demir N, Kirkagac MUC (2005) Plankton composition and water quality in a pond of spring origin in Turkey. Limnology 6:189–194

Dongmeza E, Steinbronn S, Francis G, Focken U, Becker K (2009) Investigations on the nutrient and antinutrient content of typical plants used as fish feed in small scale aquaculture in the moutainous regions of Northern Vietnam. Animal Feed Science and Technology 149:162–178

Dongmeza EB, Francis G, Steinbronn S, Focken U, Becker K (2010) Investigation on the digestibility and metabolizability of the major nutrients and energy of maize leaves and barnyard grass in grass carp (*Ctenopharyngodon idella*). Aquaculture Nutrition 16:313–326

Dumont HJ, Velde I Van de, Dumont S (1975) The dry weight estimate of biomass in a selection of cladocera, copepoda and rotifera from the plankton, periphyton and benthos of continental waters. Oecologia 19:75–97

Edwards P, Pacharaprakiti C, Yomjinda M (1994) An Assessment of the role of buffalo manure for pond culture of tilapia. I. On-station experiment. Aquaculture 126:83–95

Edwards P, Demaine H, Innes-Taylor N, Turongruang D (1996) Sustainable aquaculture for small-scale farmers: need for a balanced model. Outlook on Agriculture 25:19–26

Ejsmont-Karabin J, Gorelysheva Z, Kalinowska K, Weglenska T (2004) Role of zooplankton (Ciliata, Rotifera and Crustacea) in phosphorus removal from cycling: Lakes of the River Jorka watershed (Masuria Lakeland, Poland). Polish Journal of Ecology 52:275–284

Elser JJ, Marzolf ER, Goldman CR (1990) Phosphorus and nitrogen limitation of phytoplankton growth in the freshwaters of North America: a review and critique of experimental enrichments. Canadian Journal of Fisheries and Aquatic Sciences 47:1468–1477

Emerson K, Russo RC, Lund RE, Thurston RV (1975) Aqueous ammonia equilibrium calculations: effect of pH and temperature. Journal of the Fisheries Research Board of Canada 32:2379–2383

FAO (2012) AGP - Fertilizer Specifications. http://www.fao.org/agriculture/crops/core-themes/theme/spi/plantnutrition/fertspecs/en/#c29989, Date of Access: 27.11.2012

Frei M, Becker K (2005) Integrated rice-fish production and methane emission under greenhouse conditions. Agriculture, Ecosystems and Environment 107:51–56

Fröhlich HL, Ingwersen J, Schmitter P, Lamers M, Hilger T, Schad I (2013) Water and Matter Flows in Mountainous Watersheds of Southeast Asia: Processes and Implications for Management (Chapter 3). In: Sustainable Land Use and Rural Development in Southeast Asia: Innovations and Policies for Mountainous Areas (ed. by Fröhlich HL, Schreinemachers P, Clemens G, Stahr K), pp 109–148. Springer Verlag, Heidelberg, New York, Dordrecht, London

Gericke S, Kurmies B (1952) Colometric determination of orthophosphorc acid using vanadate molybdate (German). Fresenius Journal of Analytical Chemistry 137:15–22

Hellung-Larsen P, Andersen AP (1989) Cell volume and dry weight of cultured Tetrahymena. Journal of Cell Science 92:319-24

Hepher B (1988) Principles of fish nutrition. In: Fish Culture in Warm Water Systems: Problems & Trends (ed. by Shilo M & Sarig S), pp 121–142. CRC Press, NY, USA

Hussenot J, Martin J-LM (1995) Assessment of the quality of pond sediment in aquaculture using simple, rapid techniques. Aquaculture International 3:123–133

Jana BB, Sahu SN (1993) Relative performance of three bottom grazing fishes (*Cyprius carpio*, *Cirrhinus mrigala*, *Heteropneustes fossilis*) in increasing the fertilizer value of phosphate rock. Aquaculture 115:19–29

Knud-Hansen CF, Hopkins KD, Guttman H (2003) A comparative analysis of the fixed-input, computer modeling, and algal bioassay approaches for identifying pond fertilization requirements for semi-intensive aquaculture. Aquaculture 228:189–214

Kolar CS, Rahel FJ (1993) Interaction of a biotic factor (predator presence) and an abiotic factor (low oxygen) as an influence on benthic invertebrate communities. Oecologia 95:210–219

Köthe J, Bitsch R (1992) Oxisolv plus microwave - a new way for sample pretreatment and sample preparation. Fresenius Journal of Analytical Chemistry 343:717–718

Kumar MS, Binh TT, Luu LT, Clarke SM (2005) Evaluation of fish production using organic and inorganic fertilizer: Application to grass carp polyculture. Journal of Applied Aquaculture 17:19–34

Kumar MS, Burgess SN, Luu LT (2004) Review of nutrient management in freshwater polyculture. Journal of Applied Aquaculture 16:17–44

Luu LT (2001) The VAC system in Northern Vietnam. In: Integrated agriculture-aquaculture - A primer. Fisheries Technical Paper 407, pp 29–32. FAO/IIRR/WorldFish Center, Rome

Milstein A (1992) Ecological aspects of fish species interactions in polyculture ponds. Hydrobiologia 231:177–186

Milstein A, Wahab MA, Rahman MM (2002) Environmental effects of common carp *Cyprinus carpio* (L.) and mrigal *Cirrhinus mrigala* (Hamilton) as bottom feeders in major Indian carp polycultures. Aquaculture Research 33:1103–1117

Minh T (2009) Agriculture innovation system in Vietnam Northern mountainous region - Six decade shift from a supply driven to a diversification oriented system. PhD thesis, University of Hohenheim, pp 129–131. Margraf Publishers, Hohenhein- Stuttgart

Minot N, Epprecht M, Anh TTT, Trung LQ (2006) Income diversification and poverty in the Northern Uplands of Vietnam. International Food Policy Research Institute (IFPRI), Washington, DC

Miserendino ML (2001) Length-mass relationships for macroinvertebrates in freshwater environments of Patagonia (Argentina). Ecologia Austral 11:3–8

Miyasaka H, Genkai-Kato M, Miyake Y, Kishi D, Katano I, Doi H, Ohba S-Y, Kuhara N (2008) Relationships between length and weight of freshwater macroinvertebrates in Japan. Limnology 9:75–80

Nauwerck A (1963) Die Beziehung zwischen Zooplankton und Phytoplankton im See Erken (German). Symbolae Botanicae Upsalienses 17:1–163

Nhan DK, Verdegem MCJ, Binh NT, Duong LT, Mistein A, Verreth AJ (2008) Economic and nutrient discharge tradeoffs of excreta-fed aquaculture in the Mekong Delta, Vietnam. Agriculture, Ecosystems and Environment 124:259–269

Nikolic N, Schultze-Kraft R, Nikolic M, Böcker R, Holz I (2008) Land degradation on barren hills: A case study in northeast Vietnam. Environmental Management 42:19–36

Pucher J, Focken U (2013) Intensification of common carp culture in rural areas of northern Vietnam by plant-based feeds with protein sources of different qualities. In: 6th International

Conference "Water & Fish" (ed. by Poleksic V). pp. 44-49. June 12th-14th. University of Belgrad, Faculty of Agriculture, Belgrad-Zemun, Serbia

Pucher J, Steinbronn S, Mayrhofer R, Schad I, El-Matbouli M, Focken U (2013) Improved Sustainable Aquaculture Systems for Small-Scale Farmers in Northern Vietnam (Chapter 8). In: Sustainable Land Use and Rural Development in Southeast Asia: Innovations and Policies for Mountainous Areas (ed. by Fröhlich HL, Schreinemachers P, Clemens G, Stahr K), pp 281-317. Springer Verlag, Heidelberg, New York, Dordrecht, London

Pucher J, Gut T, Mayrhofer R, El-Matbouli M, Viet PH, Ngoc NT, Lamers M, Streck T, Focken U (2014) Pesticide-contaminated feeds in integrated grass carp aquaculture: toxicology and bioaccumulation. Diseases of Aquatic Organisms 108:137-147

Rahman MM, Nagelkerke LAJ, Verdegem MCJ, Wahab MA, Verreth JAJ (2008) Relationships among water quality, food resources, fish diet and fish growth in polyculture ponds: A multivariate approach. Aquaculture 275:108–115

Rahman MM, Meyer CG (2009) Effects of food type on diel behaviours of common carp *Cyprinus carpio* in simulated aquaculture pond conditions. Journal of Fish Biology 74:2269–2278

Reinhardt N, Gut T, Lamers M, Streck T (2012) Water Regime in paddy rice systems in Vietnam: Importance of infiltration and bund flow. International Scientific Conference "Sustainable Land Use and Rural Development in Moutain Areas" (ed. Stahr K, Fröhlich H, Clemens G, Schreinemachers P), pp. 221-222. April 16th-18th. University of Hohenheim, Stuttgart, Germany

Ross LG (2000) Environmental physiology and energetics. In: Tilapias: biology and exploitation (Beveridge MCM & McAndrew BJ), pp 89–128. Kluwer Academic Publishers, Great Britain

Ruttner F (1938) Limnologische Studien an einigen Seen der Ostalpen (German). Archiv fuer Hydrobiologie 32:167–319

Schad I, Schmitter P, Saint-Macary C, Neef A, Lamers M, Nguyen L, Hilger T, Hoffmann V (2012) Why do people not learn from flood disasters? Evidence from Vietnam's Northwestern mountains. Natural Hazards 62:221-241

Schmitter P, Dercon G, Hilger T, Hertel M, Treffner J, Lam N, Vien TD, Cadisch G (2010a) Linking spatio-temporal variation of crop response with sediment deposition along paddy rice terraces. Agriculture, Ecosystems and Environment 155:34-45

Schmitter P, Dercon G, Hilger T, Ha TTL, Thanh NH, Lam N, Vien TD, Cadisch G (2010b) Sediment induced soil spatial variation in paddy fields of Northwest Vietnam. Geoderma 155:298-307

Schmitter P, Fröhlich HL, Dercon G, Hilger T, Thanh NH, Lam NT, Vien TD, Cadisch G (2012) Redistribution of carbon and nitrogen through irrigation in intensively cultivated tropical mountainous watersheds. Biogeochemistry 109:133–150

Schroeder GL, Wohlfarth GW, Alkon A, Halevy A, Krueger H (1990) The dominance of algal-based food webs in fish ponds receiving chemical fertilizers plus organic manures. Aquaculture 86:219–229

Schwoerbel J (1994) Methoden der Hydrobiologie, Süßwasserbiologie (German), 4th edition. Gustav Fischer Verlag, Stuttgart, Jena, New York

Seenayya G (1973) Ecological studies in the plankton of certain freshwater ponds of Hyderabad - India III. Zooplankton and bacteria. Hydrobiologia 41:529-540

Sevilleja R, Torres J, Sollows J, Little D (2001) Using animal wastes in fishponds. In: Integrated agriculture-aquaculture, a primer. Fisheries Technical Paper No 407:121–124

Sládecek V (1983) Rotifers as indicators of water quality. Hydrobiologia 100:169–201

Somsiri T, Oanh DTH, Chinabut S, Phuong NT, Shariff M, Yusoff FM, Bartie K, Giacomini M, Robba M, Bertone S, Huys G, Teale A (2006) A simple device for sampling pond sediment. Aquaculture 258:650–654

Spataru P (1977) Gut content of silver carp - *Hypophthalmichthys molitrix* (Val.) - and some trophic relations to other fish species in a polyculture system. Aquaculture 11:137–146

Steinbronn S (2009) A case study: Fish production in the integrated farming system of the Black Thai in Yen Chau district (Son La province) in mountainous north-western Vietnam - Current state and potential. Ph.D. Diss. Institute for Animal Production in the Tropics and Subtropics, Universität Hohenheim, Stuttgart-Hohenheim, Germany

Teichert-Coddington DR, Green BW (1993) Influence of daylight and incubation interval on water column respiration in tropical fish ponds. Hydrobiologia 250:159–165

Thai BT, Burridge CP, Austin CM (2007) Genetic diversity of common carp (*Cyprinus carpio* L.) in Vietnam using four microsatellite loci. Aquaculture 269:174–184

Thai BT, Pham TA, Austin CM (2006) Genetic diversity of common carp in Vietnam using direct sequencing and SSCP analysis of the mitochondrial DNA control region. Aquaculture 258:228–240

Turker H, Eversole AG, Brune DE (2003). Comparative Nile tilapia and silver carp filtration rates of partitioned aquaculture system phytoplankton. Aquaculture 220:449-457

Van TN (2003) Diversity of phytoplankton in Vietnamese inland water - Potential and challenges. Ho Chi Minh City, Agricultural Publishing House

Wezel A, Luibrand A, Thanh LQ (2002) Temporal changes of resource use, soil fertility and economic situation in upland Northwest Vietnam. Land Degradation and Development 13:33–44

4 Pond management strategies for small-scale aquaculture in northern Vietnam: Fish production and economic performance

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Abstract

The traditional pond aquaculture in northern Vietnam is a plant-based integrated aquaculture system using poor quality pond inputs (macrophytes, farm by-products, manures). Most applied feeds are palatable solely to the grass carp (*Ctenopharyngodon idella*), which is the main species in traditional fish polyculture. Secondary species are malnourished as the natural food productivity is diminished by uncontrolled water flow-through and high turbidity. Mass mortalities of grass carp lead to high financial losses for the farmers. To improve the fish production, researchers developed a semi-intensive pond management in stagnant water in which common carp was cultured as the main species with supplemental fertilization and pelleted feeds based mainly on locally available resources. In this study, the traditional and semi-intensive pond management was compared in six ponds for fish production, nutrient efficiencies and economic net-benefit. The use of higher quality feed and fertilizer inputs under semi-intensive pond management resulted in higher fish yields of 228 ± 42 kg 1000 m^{-2} compared to 88 ± 44 kg 1000 m^{-2} under traditional management and higher net economic benefit of 3,848,000 ± 1,469,000 VND 1000 m^{-2} under semi-intensive compared to 846,000 ± 3,753,000 VND 1000 m^{-2} under traditional management. Under semi-intensive management, 11.5% of applied total nitrogen was transferred into fish biomass while under traditional management, 4.4% of applied total nitrogen converted into fish biomass.

4.1 Introduction

Freshwater fish is an important part of the supply of animal based protein, amino acids, fatty acids, minerals and vitamins in the diet of humans, especially that of the poor in developing countries of South-East Asia (Tacon 1997; Prein *et al.* 2000; Dey and Ahmed 2005; Dey *et al.* 2005; Mishra and Ray 2009). In Vietnam, freshwater fish contributes 12.4% of the 29 g $capita^{-1}$ day^{-1} animal based protein supply (FAO 2013) of which 37% is supplied by cyprinid and cichlid species mainly produced by aquaculture (FAO 2011). It has been widely recognized that farm product diversification by aquaculture can contribute in a sustainable manner to food security and poverty alleviation (Tacon 1997; Edwards 2000; Prein *et al.* 2000) and may increase resilience to financial shocks in developing countries such as Vietnam. While in the lowlands of Vietnam, aquaculture practices improved greatly in the past decades, little improvements were achieved in the mountainous remote regions such as the northern mountainous region.

Even if improved infrastructure and the green revolution increased farmers' access to higher quality pond inputs and technologies as well as providing markets for selling higher value products such as fish (Anrooy 2001), these improvements seem not to ameliorate the fish supply in rural areas. As an example of this situation, the change in prices of several fish species on a rural market is shown in Figure 4.1.

The increase in fish prices can be attributed to a fall in fish supply and an increase in demand rather than to inflation. These higher demands in rural markets may provide an opportunity for small-scale fish farmers to increase their income by improving their traditional pond aquaculture.

Mountainous northern Vietnam is one of the poorest region of Vietnam (Mishra and Ray 2009) and is populated by high share of small-scale farmers often belonging to ethnic minorities (Minot *et al.* 2006; IFAD 2012). These small-scale farmers operate a subsistent oriented integrated farming system and are especially dependent on cash crops such as maize and cassava which are intensively cultivated on sloped fields and causes high levels of erosion (Wezel *et al.* 2002; Nikolic *et al.* 2008; Saint-Macary *et al.* 2010). The traditional pond aquaculture is integrated into the farming system similarly to the upland VAC (Vietnamese acronym "garden", "fishpond" and "livestock pen") system, which combines gardening, with fishpond aquaculture and livestock production as described by Luu (2001).

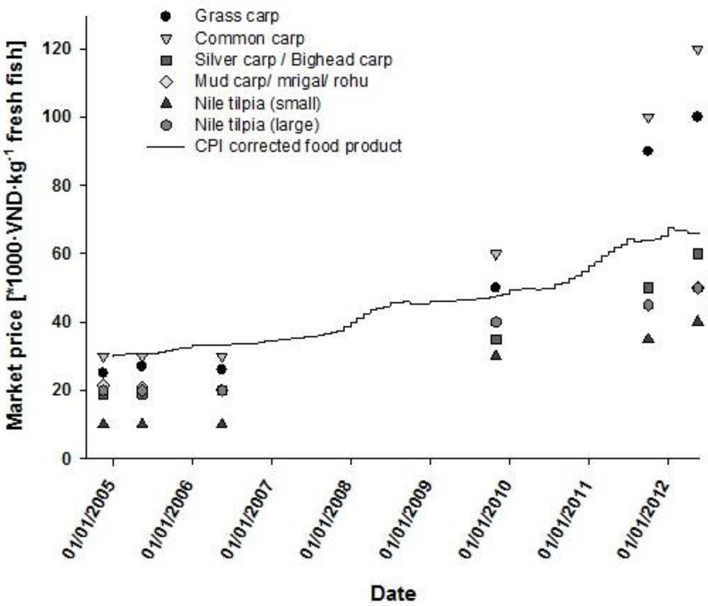

Figure 4.1: Prices of fresh fish of different species (*1000 VND kg^{-1}) at the local market of Yen Chau city (Steinbronn 2009 and own observations) and price development of a CPI correlated food product of a product with initial value of 50000 VND kg^{-1} (GSO 2013). CPI: Consumer Price Index; VND: Vietnamese Dong.

Farmers stock their ponds with a polyculture of the macro-herbivorous grass carp, *Ctenopharyngodon idella* (50-60% of fish biomass), as the main species with 3-5 secondary species including the omnivorous common carp (*Cyprinus carpio*), mud carp (*Cirrhinus cirrhosis*), Mrigal (*Cirrhinus mrigala*), and Nile tilapia (*Oreochromis niloticus*) as well as the filter feeders silver carp (*Hypophthalmichthys molitrix*) and bighead carp (*Aristichthys nobilis*) (Steinbronn 2009). Weeds from rice paddies, leaves from maize, cassava, rice and banana as well as farm by-products (rice bran, cassava peels…) and ruminant manure traditionally serve as pond inputs (Steinbronn 2009). Ponds are operated with a high water flow-through, which is transporting eroded particles from the upland fields into to ponds and results in ponds with high turbidity, high loss of nutrients and low productivity of natural food resources (Steinbronn 2009). The level of fish production is rather low for an external feed-based aquaculture and is caused by poor quality of external feeds and low availability of natural food resources (Thai *et al.* 2006, 2007; Steinbronn 2009), mass mortalities of grass carp caused by a species specific disease (Van *et al.* 2002; Steinbronn 2009), shortage of water, high labour requirements, poor water quality and pollution by pesticides (Steinbronn 2009).

To overcome these limitations of traditional aquaculture, modifications towards a semi-intensive pond management were developed. The modifications were based on the local knowledge of the farmers and locally available feed and fertilizer resources using a participatory approach. The modifications included the replacement of disease prone grass carp by common carp as the main species, improved fertilization, water-flow control to give a much lower turnover of water in the ponds and supplemental feeding of the common carp with pelleted feed locally produced and based on mainly locally available feed resources (Pucher *et al.* 2013). The objective of this study was to compare the traditional and the semi-intensive pond management for their fish production, feed utilization, nutrient efficiency and economic net benefit under local field conditions.

4.2 Material and methods

Study area and site selection

The study was carried out as an action-research trial in three neighbouring pairs of ponds (total six ponds) belonging to Black Thai farmers in Yen Chau district, Son La province, northern Vietnam and was conducted between April and October 2009. The chosen ponds were situated at altitudes between 420 and 470 m height above mean sea level, had a pond area between 540 and 990 m and had a pond depth between 0.9 and 1.3 m. The pairs of ponds were selected to cover the typical local environmental circumstances by differing with respect to surrounding land use (sloped maize fields, forest and rice paddies) and water sources (rain fed, irrigation water channelled from a reservoir) The ponds of each pair were less than 50 m away from each other. For more details on location (GPS), shape and surrounding land use and soil quality, see the description published in Pucher *et al.* (2014a).

Before starting the trial, all ponds were completely drained, harvested, filled with fresh water and fertilized with ruminant manure (230 kg 1000 m^{-2} FM). 10 days after filling and fertilizing, ponds were stocked at a density of 1.5 fingerlings per m^2. The following fish species and fish sizes (total body length; body masses; number of fish measured for total body length and body mass) were stocked to the ponds: grass carp (64.9±7.9 mm; 3.6±2.3 g; n=66), common carp (47.9±8.5 mm; 1.4±0.9 g; n=38), bighead carp (46.1±3.8 mm; 1.0±0.3 g; n=35), silver carp (78.8±8.7 mm; 3.7±1.3 g; n=19) and Nile tilapia (43.4±7.6 mm; 1.4±0.9 g; n=47). All ponds were stocked with the same stocks of fingerlings. Fingerlings were bought from two local nurseries, which are supplied with fry from the only hatchery in the Son La province located in the provincial capital Son La city.

The farmers following the traditional management scheme (see Table 4.1) managed one pond of each pair of ponds. Farmers following the modifications towards a semi-intensive aquaculture as advised by researchers (see Table 4.1) managed the second pond of each pair of ponds.

Table 4.1: Management characteristics of the traditional and semi-intensive pond management.

	Traditional pond management	**Semi-intensive pond management**
Pond preparation:		
Greening of dikes and channels around the pond*	No	Yes
Installation of pipes at water in- and outflow	No	Yes
Liming [kg $CaCO_3$ 1000 m^{-2}]	-	90
Fish stocking:		
Main species (60% of total fish number or 0.9 fish m^{-2})	Grass carp	Common carp
Secondary species (10% of total fish number or 0.15 fish m^{-2}, each)	Common carp, silver carp, bighead carp, Nile tilapia	Grass carp, silver carp, bighead carp, Nile tilapia
Water management scheme:	No water flow control, dependent on farmers´ practice and water availability	Minimized water inflow**
Fertilization [kg 1000 m^{-2} $month^{-1}$]:		
Dry matter of ruminant manure	49 ± 13	56 ± 16
Urea		6.3 ± 0.2
Inorganic phosphorus		2.3 ± 0.2
Total fertilizer nitrogen input	1.7 ± 0.4	2.6 ± 0.7
Total fertilizer phosphorus input	0.1 ± 0.0	0.5 ± 0.1
Feed management scheme:	Leaves, grasses and by-products	Leaves, grasses, by-products, supplemental feeding of pellets

* Greening of dikes and surrounding channels were installed to reduce the inflow of runoff water from surrounding slopes.
**Water inflow pipes were generally closed and were just occasionally opened to top up the ponds with fresh water. Inflow pipes were closed altogether during rain events. Times of water inflow were scheduled to coincide with days without rain and when pesticides had not been applied upstream for more than a week.

Feed preparation and feeding management

Under both pond managements, traditionally used feed resources were applied. Under traditional pond management, the farmers used significantly higher amounts of leaf material as feed inputs to feed the high stocking level of grass carp. To apply leaf material, all farmers used floating bamboo feeding frames situated in a deep area of the pond. All plant based feeds were applied within this feeding frame to keep the feeds easily accessible for the grass carp and to facilitate the removal of the larger unconsumed plant parts such as middle ribs of banana leaves or branches of bamboo and cassava. Even though farmers removed some of the larger pieces of unconsumed plant material, substantial masses of this fraction were not recovered by this practice and were left in the pond (Table 4.2, see "unconsumed fraction"). The quantity of this fraction was found to be significantly greater under traditional than semi-intensive pond management.

Under the traditional pond management, no compound feeding is utilized. For the semi-intensive pond management, pelleted feed was produced and provided by researchers. It contained heat-treated full-fat soybean meal (43%), corn meal (35%), fishmeal (10%), cassava meal (7%), rice bran (3.3%), mineral mix (1%), vitamin mix (0.5%), and sunflower oil (0.2%). This feed formulation had shown to be the most economical mix for common carp culture under the local market situation and feed ingredient availability (Tuan 2010). All feed ingredients, except fishmeal, are farm or by-products and are locally available. The fishmeal was purchased from a large feed ingredient retailer in Hanoi and contained 48% crude protein, 40% crude ash and 6% crude lipid of dry matter and contained 14 kJ kg^{-1} gross energy. The feed was pelleted using a small mincing machine and was sun or heat dried. Farmers were advised to split the daily pelleted feed ration into two portions; one for the morning and one for the evening. Every month, the feeding rate was adjusted to the current fish biomass by means of monthly catching and weighing of fish in each pond. Supplemental feeding rate was targeted to amount 2.7-3% of total fish biomass which was evaluated as the most efficient feeding level in a polyculture stocked with more than 50% omnivorous fish species (Abdelghany and Ahmad 2002). However, it was not possible to catch a representative number of all stocked species without significantly increasing pond turbidity. Therefore, the feeding rate was adjusted to daily 6% of common carp biomass by using common carp (60% of the numbers of stocked fish) as an estimate of the total fish biomass under the assumption that growth of common carp will be proportional to the growth of other stocked species.

The daily feeding and fertilization with commonly used on-farm feed resources, such as leaves, manure and farm by-products, was decided by farmers themselves with respect to availability and acceptance by fish in both systems. Each farmer recorded daily the types and masses of inputs. These input data were used to calculate budgets and efficiencies on a pond basis. No fish were harvested during the period of cooperation between farmers and researchers.

Sampling and chemical analysis

In October, the ponds were completely harvested by draining the water and catching all fish using beach seine, cast nets and drop nets. The fishes were weighed in groups of 20. Based on body masses at stocking and final harvest, specific growth rates (SGRs) were calculated for each fish species by using the formula:

SGR = 100 x [(ln final fish mass - ln initial fish mass)/days of experiment].

Six individuals of each stocked fish species from each pond were killed and divided into two groups of 3 fish for analysis. The groups of fish were autoclaved, homogenized and freeze-dried. All pond inputs (leaves, by-products, fertilizers and pellet feed) used by farmers were sampled once a month in each pond. All feed items were separated into consumed and unconsumed fractions based on observations of uneaten fractions remaining in the pond after 24 hours. The two fractions were dried and ground.

Samples of fish and feeds were analysed at the University of Hohenheim (Germany) for DM, crude lipid (CL), crude ash (CA) and total phosphorus (TP) according the AOAC (1990). Total nitrogen (TN) and Crude protein (CP) was determined using a C/N analyser (Vario MAX CN, Elementar Analysensysteme GmbH, Germany) with a protein/nitrogen conversion factor of 6.25. Gross energy (GE) was determined with a bomb calorimeter (IKA C 7000, Janke & Kunkel IKA-Analysentechnik, Germany) using a benzoic acid standard. In feed samples, neutral detergent fibre (NDF), acid detergent fibre (ADF) and lignin were determined according to Van Soest *et al.* (1991) with the modification that NDF was assayed without sodium sulphite and amylase.

Data description and statistical analysis

The fish yield and feeding data are shown in kg 1000 m^{-2} as this area is close to the average pond area owned and managed by a Black Thai farmer household in the research area (Steinbronn 2009). The following budget estimates were calculated based on input and output data gained from the record books of farmers and the final harvest as well as the proximate composition of feed, fertilizers and fish:

Protein efficiency ratio (PER) = fish FM gain / CP fed

Protein productive value (PPV) = (fish CP gain / CP fed) x 100

Apparent lipid conversion (ALC) = (fish CL gain / CL fed) x 100

TN efficiency ratio (TNER) = fish FM gain / TN feed and fertilizer

TN productive value (TNPV) = (fish TN gain / TN feed and fertilizer) x 100

TP efficiency ratio (TPER) = fish FM gain / TP feed and fertilizer

TP productive value (TPPV) = (fish TP gain / TP feed and fertilizer) x 100.

In the calculated efficiencies, only recovered/harvested fish were included. Fish that were lost due to mortality and other losses were not included as time and amount of these losses were not measureable and do not reflect the practical value for the farmers. As these losses of fish were not included, the efficiencies calculated here are lower than the actual efficiencies. Based on local market prices of tradable feeds, fertilizers and fish, the economic net benefit was calculated.

Data were processed and depicted using SigmaPlot 11.0 and statistically analysed using STATISTICA 8 (StatSoft®, USA). The two pond managements were compared according to the added feed amounts, fish yields and crude nutrient turnover by one-way ANOVAs. Data sets were tested for homogeneity by the Levene test. Data distributions were checked visually for normality. To meet the assumptions necessary for ANOVAs, data sets were log or square root transformed, if needed. Data sets, which strongly violated the assumptions of one-way ANOVA, were analysed by a non-parametric equivalent test for pond management effects (Mann-Whitney U Test). All statistical tests were performed at a level of significance at 5%.

4.3 Results

The amount and kind of leaf material used as feed inputs under both pond managements was left to each farmer and differed from farm to farm and depended on the availability of on-farm resources, the effort required to transport these resources from the fields to the ponds and the time availability of the farmer concerned (Table 4.2).

Plant material from maize, banana, cassava, elephant grass and mixed weeds was consumed to the extent of about 65% to 80% of FM, while the unconsumed fractions were deposited on the pond bottom where they decomposed. Compared to the consumed fractions, unconsumed fractions of plant material had an overall higher content of NDF, ADF and lignin and a lower content of CL and CP. Under traditional pond management, rice bran, cassava tuber and other by-products were used more extensively than under semi-intensive pond management because farmers managing semi-intensively used the compound feed instead (Table 4.2).

In Table 4.3, fish yields, retrieval rates and specific growth rates (SGR) are shown for both pond managements. The retrieval rates of stocked fish were not significantly different between the two pond managements but were numerically higher for all species under semi-intensive pond management. Planktivorous fish showed higher retrieval rates than other species especially under semi-intensive pond management. Nile tilapia had a retrieval rate above 100% due to their high reproduction rates. Poor retrieval rates were found for common carp and grass carp. The fish losses cannot be explained by mass mortalities as farmers only occasionally observed single dead fish. However, owing to the presence of many naturally occurring and self-recruiting scavenger species in the ponds (crabs, shrimps) occasional mortalities of fish may not be observable for farmers because the dead fish are quickly eaten.

The semi-intensive pond management resulted in a significantly higher fish yield and higher SGRs for all species, except for common carp, in comparison to the traditional pond management.

The species-specific chemical composition of the harvested fish is shown in Table 4.4. Under semi-intensive pond management, all fish species, and especially common carp, showed higher contents of CL as well as GE and a lower content of CA than the fish harvested under traditional pond management. This effect was significant for grass carp, common carp and silver carp.

Protein efficiency ratios (PER), protein productive values (PPV) and apparent lipid conversion (ALC) under both pond managements are shown in Table 4.5. For these calculations, only the consumed fractions of plant material were taken into account. The net conversions of CP and CL from the applied feeds were significantly higher under semi-intensive than under traditional pond management. In Table 4.5, the efficiency ratios are shown for TN (TNER, TNPV) and TP (TPER, TPPV). These calculations include the inputs of chemical and organic fertilizer, consumed feed fractions and the unconsumed feed fractions used as pond inputs. Under semi-intensive pond management, the input of 1 kg of TN produced on average 4.9 kg fresh fish while on average 11.5% of TN was transferred into fish biomass. Under traditional pond management, on average 1.9 kg of fish fresh mass was produced by each kg of TN added to the pond while on average only 4.4% of the added TN was transferred into fish biomass.

Table 4.2: Amount of feed inputs in dry matter (DM), crude protein (CP), crude lipid (CL), total carbon (TC) and total phosphorus (TP) used under traditional and semi-intensive pond management during the 6 month production cycle. Based on observations in the ponds, plant materials were separated into consumable and unconsumable fractions and were determined on a fresh matter (FM) basis.

	Fraction	Fraction of FM [%]	Traditional management [kg 1000 m^{-2}]					Semi-intensive management [kg 1000 m^{-2}]				
			DM	CP	CL	TC	TP	DM	CP	CL	TC	TP
Maize leaves	Consumed	72±6	155±102	17±11	3±2	69±46	0.3±0.2	95±62	11±7	2±1	42±27	0.2±0.1
	Unconsumed	28±6	58±39	3±2	1±1	26±18	0.1±0.0	36±23	2±1	1±0	16±11	0.0±0.0
Banana leaves	Consumed	67±4	100±60	17±10	5±3	46±28	0.2±0.1	28±19	5±3	2±1	13±9	0.1±0.0
	Unconsumed	33±4	17±11	0±0	0±0	7±4	0.0±0.0	5±3	0±0	0±0	2±1	0.0±0.0
Cassava leaves	Consumed	80±17	230±19[a]	57±5[a]	13±1[a]	114±9[a]	0.7±0.1[a]	60±83[b]	15±20[b]	4±5[b]	30±41[b]	0.2±0.2[b]
	Unconsumed	21±17	35±3[a]	3±0[a]	1±0[a]	16±1 [a]	0.1±0.0 [a]	9±13[b]	1±1[b]	0±0[b]	4±6[b]	0.0±0.0[b]
Elephant grass	Consumed	65±8	69±55[a]	10±8[a]	1±1[a]	30±24[a]	0.2±0.2[a]	5±4[b]	1±1[b]	0±0[b]	2±2[b]	0.0±0.0[b]
	Unconsumed	35±8	34±27[a]	4±3[a]	0±0[a]	14±11[a]	0.1±0.1[a]	2±2[b]	0±0[b]	0±0[b]	1±1[b]	0.0±0.0[b]
Mixed weeds	Consumed	78±1	155±55[a]	29±10[a]	1±1	66±23[a]	0.5±0.2	56±35[b]	10±7[b]	1±1	24±15[b]	0.2±0.1
	Unconsumed	22±1	50±18	4±2	0±0	22±8	0.1±0.0	18±11	2±1	0±0	8±5	0.0±0.0
Duckweed	Consumed	100	4±4	1±0	0±0	1±1	0.0±0.0	5±6	1±1	0±0	1±2	0.0±0.0
Rice bran	Consumed	100	398±292	32±24	3±2	181±132	2.8±2.0	58±40	5±3	3±2	26±18	0.4±0.3
Cass. tuber	Consumed	100	239±266	3±4	1±0	110±123	0.2±0.3	118±29	2±0	1±0	56±13	0.1±0.0
Comp. feed	Consumed	100	-	-	-	-	-	323±102	87±28	33±0	152±48	1.6±0.5
Total	**Consumed**		**1350±725**	**166±55**	**47±21**	**616±329**	**4.9±2.7**	**748±177**	**136±40**	**44±14**	**345±85**	**2.8±0.8**
Total	**Unconsumed**		**195±76[a]**	**15±6[a]**	**3±1**	**86±34[a]**	**0.3±0.1**	**70±38[b]**	**5±2[b]**	**1±1**	**31±17[b]**	**0.1±0.1**
Grand Total			**1545±787**	**181±60**	**50±22**	**702±356**	**5.3±2.8**	**818±205**	**140±41**	**45±14**	**376±97**	**2.9±0.9**

Mean values in the rows that do not share the same superscript(s) differ significantly at $p \leq 0.05$. Mean values in the rows without superscripts did not differ significantly.

Table 4.3: Final fish net yields, retrieval rates and specific growth rates (SGR) in means (± standard deviations of the pond means) of the two pond managements for a 6 month production cycle

	Traditional pond management			Semi-intensive pond management		
	Fish yield [kg 1000 m^{-2} FM]	Retrieval rate [%]	SGR [%]	Fish yield [kg 1000 m^{-2} FM]	Retrieval rate [%]	SGR [%]
Grass carp	37±19	41±27	1.8±0.1^{b}	46±12	67±25	2.6±0.2^{a}
Common carp	4±6^{b}	20±17	1.9±0.7	36±13^{a}	38±8	2.4±0.3
Silver carp	17±4^{b}	89±14	1.7±0.0^{b}	61±3^{a}	92±5	2.5±0.1^{a}
Bighead carp	8±4^{b}	78±25	2.0±0.0^{b}	40±13^{a}	93±0	2.9±0.2^{a}
Nile tilapia	16±15	138±56	2.5±0.4*	36±22	227±182	3.4±0.6*
SRS	5±4			10±15		
Total fish	**88±44^{b}**	**60±21**		**228±42^{a}**	**76±22**	

Mean values in the rows that do not share the same superscript(s) differ significantly at p≤0.05. Mean values in the rows without superscripts did not differ significantly. FM: fresh mass, SGR: specific growth rate, SRS: self-recruiting species.

*Calculated only for stocked cohorts (reproduction excluded).

The two pond managements did not significantly differ in the efficiency of TP added. However, under semi-intensive pond management, on average 29.5 kg of fresh fish was produced per kg of added TP while under traditional pond management, the fish production per kg of added TP amounted to an average of 16.2 kg with a high variation between ponds.

In Table 4.6, the costs of the used input resources and benefits associated with the two pond managements are shown. For calculation of financial benefit, only resources that are tradable in the region were taken into account. Pond operation was carried out by family labour. Leaves and grasses as feed, manure as fertilizer and opportunity costs of labour for collection of these feeds and fertilizers were not included here, as these resources had no monetary value in the research area. Under traditional pond management, only rice bran was a resource with a market value and showed a high variation in the amount used as pond input. Under semi-intensive pond management, compound feed was the highest financial input comprising on average 70% of the total value of pond inputs. The costs of feed ingredients, electricity and production labour are included in the price of the compound feed. Chemical fertilizer accounted for 8% of the financial input. The value of the produced fish was significantly higher under semi-intensive management and resulted on average in a higher net benefit than under traditional pond management. The latter showed a highly diverse net benefit and even a net financial deficit in one of the ponds which was rain fed, situated in the upland fields and highly effected by eroded particles. This pond was fed with higher amounts of rice bran than the other ponds (see high variation of rice bran input in Table 4.2) and showed the lowest retrieval rates, fish growth, natural food resources and oxygen supply.

Table 4.4: Dry matter content and chemical composition of fish harvested under traditional and semi-intensive pond management.

	Traditional pond management					**Semi-intensive pond management**				
	DM [% FM]	CA [% DM]	CP [% DM]	CL [% DM]	GE [MJ kg^{-1} DM]	DM [% FM]	CA [% DM]	CP [% DM]	CL [% DM]	GE [MJ kg^{-1} DM]
Grass carp	22.0±1.6^{b}	17.0±2.7^{a}	65.6±2.9^{a}	6.2±1.8^{b}	20.9±0.9^{b}	27.6±2.4^{a}	10.8±0.3^{b}	53.0±1.9^{b}	20.2±5.6^{a}	25.2±0.7^{a}
Common carp	35.8±12.6	15.9±5.7^{a}	61.7±3.4^{a}	16.1±7.2^{b}	22.5±2.3^{b}	34.4±3.3	5.6±2.2^{b}	43.0±7.5^{b}	46.0±10.3^{a}	29.9±2.2^{a}
Silver carp	23.2±1.5^{b}	19.8±0.6^{a}	63.4±1.6^{a}	10.3±1.6	20.7±0.2^{b}	29.2±1.1^{a}	16.0±2.6^{b}	56.8±0.6^{b}	14.4±5.7	22.8±1.3^{a}
Bighead carp	18.6±2.2^{b}	25.2±1.3	66.1±4.5	5.9±4.3	18.3±1.0	24.8±2.8^{a}	18.4±6.5	59.1±6.1	13.8±9.3	21.7±3.1
Nile tilapia	28.5±4.4	16.2±7.0	55.5±3.2	23.8±8.6	23.1±3.0	31.3±2.0	11.0±1.2	51.8±1.0	30.1±0.2	25.6±0.6

Mean values in the rows that do not share the same superscript(s) differ significantly at p≤0.05. Mean values in the rows without superscripts did not differ significantly. CA: crude ash, CL: crude lipid, CP: crude protein, DM: dry matter, FM: fresh matter, GE: gross energy.

Table 4.5: Conversion indicators of crude protein, crude lipid, gross energy, total nitrogen (TN) and total phosphorus (TP) of applied feeds and fertilizers to fish in means (± standard deviations of the pond means) under both pond managements.

		Traditional pond management	**Semi-intensive pond management**
Feed efficiencies	PER	0.48±0.35^{b}	1.72±0.35^{a}
	PPV [%]	6.9±5.2^{b}	25.6±6.0^{a}
	ALC [%]	6.1±5.3^{b}	37.2±2.6^{a}
TN efficiencies:	TNER	1.9±1.3^{b}	4.9±0.8^{a}
	TNPV [%]	4.4±3.2^{b}	11.5±1.3^{a}
TP efficiencies:	TPER	16.2±11.6^{a}	29.5±4.7^{a}
	TPPV [%]	6.8±5.7^{a}	16.5±5.9^{a}

Mean values in the rows that do not share the same superscript(s) differ significantly at p≤0.05. ALC: Apparent lipid conversion, PER: Protein efficiency ratio, PPV: Protein productive value, TNER: Total nitrogen efficiency ratio, TNPV: Total nitrogen productive value, TPER: Total phosphorus efficiency ratio, TPPV: Total phosphorus productive value.

Table 4.6: Financial analysis: Costs and benefits of tradable pond inputs in x 1000 VND under both pond managements. Pond inputs which are not traded in the region (leaves, organic fertilizer) and family labour are not taken into account.

			Value of in- & outputs [1000 VND 1000 m^{-2} $trial^{-1}$]	
		Price [kg^{-1}]	**Traditional management**	**Semi-intensive management**
Costs	Fish fingerlings		948±1[a]	788±8[b]
	Lime	0.8	0±0[b]	19±2[a]
	Urea	7	0±0[b]	207±23[a]
	Super phosphate	3.1	0±0[b]	233±4[a]
	Rice bran	3	1825±2047	391±138
	Compound feed	11	0±0[b]	3875±1222[a]
	Total pond inputs		**2773±2048**	**5513±1052**
Benefit	Grass Carp	50	1842±942	2273±591
	Common Carp	60	258±328[b]	2146±764[a]
	Silver Carp	35	586±134[b]	2127±99[a]
	Bighead Carp	35	289±156[b]	1385±454[a]
	Nile Tilapia	40	644±617	1428±876
	Total fish		**3619±2112[b]**	**9360±1139[a]**
Net benefit			**846±3753**	**3848±1469**

Mean values in the rows that do not share the same superscript(s) differ significantly at p≤0.05. The cost-benefit calculation is based on local prices in the year of the trial (2009). VND: Vietnamese Dong (US$ 1.00 = VND 18.000).

4.4 Discussion

The leaf material and organic fertilizer used as pond inputs in this study had similar proximate compositions to those reported previously for this region (Dongmeza *et al.* 2009; Steinbronn 2009) and were applied under traditional pond management at rates similar to the daily rate of 3.7 kg 1000 m^{-2} DM as reported by Steinbronn (2009). All plant feed resources showed high contents of NDF, ADF and lignin that far exceeded the suggested maximal crude fibre content of feeds for grass carp (Liao *et al.* 1980; Mao *et al.* 1985) and is known to reduce the digestibility of nutrients and energy (Steffens 1989; Anderson *et al.* 1991; NRC 2011). Most plant materials traditionally used as fish feed in the research region were identified as unsuitable by Dongmeza *et al.* (2009), due to the high levels of crude fibre and/or high levels of anti-nutrients such as cyanide, phytic acid and tannins, which were shown to have adverse effects on growth and/or feed utilisation (Ng and Wee 1989; Becker and Makkar 1999; Francis *et al.* 2001). In the polyculture systems, the plant-derived feed resources are only utilizable by the grass carp which is widely reported to feed on macrophytes after it reaches a body length of 25-30 mm (Opuszynski 1972; De Silva and Weerakoon 1981). Feed intake of grass carp is known to be dependent on fish size, temperature and kind of feed (Opuszynski 1972; Cai and Curtis 1990; Osborne and Riddle 1999). Under traditional pond management, the SGR of grass carp was in the range of reported growth rates of grass carp that had been fed on several terrestrial plants: Feeding of Cassava leaves or Napier grass to grass carp resulted in SGRs of 0.5 and 1.0, respectively (Tan 1970), while in other studies Napier grass resulted in SGRs of 1.8 and 2.7 (Venkatesh and Shetty 1978; Shrestha and Yadac 1998). The significantly increased production and SGRs of grass carp under semi-intensive pond management in this study suggest that they were freely consuming the supplemental pellet feed.

The higher quality feed and fertilizer input significantly affected the overall fish production under semi-intensive pond management. Higher quality fertilization and a water management regime that led to more static conditions in the ponds enhanced primary and secondary production (as shown in Pucher *et al.* 2014b) which in turn favoured those species generally considered to be filter feeders especially silver carp and bighead carp (Spataru 1977; Burke *et al.* 1986; Milstein 1992; Turker *et al.* 2003; Milstein *et al.* 2006; Zhang *et al.* 2006; Cooke *et al.* 2009). Nile tilapia showed high production and SGRs under both pond managements which reflects their wide range of external and natural food resources (Beveridge *et al.* 1989; Schroeder *et al.* 1990; Beveridge and Baird 2000). In other studies on semi-intensive management, common carp showed preference to feed on pelleted feed, macro-zoobenthos and too a little extent on zooplankton (Schroeder 1983; Rahman *et al.* 2008). In a polyculture of silver carp (1 fish m^{-2}), Nile tilapia (1 fish m^{-2}) and common carp (0.2 fish m^{-2}), Abdelghany and Ahmad (2002) reported a daily supplemental pellet feeding rate of 2.7% of total fish biomass combined with intensive fertilization as being optimal for fish growth and financial net benefit. In their study, Nile tilapia, silver carp and common carp showed SGRs of 1.9, 3.0 and 3.6%, respectively, at a supplemental feeding rate of commercial pellets (full feed) of 3% total fish mass. These high SGRs were not reached in this present study as the feeds and fertilizers applied were of lower quality and quantity. The initial assumption for the calculation of feeding rates in semi-intensively managed ponds, namely that common carp make up 50% of the total fish biomass, turned out to be wrong as the relative growth of other stocked species was much higher. Over time, this resulted in an increasingly lower pellet feed supply than the targeted rate of 3% of total fish biomass. The effective feeding rate towards the end of the experiment has been recalculated as about 1% of the total fish biomass. This may explain the relative poor growth rate of common carp that is the most dependant on pelleted feed.

The proximate composition of fish under semi-intensive pond management suggests an improved nutritional status compared to fish under traditional management (Table 4.4). Under semi-intensive management, the high CL content of common carp, as the main consumer of the pellet feed, indicates an oversupply of energy resources in the feeds. This requires a future optimization of the feeding management either by increasing pond fertilization to enhance the availability of protein from natural food resources or by increasing the level of CP in the pelleted feeds as suggested by De Silva (1993).

The low retrieval rates of common carp and grass carp, which are the main species in both management regimes and which have the highest market value, negatively affected the benefit. It is widely known in northern Vietnam that grass carp is a sensitive fish species susceptible to disease and that it suffers from mass mortalities (Van *et al.* 2002; Steinbronn 2009; Pucher *et al.* 2013). No mass mortalities were observed in the present study but there does seem to have been unobserved mortality of grass carp which may have been caused by a multi-factorial disease with opportunistic bacteria being the primary agents combined with unsuitable environmental circumstances, malnutrition or pesticide contaminated plant based feeds as described by Pucher *et al.* (2013, 2014c). Pouching and predation by birds and reptiles cannot completely been ruled out, as the experiment was carried out in real farmers ponds in an open landscape and not in the more controlled environment of a research station. In any case, our data demonstrate a positive correlation of nutritional status and retrieval rate of grass carp, making normal mortality a more likely reason for the disappearance of grass carp.

Common carp has only seldom been mentioned by farmers as being susceptible to diseases (Steinbronn 2009) but are potentially disease-prone as was demonstrated in 2011 when the Koi Carp Virus CyHV-3 was identified in the province´s largest hatchery (Pucher *et al.* 2013) where the fingerlings for this trial originated from.

The net benefit from the traditional pond management varied greatly with even financial losses occurring under the assumption that rice bran is tradable regardless of the season. Although semi-intensive pond management showed higher net-benefits in all ponds, the higher expenses for pond modifications, fish feed and fertilization, equivalent to 20% of a poor households' annual income (national poverty line), are more likely to be made by better-off and more educated farmers. Poor farmers were shown to be more risk averse (Saint-Macary *et al.* 2013) and the farmer's willingness to adopt innovations or modifications in their farming system is inversely correlated to the labour or financial inputs required (Reardon 1995). Since in the study area innovations are traditionally transferred horizontally between farmers (Schad *et al.* 2011), successful adopters may spread the innovations in future especially if the cost of pelleted feed can be reduced. Fishmeal is the only feed ingredient not being locally available and is therefore an expensive resource. Fishmeal may be replaced in the pelleted feeds by earthworms produced on-farm using available ruminant manure resources (Tuan 2010; Müller *et al.* 2012; Pucher *et al.* 2012a,b) which would reduce the operational costs for the supplemental pellet feeds and the risks (price, availability) of being dependent from external supplies.

With respect to household food security, the production of species mainly used for home consumption, i.e. Nile tilapia and self-recruiting species such as small indigenous fish, crabs, shrimps, snails etc. was also more than doubled under semi-intensive pond management. This results in a higher supply of animal protein from the pond to the respective households, in addition to the increased cash income.

4.5 Conclusion

The change towards a semi-intensive pond management that used higher quality feeds and fertilizer resources with reduced water flow and lower turbidity in ponds producing an omnivorous main fish species was shown to increase fish yields, nutrient efficiencies and economic net-benefit. This set of pond management modifications gives those farmers who adopt the possibility to diversify their farming products by using farm products as feed ingredients and to produce higher valued fish products. This may increase income, and the ability of farmers to adapt to sudden changes as well as improving the nutritional status of small-scale farming households. However, the implementation of semi-intensive pond management requires knowledge of investments, feed/fertilizer ingredients, and the technology of feed mincers / pelleters. Farmers may therefore need basic training to spread information, establish fish farmer unions or/and to get micro-credits to cover the initial cost of pond modification and the operational cost of feeds for the first year(s).

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4.6 References

Abdelghany AE, Ahmad MH (2002) Effects of feeding rates on growth and production of Nile tilapia, common carp and silver carp polycultured in fertilized ponds. Aquaculture Research 33:415-423

Anderson J, Capper BS, Bromage NR (1991) Measurement and prediction of digestible energy values in feedstuffs for the herbivorous fish tilapia (*Oreochromis niloticus* Linn.). British Journal of Nutrition 66:37–48

Anrooy R van (2001) Fish marketing in the northern mountains of Viet Nam. FAO Aquaculture Newsletter 28:8-10

AOAC (1990) Official Methods of Analysis, 15th ed. Association of Official Analytical Chemists, Arlington, VA.

Becker K, Makkar HPS (1999) Effects of dietary tannic acid and quebracho tannin on growth performance and metabolic rates of common carp (*Cyprinus carpio* L.). Aquaculture 175:327–335

Beveridge MCM, Begum M, Frerichs GN, Millar S (1989) The ingestion of bacteria in suspension by the tilapia *Oreochromis niloticus*. Aquaculture 81:373-378

Beveridge MCM, Baird DJ (2000) Diet, feeding and digestive physiology. In: Beveridge MCM, MacAndrew BJ (eds.) Tilapias: Biology and Exploitation. Kluwer Academic Publishers, Dordrecht, pp. 59-87

Burke JS, Bayne DR, Rea H (1986) Impact of silver and bighead carp on plankton communities of channel catfish ponds. Aquaculture 55:59-68

Cai Z, Curtis LR (1990) Effects of diet and temperature on food consumption, growth rate and tissue fatty-acid composition of triploid grass carp. Aquaculture 88:313-327

Cooke SE, Hill WR, Meyer KP (2009) Feeding at different plankton densities alters invasive bighead carp (*Hypophthalmichthys nobilis*) growth and zooplankton species composition. Hydrobiologia 625:185-193

De Silva SS, Weerakoon DEM (1981) Growth, food intake and evacuation rates of grass carp, *Ctenopharyngodon idella* fry. Aquaculture 25:67-76

De Silva SS (1993) Supplemental feeding in semi-intensive aquaculture systems. In: New MB, Tacon AGJ, Csavas I (eds.) Farm-made aquafeeds. Proceedings of the FAO/AADCP Regional Expert Consultation on Farm-Made Aquafeeds, 14-18 December 1992. FAO-RAPA/AADCP, Bangkok, Thailand, 434p., pp. 24-60

Dey MM, Ahmed M (2005) Aquaculture - Food and livelihood for the poor in Asia: A brief overview of this issues. Aquaculture, Economics and Management 9:1-10

Dey MM, Rab MA, Paraguas FJ, Bhatta R, Alam F, Koeshendrajana S, Ahmed M (2005) Status and economics of freshwater aquaculture in selected countries of Asia. Aquaculture, Economics and Management 9:11-37

Dongmeza E, Steinbronn S, Francis G, Focken U, Becker K (2009) Investigations on the nutrient and antinutrient content of typical plants used as fish feed in small scale aquaculture in the moutainous regions of Northern Vietnam. Animal Feed Science and Technology 149:162-178

Edwards P (2000) Aquaculture, poverty impacts and livelihoods. ODI Natural Resource Perspectives 56:1-4

FAO (2011) Aquaculture production 1950 – 2009. (Data set for Fishstat Plus). FAO Fisheries Department, Fishery Data and Statistics Unit.

FAO (2013) Food balance sheets. Vietnam. http://faostat.fao.org/site/368/DesktopDefault.aspx?PageID=368#ancor. Accessed 14 Feb 2013

Francis G, Makkar HPS, Becker K (2001) Antinutritional factors present in plant-derived alternate fish feed ingredients and their effects in fish. Aquaculture 199:197-227

GSO (2013) General Statistics Office of Vietnam, North West Region of Vietnam. http://www.gso.gov.vn. Accessed on 15 May 2013

IFAD (2012) Rural Poverty Portal: Rural poverty in Viet Nam. http://www.ruralpovertyportal.org/country/home/tags/viet_nam. Accessed on 15th Feb 2013

Liao X, Lin D, Mao Y, Cai F (1980) Experiments on enzymatic fiber pellets used as fish feed and protein requirements of grass carp (*Ctenopharyngodon idella* C. et V.). Journal of Fish China 4:217

Luu LT (2001) The VAC system in Northern Vietnam. In: Integrated agriculture-aquaculture: A primer. FAO/IIRR/WorldFish Center, Fisheries Technical Paper 407, Rome, pp. 29-32

Mao Y, Cai F, Lin D (1985) Studies on the daily requirements of protein, carbohydrates, fat, minerals and fiber of juvenile grass carp (*Ctenopharyngodon idella* C. et V.). Transactions of the Chinese Ichthyol Society 4:81

Milstein A (1992) Ecological aspects of fish species interactions in polyculture ponds. Hydrobiologia 231:177-186

Milstein A, Ahmed AF, Masud OA, Kadir A, Wahab MA (2006) Effects of the filter feeder silver carp and the bottom feeders mrigal and common carp on small indigenous fish species (SIS) and pond ecology. Aquaculture 258:439-451

Minot N, Epprecht M, Anh TTT, Trung LQ (2006) Income diversification and poverty in the Northern Uplands of Vietnam. International Food Policy Research Institute (IFPRI), Research Report 45, Washington, D.C., 137p.

Mishra V, Ray R (2009) Dietary diversity, food security and undernourishment: The Vietnamese evidence. Asian Economic Journal 23:225-247

Müller JL, Pucher J, Tran TNT, Focken U, Kreuzer M (2012) The potential of vermiculture to produce on-farm feed resources for aquaculture in mountainous areas of North Vietnam In: Stahr K, Fröhlich H, Clemens G, Schreinemachers P (eds.) International Scientific Conference "Sustainable Land Use and Rural Development in Mountain Areas", 16-18 April, University of Hohenheim, Stuttgart, Germany, pp. 113-114

Ng WK, Wee KL (1989) The nutritive value of cassava leaf meal in pelleted feed for Nile tilapia. Aquaculture 83:45-58

Nikolic N, Schultze-Kraft R, Nikolic M, Boecker R, Holz I (2008) Land degradation on barren hills: A case study in northeast Vietnam. Environmental Management 42:19-36

NRC (National Research Council) (2011) Nutrient Requirements of Fish and Shrimp. National Research Council of the National Academics, The National Academies Press, Washington D.C.

Opuszynski K (1972) Use of phytophagous fish to control aquatic plants. Aquaculture 1:61-74

Osborne JA, Riddle RD (1999) Feeding and growth rates for triploid grass carp as influenced by size and water temperature. Journal of Freshwater Ecology 14:41-45

Prein M, Ahmed M (2000) Integration of aquaculture into smallholder farming systems for improved food security and household nutrition. Food and Nutrition Bulletin 21:466-471

Pucher J, Mayrhofer R, El-Matbouli M, Focken U (2014a) Effects of modified pond management on limnological parameters in small-scale aquaculture ponds in mountainous Northern Vietnam. Aquaculture Research 1-15; doi:10.1111/are.12465

Pucher J, Mayrhofer R, El-Matbouli M, Focken U (2014b) 15N tracer application to evaluate nitrogen dynamics of food webs in two subtropical small-scale aquaculture ponds under different managements. Isotopes in Environmental and Health Studies 1-14; doi: 10.1080/10256016.2014.922963

Pucher J, Gut T, Mayrhofer R, El-Matbouli M, Viet PH, Ngoc NA, Lamers M, Streck T, Focken U (2014c) Pesticide contaminated feeds in integrated grass carp aquaculture: Toxicology and bioaccumulation. Diseases of Aquatic Organisms 108:137-147

Pucher J, Tuan NN, Yen TTH, Mayrhofer R, El-Matbouli M, Focken U (2012a) Earthworm meal as alternative animal protein source for full and supplemental feeds for Common Carp (*Cyprinus carpio* L.). In: Stahr K, Fröhlich H, Clemens G, Schreinemachers P (eds.) International Scientific Conference "Sustainable Land Use and Rural Development in Mountain Areas", University of Hohenheim, Stuttgart, Germany, 16-18 April, pp. 167-168

Pucher J, Tuan NN, Yen TTH, Mayrhofer R, El-Matbouli M, Focken U (2012b) Feeding Fish Without Fishmeal: Earthworm Meal as Alternative Animal Protein Source in Rural Areas. In: Tielkes E (ed.) Tropentag 2012 "Resilience of agricultural systems against crises", Göttingen - Kassel/Witzenhausen, Germany, 19-21 September. Cuvillier, pp. 75

Pucher J, Steinbronn S, Mayrhofer R, Schad I, El-Matbouli M, Focken U (2013) Improved Sustainable Aquaculture Systems for Small-Scale Farmers in Northern Vietnam. In: Fröhlich HL, Schreinemachers P, Clemens G, Stahr K (eds.) Sustainable Land Use and Rural Development in Southeast Asia: Innovations and Policies for Mountainous Areas. Springer Verlag, Heidelberg, New York, Dordrecht, London, pp. 281-317

Rahman MM, Jo Q, Gong YG, Miller SA, Hossain MY (2008) A comparative study of common carp (*Cyprinus carpio* L.) and calbasu (*Labeo calbasu* Hamilton) on bottom soil resuspension, water quality, nutrient accumulations, food intake and growth of fish in simulated rohu (*Labeo rohita* Hamilton) ponds. Aquaculture 285:78-83

Reardon T (1995) Sustainability issue for agricultural research strategies in semi arid tropics: Focus on the Sahel. Agricultural Systems 48:345-359

Saint-Macary C, Keil A, Nielsen T, Birkenberg A, Van LTA, Van DTT, Ufer S, Dung PTM, Heidhues F, Zeller M (2013) Linkages Between Agriculture, Poverty and Natural Resource Use in Mountainous Regions of Southeast Asia. In: Fröhlich HL, Schreinemachers P, Clemens G, Stahr K (eds.) Sustainable Land Use and Rural Development in Southeast Asia: Innovations and Policies for Mountainous Areas. Springer Verlag, Heidelberg, New York, Dordrecht, London, pp. 175-212

Saint-Macary C, Keil A, Zeller M, Heidhues F, Dung PTM (2010) Land titling policy and soil conservation in the Northern uplands of Vietnam. Land Use Policy 27:617-627

Schad I, Roessler R, Neef A, Zarate AV, Hoffamnn V (2011) Group-based learning in an authoritarian setting? Novel extension approaches in Vietnam's Northern uplands. Journal of Agricultural Education and Extension 17:85-98

Schroeder GL (1983) Sources of fish and prawn growth in polyculture ponds as indicated by dC analysis. Aquaculture 35:29-42

Schroeder GL, Wohlfarth G, Alkon A, Halevy A, Krueger H (1990) The dominance of algal-based food webs in fish ponds receiving chemical fertilizers plus organic manures. Aquaculture 86:219-229

Shrestha MK, Yadav CNR (1998) Feeding of napier (*Pennisetum purpureum*) to grass carp in polyculture: A sustainable fish culture practice for small farmers. Asian Fisheries Science 11:287-294

Spataru P (1977) Gut content of silver carp - *Hypophthalmichthys molitrix* (Val.) - and some trophic relations to other fish species in a polyculture system. Aquaculture 11:137-146

Steffens W (1989) Principles of Fish Nutrition. Ellis Horwood Ltd, Chichester, UK, 384p.

Steinbronn S (2009) A case study: Fish production in the integrated farming system of the Black Thai in Yen Chau district (Son La province) in mountainous North-Western Vietnam - Current state and potential (PhD thesis). Institute for Animal Productions in the Tropic and Subtropics, Hohenheim Stuttgart, Germany, University of Hohenheim, 207p.

Tacon AGJ (1997) Contribution to food fish supplied. In: Review of the State of World Aquaculture. FAO Fisheries Circular No. 886, FAO, Rome, Italy, 163p.

Tan YT (1970) Composition and nutritive value of some grasses, plants and aquatic weeds tested as diets. Journal of Fish Biology 2:253-257

Thai BT, Pham TA, Austin CM (2006) Genetic diversity of common carp in Vietnam using direct sequencing and SSCP analysis of the mitochondrial DNA control region. Aquaculture 258:228–240

Thai BT, Burridge CP, Austin CM (2007) Genetic diversity of common carp (*Cyprinus carpio* L.) in Vietnam using four microsatellite loci. Aquaculture 269:174-186

Tuan NN (2010) Development of supplemental diets for carp in Vietnamese upland ponds based on locally available resources (PhD thesis). Institute for Animal Productions in the Tropic and Subtropics, Hohenheim Stuttgart, Germany, University of Hohenheim, 159p.

Turker H, Eversole AG, Brune DE (2003) Comparative Nile tilapia and silver carp filtration rates of Partitioned Aquaculture System phytoplankton. Aquaculture 220:449-457

Van PT, Khoa LV, Lua DT, Van KV, Ha NT (2002) The impacts of red spot disease on small-scale aquaculture in Northern Vietnam. In: Arthur JR, Phillips MJ, Subasinghe RP, Reantaso MB, MacRae IH (eds.) Primary aquatic animal health care in rural, small-scale aquaculture development. FAO Fisheries Technical Paper 406, FAO, Rome, pp. 165-176

Van Soest PJ, Robertson JB, Lewis BA (1991) Methods of dietary fiber, neutral detergent fiber and non starch polysaccharides in relation to animal nutrition. Journal of Dairy Science 74:3583–3597

Venkatesh B, Shetty HPC (1978) Studies on the growth rate of grass carp *Ctenopharyngodon idella* (Valenciennes) fed on two aquactic weeds and a terrestrial grass. Aquaculture 13:45-53

Wezel A, Luibrand A, Thanh LQ (2002) Temporal changes of resource use, soil fertility and economic situation in upland Northwest Vietnam. Land Degradation and Development 13:33-44

Zhang X, Xie P, Hao L, Guo N, Gong Y, Hu X, Chen J, Liang G (2006) Effects of the phytoplanktivorous silver carp (*Hypophthalmichthys molitrixon*) on plankton and the hepatotoxic microcystins in an enclosure experiment in a eutrophic lake, Lake Shichahai in Beijing. Aquaculture 257:173-186

5 ^{15}N tracer application to evaluate nitrogen dynamics of food webs in two subtropical small-scale aquaculture ponds under different managements

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Abstract

Small, semi-intensively managed aquaculture ponds contribute significantly to the food security of small-scale farmers around the world. However, little is known about nutrient flows within natural food webs in such ponds in which fish production dependent on the productivity of natural food resources. ^{15}N was applied as ammonium at 1.1% and 0.4% of total nitrogen in a traditionally managed flow-through pond and a semi-intensively managed stagnant pond belonging to small-scale farmers in northern Vietnam and traced through the natural food resources over 7 days. Small-sized plankton (1-60 µm) were the dominant pelagic biomass in both ponds with higher biomass in the stagnant pond. This plankton assimilated major portions of the applied tracer and showed a high sedimentation and turnover rate. High re-activation of settled nutrients into the pelagic food web was observed. The tracer was removed more quickly from the flow-through pond than from the stagnant pond. A steady nutrient supply could increase fish production.

5.1 Introduction

Aquaculture plays an important role in the supply of animal-derived protein for human nutrition and contributes significantly to food security in many parts of the world. Nowadays, more than half the fish being consumed worldwide is produced by aquaculture (Naylor *et al.* 2009). In 2012, aquaculture production overtook global beef production for the first time (Larsen and Roney 2013). Nowadays, there is a wide variety of technologies in aquaculture production ranging from extensively managed water systems to highly intensive indoor and outdoor production systems. This fast development in the supply of fish for human consumption necessitates a sustainable and efficient use of key resources such as feeds, fertilizers and water.

Analysis of the natural signatures of stable isotopes and the application of stable isotopes as tracers are well established in science as means of evaluating aquatic food webs and measuring the effects of pollution in streams (Zah *et al.* 2001; Merriam *et al.* 2002; Kerner *et al.* 2004; IAEA 2008; O´Brien *et al.* 2012), lakes (Bootsma *et al.* 1996; Gu *et al.* 1996; Keough *et al*. 1996; Rezanka and Hershey 2003; Ventura and Catalan 2008; Borderelle *et al.* 2009; Epstein *et al.* 2012; France 2012; Gondwe *et al.* 2012) and marine systems (Redmond *et al.* 2010; Primavera 1996; Bode *et al.* 2011; Galván *et al.* 2011; Herbeck and Unger 2013). In semi-intensive aquaculture systems, in which both natural food and supplemental feed may contribute to the growth of fish or other cultivated animals, Schroeder (1983a, b) suggested using the naturally occurring differences in carbon isotopes (□13C) between natural food from the pond environment and feeds from terrestrial or marine environments to estimate the sources of fish growth. This approach has since been applied with various modifications (Anderson *et al.* 1987; Cam *et al.* 1992; Lochmann and Phillips 1996; Focken *et al.* 1999; Focken 2007). Nitrogen isotopes in their natural abundance were occasionally included in this approach (Parker *et al.* 1989; Gu *et al.* 1996; Focken *et al.* 1999). Similarly, natural abundances of carbon and nitrogen isotopes have been used to study the effects of effluents originating from aquaculture enterprises (Redmond *et al.* 2010; Gondwe *et al.* 2012; Herbeck and Unger 2013). ^{15}N -enriched compounds have been used as tracers within intensive aquaculture systems (Burford *et al.* 2002; Burford *et al.* 2004; Avnimelech 2007; Avnimelech and Kochba 2009), to study denitrification rates in ponds (Riise and Roos 1997) or to study the effects of nutrients released from intensive aquaculture systems on the environment (Felsing *et al.* 2006). Here, we present a ^{15}N -tracer experiment to compare the food webs in two tropical aquaculture ponds under different management regimes.

In mountainous Northern Vietnam, pond aquaculture is integrated into the farming systems of small-scale farmers and is traditionally run as a flow-through system with constant water inflow and outflow from rice paddies and irrigation streams (Luu 2001; Steinbronn 2009; Anyusheva *et al.* 2012). Leaf material, farming by-products, and manure from ruminants and pigs are applied as external feed and fertilizer inputs to feed the traditional fish-polyculture. This system is dominated by grass carp (*Ctenopharyngodon idella*), which are capable of digesting leaf material, and allows a beneficial production based on low cost and low quality feed resources (Prowse 1971; Opuszynski 1972). The ponds are also stocked with secondary species such as silver carp (*Hypophthalmichthys molitrix*), bighead carp (*Hypophthalmichthys nobilis*), common carp (*Cyprinus carpio*), tilapia (*Oreochromis niloticus*), and mud carp (*Cirrhinus cirrhosus*) which feed on different natural food resources thereby minimizing interspecies competition (Tacon and De Silva 1997). Traditional aquaculture in this region is limited by loss of pond productivity due to high turbidity in the pond caused by the constant inflow of eroded particles from surrounding intensive agriculture. Further, the production of grass carp is endangered by the occurrence of a species-specific disease that causes mass mortalities of grass carp in Northern Vietnam (Steinbronn 2009; Pucher *et al.* 2013).

Modifications of traditional pond management towards a more semi-intensive pond management were introduced to farmers to improve their aquaculture production. The modifications consisted of a change of water flow management, tending towards a more static pond, to inhibit the introduction of eroded particles and thereby reduce turbidity. Supplementary chemical fertilizers were applied to increase pond productivity. The disease prone grass carp were replaced by common carp and supplemental feeding of pellets based on locally available ingredients was applied. The traditional and modified pond management systems are described in more detail by Steinbronn (2009) and Pucher *et al.* (2013).

The objective of this study was to measure the nutrient flow of applied ^{15}N tracer within the natural food web in ponds to evaluate the nitrogen dynamics under traditional flow-through management and semi-intensive, modified management in static water.

5.2 Materials and methods

Study site and experimental design

The tracer experiment was performed from 1st till 8th of October 2011 in two ponds belonging to Black Thai farmers in mountainous Northern Vietnam (Son La province, Yen Chau district, Chieng Khoi commune). One pond was managed traditionally and the second semi-intensively.

Table 5.1: Means (± standard deviation) of nitrogen masses [mg N m^{-2}] of seston, pelagic plankton, zoobenthos, stocked fish species, and pond bottom (2 cm top layer) as well as sedimentation rate [mg N m^{-2} d^{-1}] under the two pond managements

	Size class	n	Traditional management	Semi-intensive management
Seston [mg N m^{-2}]	1-15 µm	7	240.1±87.0^b	573.9±106.3^a
Plankton [mg N m^{-2}]	15-60 µm	7	5.8±2.3^b	22.2±2.8^a
	60-200 µm	7	2.5±0.6	4.0±1.1
	>200 µm	7	3.1±0.8	3.5±2.1
Zoobenthos [mg N m^{-2}]	>500 µm	4	35.7±27.5	15.6±12.3
Sum natural food [mg N m^{-2}]			**287.2±118.2^b**	**619.2±124.6^a**
Grass carp [mg N m^{-2}]		*	1919.7	3217.0
Common carp [mg N m^{-2}]		*	107.6	2755.9
Silver carp [mg N m^{-2}]		*	359.6	1516.3
Bighead carp [mg N m^{-2}]		*	238.3	426.7
Mrigal [mg N m^{-2}]		*	697.9	-
Nile tilapia [mg N m^{-2}]		*	164.1	2468.9
Sum fish [mg N m^{-2}]		*	**3487.4**	**10404.8**
Pond bottom [mg N m^{-2}]	< 1000 µm	5	28572±3150	26402±1624
Sedimentation rate [mg N m^{-2} d^{-1}]	< 1000 µm	6	2614±344^b	4257±644^a

Mean values in the rows that do not share the same superscript(s) differ significantly with respect to pond management at $p \leq 0.05$. Mean values in the rows without superscripts did not differ significantly.

* Nitrogen masses of stocked fish were determined after complete harvest and are based on 46 and 59 sampled and singly analysed fish for traditional and semi-intensive pond management, respectively.

The traditionally managed pond was 580 m^2 in size (estimated volume 464 m^3) and was stocked at 1.1 fish m^{-2} with macro-herbivorous grass carp as the main species (> 50%) and bottom feeders (common carp, mrigal), filter feeders (silver carp, bighead carp) and grazers (Nile tilapia) as secondary species. The fish biomass (expressed as mg nitrogen m^{-2}) of each stocked species is shown in Table 5.1. The pond had uncontrolled flow-through of water from the surrounding rice paddies at a rate of 83.4 L m^{-2} d^{-1} (equivalent to the theoretical retention time of the pond of 9.6 days). Leaves of maize, banana, and cassava as well as weeds from rice paddies were used as external feed resources. The pond was fertilized by ruminant manure at about 500 kg ha^{-1} $month^{-1}$.

The semi-intensively managed pond was 545 m^2 in size (estimated volume 436 m^3) stocked at 1.9 fish m^{-2} with omnivorous common carp as main species (50%) and macro-herbivorous grass carp, bottom feeders (mrigal), filter feeders (silver carp, bighead carp) and grazers (Nile tilapia) as secondary species. The biomass of each stocked fish species is shown in Table 5.1. The pond had no water inflow or outflow during the experimental period. Grass carp were fed by leaves of maize, banana and cassava as well as weeds from rice paddies were used as external feed resources. Fish were fed on plant protein based pellets at a level of 6% of the common carp fresh biomass daily. Ponds were fertilized by ruminant manure at a rate of 500 kg ha^{-1} $month^{-1}$ and chemical fertilizers (urea and single superphosphate) by monthly applying about 10.6 kg nitrogen ha^{-1} and 3.1 kg phosphorus ha^{-1}. During the tracer study, no chemical fertilizers were applied. At the end of the experiment, the ponds were harvested by draining all the water.

Tracer application

The amount of ^{15}N was calculated to provide 0.1 to 1% of total N in the system. Based on total N estimations, ^{15}N was applied at a rate of 47.64 mg m^{-2} in the form of ammonium sulfate (98.2 At% enrichment, Chemotrade, Düsseldorf, Germany). The ^{15}N was applied as ammonium to mimic the effect of applied chemical nitrogen fertilizers, which is available as urea in this region. For each pond, the ^{15}N enriched ammonium sulfate was dissolved in five liters of pond water in a bucket and spread over the entire pond surface in 100 mL aliquots. The ^{15}N tracer was applied to the ponds at 10 am on day 0 (1^{st} of October).

Sampling design

To trace the applied ^{15}N in the natural food web, samples of phytoplankton, zooplankton and zoobenthos were taken one hour before tracer application and daily at 10 am over a period of seven days after tracer application. For plankton sampling, five x 10 L samples of pond water were taken at different sites in the ponds with a plastic bucket and pooled in a 60 L barrel. The 50 L pond water were filtered through a cascade of meshes of sizes 200 µm, 60 µm and 15 µm. The filtered plankton fractions (meso-plankton: > 200 µm; large micro-plankton: 60-200 µm; small micro-plankton: 15-60 µm) were flushed quantitatively into pre-weight 50 mL FalconTM tubes and were stored at -18°C. For the determination of seston (1-15 µm in size), 1.5 L of water passing the mesh cascade were taken to the laboratory and sucked under weak vacuum through pre-dried (60°C) and pre-weighed glass fibre filters (mean retention limit of 1.2 µm; GF/C; Whatman). This sampling procedure was performed twice for sample replication. All samples were freeze dried and weighed to determine the dry mass of the respective fraction.

For zoobenthos sampling, 10 sediment cores per pond were taken at different sites of each pond using a PVC pipe (Ø 4.5 cm) (Somsiri *et al.* 2006). The pond sediment cores were washed through a 500 µm mesh and macro-zoobenthos were segregated and stored in buffered 5% formalin solution. Zoobenthos were freeze dried and weighed.

Tracer accumulation in the pond bottom was determined by sampling the top layer (2 cm) of the pond bottom on days 0, 2, 3, 4 and 7. Per day and pond, five replicated cores (taken by a corer; see above) were pooled, dried and sieved through a mesh with 0.5 mm mesh size for analysis.

For determination of the sedimentation rates in the ponds, five plastic jars with screw lids (opening of 2.74 cm diameter) were attached, with the open mouths pointing upwards, to sticks in the ponds 3 cm above the pond bottom. The following day, the jars were closed by screwing on the lid under water, removed and replaced by empty ones. The contents of the jars (water and sediments) were transferred to pre-weighed 50 mL FalconTM tubes, stored at -18°C, and freeze dried.

Chemical analysis

For photometrical analyses, filtered water samples were prepared in duplicates for dissolved total ammonia nitrogen (TAN), nitrite nitrogen (NO_2-N), nitrate nitrogen (NO_3-N) and ortho-phosphate phosphorus (PO_4-P) using the test kits (Spectroquant, Catalogue number 14752, 14776, 09713 and 14848, respectively, Merck Chemicals, Darmstadt, Germany). Total nitrogen (TN) and total phosphorus (TP) in raw pond water were determined in mixed water samples made up of three 0.5 L samples taken in each pond at 25, 50 and 75 cm water depth. Two x 20 mL aliquots of mixed pond water were completely oxidized by adding 2 micro-spoons of Oxisolv® (Merck, Darmstadt, Germany) and autoclaving for 10 minutes (modified method of Köthe and Bitsch (1992)). The digested water samples were prepared for the photometrical analyses for dissolved NO_3-N and PO_4-P (described above), these parameters being equivalent to TN and TP. Photometrical analyses of TAN, NO_2-N, NO_3-N, PO_4-P, TN and TP were performed using a field photometer (Photolab S12; WTW, Weilheim, Germany).

Samples were analysed for dry matter (DM) according to AOAC (1990) and were analysed for nitrogen mass and isotopic enrichment by an Elemental Analyzer (Euro Vector, HEKAtech, Wegberg, Germany) coupled to an isotope ratio mass spectrometer (IRMS) (Delta Plus Advantage, Thermo Scientific, Bremen, Germany). ^{15}N-content of all samples is reported as atom percent (At%). L-glutamic acid (USGS 41) and Low Organic Content Soil Standard OAS were used as standards.

Rates (At% d^{-1}) of uptake and turnover for the natural food fractions in the ponds under traditional and semi-intensive management are derived from regression models fitted to At% over time (Epstein *et al.* 2012). Uptake rates are expressed as the slope of the linear regression of natural log of At% from the time of tracer application till the maximum At% was reached. Turnover rates are expressed as the exponent of the exponential decay of At% following the maximum At%.

For calculation of the tracer budget, the percentages of applied tracer per area was calculated for the mass of the single pelagic size classes and zoobenthos while the enrichment before tracer application was taken as background enrichment and subtracted from total atomic percentage of the respective natural food resources.

Statistical analysis

Data were processed and depicted using SigmaPlot 11.0 (Sysstat, San Jose, CA, USA) and statistically analysed using STATISTICA 8 (StatSoft®, Tulsa, OK, USA). Biomasses of natural food resources were analysed by repeated measures ANOVAs. Data sets were tested for homogeneity by the Levene´s test and checked visually for normality.

5.3 Results

No statistically significant differences in dissolved and suspended nutrient concentrations were detected between the two pond managements (Table 5.2). Under both pond management schemes, nitrate was the main source of dissolved nitrogen while phosphorus was near the detection limit of the used method. Nitrogen fixed in suspended particles was found to be significantly higher under semi-intensive pond management.

Over the 7 days, concentrations of dry mass and nitrogen associated with seston, phytoplankton, and zooplankton were found to vary (Figure 5.1). Seston (1-15 µm) was the dominant pelagic resource in both ponds at any time. The pond under semi-intensive pond management contained significantly higher biomass in the planktonic fractions of seston (1-15µm) and small micro-plankton (15-60 µm) compared to the pond under traditional pond management (Table 5.1). Plankton fractions larger than 60 µm size did not show any difference in the abundance. Under traditional pond management, the zoobenthos resources per m2 were recorded to be very divers and on average higher than under semi-intensive pond management (Table 5.1). The pond under semi-intensive management showed a significantly higher sedimentation rate of total matter as well as of TN per area than the pond under traditional management (Table 5.1, Figure 5.3).

The atomic percentages of pond biota at day zero before application of the tracer are shown in Table 5.3. The nitrogen dynamics and the pathways through the natural food web within the two ponds were affected by differences in management, time and the fractions of natural food resources (compare Table 5.4 and Figure 5.1).

In the following section, the dynamics of the tracer within the pelagic plankton fractions will be described for the two pond management schemes separately.

In the traditionally managed pond, the maximum At% of ^{15}N was found on day one in the smallest fraction (seston, 1-15 µm), accounting for 7.7 At% which was the highest enrichment found in the trial. This fraction was 10 times more abundant than the sum of the larger pelagic plankton resources and showed a rapid nitrogen uptake rate of more than 3.0 At% d^{-1}, equivalent to 520 mg m^{-2} d^{-1} nitrogen, and an exponential turnover rate of 0.41 per day (Table 5.4). A similar tracer pattern was found in the plankton fraction 15-60 µm with the highest At% of ^{15}N on day one and an uptake of more than 2.1 At% d^{-1}, equivalent to 0.15 mg m^{-2} d^{-1} nitrogen, and turnover rate of 0.27 d^{-1}. While the smaller plankton fractions assimilated the applied tracer quickly, the uptake and turnover pattern of the larger plankton fractions (60-200 µm and >200 µm; mostly zooplankton) was slower. The At% of ^{15}N in the plankton fraction 60-200 µm reached its maximum on days 2-4 with an uptake rate of 0.96 At% d^{-1} followed by an exponential decrease of 0.17 d^{-1}. The largest plankton fraction showed the highest At% of ^{15}N on day four with an uptake rate of 0.55 At% d^{-1} and an exponential turnover of 0.22 d^{-1}.

Table 5.2: Nutrient concentrations and ratios of filtered and raw pond water over the experimental period under two pond managements based on photometrical analysis

Sample preparation	Nutrient	Traditional management	Semi-intensive management
Filtered	TAN [mg L^{-1}]	0.10 ± 0.04	0.18 ± 0.12
	NO_2^--N [mg L^{-1}]	0.05 ± 0.01	0.06 ± 0.02
	NO_3^-- N [mg L^{-1}]	0.83 ± 0.29	0.63 ± 0.18
	N_{diss} [mg L^{-1}]	0.98 ± 0.31	0.87 ± 0.28
	PO_4^+-P [mg L^{-1}]	0.02 ± 0.02	0.01 ± 0.01
	N_{diss}/PO_4-P	302 ± 362	170 ± 168
Raw	TN [mg L^{-1}]	1.67 ± 0.19	1.87 ± 0.25
	TP [mg L^{-1}]	0.10 ± 0.03	0.16 ± 0.05
	TN/TP	17 ± 4	13 ± 5

n = 7. Mean values in the rows that do not share the same superscript(s) differ significantly with respect to pond management at p≤0.05. Mean values in the rows without superscripts did not differ significantly.

Table 5.3: Atomic percent [At%] of ^{15}N of seston, plankton, benthos, and sedimented particles and pond bottom (2 cm top layer) prior the application of the ^{15}N tracer (day zero) based on duplicate measurements.

	Size class	Traditional management	Semi-intensive management
Seston	1-15 µm	0.379	0.372
Plankton	15-60 µm	0.370	0.370
	60-200 µm	0.374	0.372
	>200 µm	0.371	0.371
Zoobenthos	>500 µm	0.374	0.368
Sedimented particles	< 1000 µm	0.373	0.372
Pond bottom	< 1000 µm	0.368	0.368

Table 5.4: Rates [At% of ^{15}N d^{-1}] of uptake and turnover of seston, plankton and benthos in the ponds under traditional and semi-intensive management generated from regression models fitted to the At% of ^{15}N in dry matter. Uptake rate is defined as the slope of the linear regression of natural log of At% of ^{15}N from tracer application till maximum At% of ^{15}N in dry matter. Turnover rates are measured as the exponent of the exponential regression of At% of ^{15}N in dry matter following the maximum At% of ^{15}N in dry matter. Numbers given in parentheses represent the coefficient of determination (r^2).

Rate [d^{-1}]		Traditional management		Semi-intensive management	
Uptake (r^2):					
Seston	1-15 µm	>3.01*	(1.00)	1.01	(0.77)
Plankton	15-60 µm	>2.07*	(1.00)	0.77	(0.89)
	60-200 µm	0.96	(1.00)	0.67	(0.99)
	>200 µm	0.55	(1.00)	0.44	(0.89)
Zoobenthos	>500 µm	0.04°	(1.00)	0.00	(0.81)
Turnover (r^2):					
Seston	1-15 µm	0.41	(0.97)	0.28	(0.89)
Plankton	15-60 µm	0.27	(0.87)	0.28	(0.93)
	60-200 µm	0.17	(0.90)	0.22	(0.94)
	>200 µm	0.22	(0.89)	0.18	(0.91)
Zoobenthos	>500 µm	0.01	(0.11)	na	na

na: no data

Calculation of turnover rate was not possible as enrichment showed no stable peak (see Figure 5.2).

*: Measured peak was found one day after tracer application. Consequently, only two points are integrated in the linear regression. However, it is likely that the real maximum enrichment was reached sooner than 24 h after tracer application. °: No clear peak was identifiable for calculation of the uptake rate.

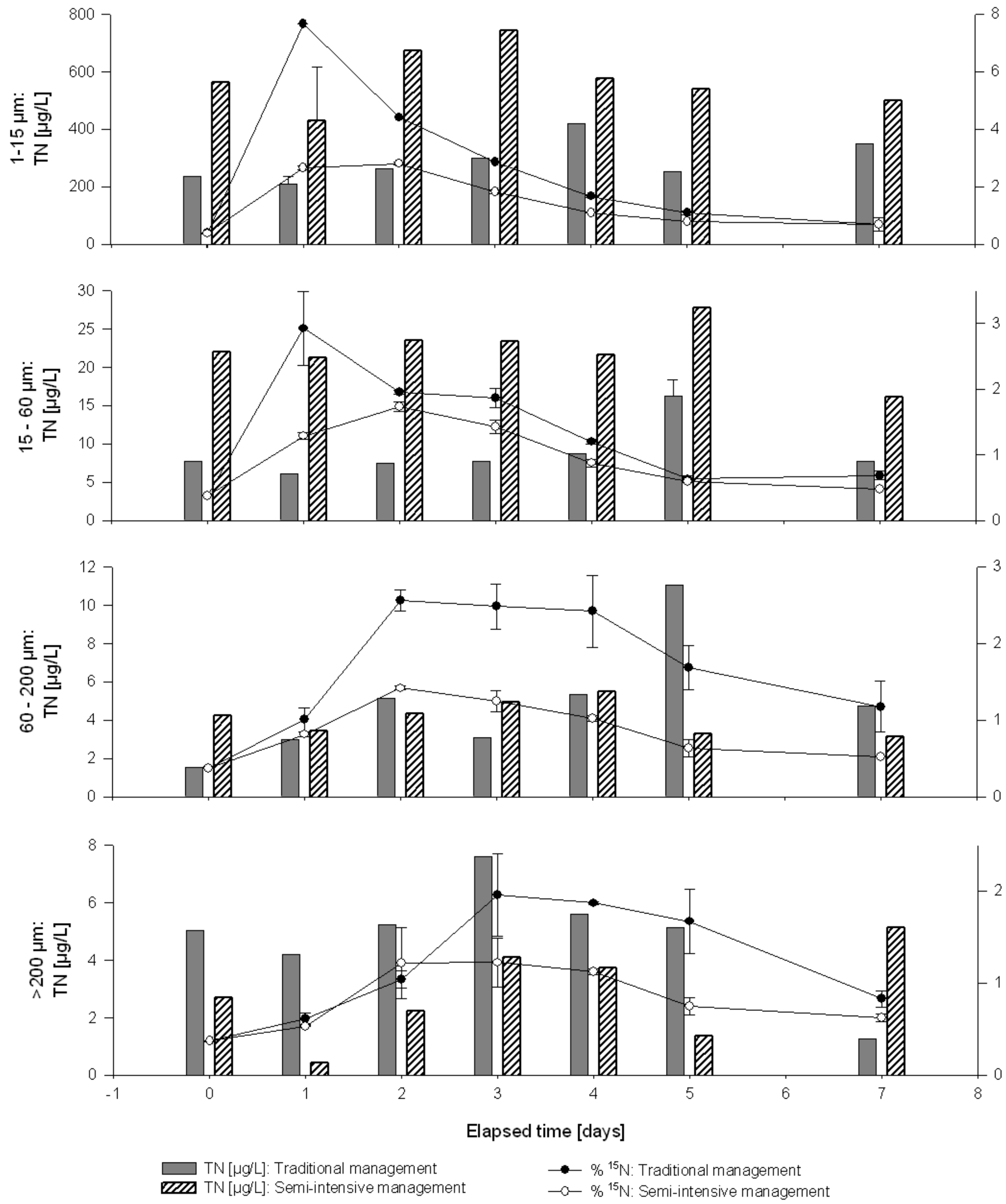

Figure 5.1: Total nitrogen concentrations (TN, bars, left y-axis) and isotopic enrichment (At% of ^{15}N, lines, right y-axis) of pelagic organisms under the two pond managements over the elapsed time.

Table 5.5: Recovery rate of the Percentage of total amount of applied ^{15}N tracer over the experimental period in days. Data are expressed as percentage of the total amount of applied ^{15}N (100% = 47.64 mg m^{-2}) and are shown for the sum of all resources and for single resources (in seston, plankton, and zooplankton (per area), pond bottom (2 cm top layer) and sedimentsation rate which settled within the day before the respective sampling day (per area and day) over the experimental period. Numbers in parentheses are sums of all sampled resources while single resources could not be quantified due to lack of samples.

Elapsed time [d]		**0**	**1**	**2**	**3**	**4**	**5**	**7**
Traditional pond management:								
Seston	1-15 µm	0.00	24.10	15.71	10.95	7.36	3.23	2.03
Plankton	15-60 µm	0.00	0.24	0.16	0.17	0.10	0.06	0.03
	60-200 µm	0.00	0.04	0.13	0.10	0.14	0.07	0.04
	>200 µm	0.00	0.02	0.05	0.10	0.13	0.08	0.02
Zoobenthos	>500 µm	0.00	na	0.06	na	0.00	na	0.03
Sedimentation		0.00	49.50	49.50	37.90	25.74	15.13	4.76
Pond bottom	>1000 µm	0.00	na	0.24	0.39	0.22	na	1.23
Sum		**0.00**	**(73.90)**	**65.85**	**49.61**	**33.69**	**(18.57)**	**8.14**
Semi-intensive pond management:								
Seston	1-15 µm	0.00	24.77	33.79	20.76	8.51	4.63	2.09
Plankton	15-60 µm	0.00	0.42	0.67	0.48	0.24	0.12	0.04
	60-200 µm	0.00	0.03	0.08	0.10	0.07	0.01	0.01
	>200 µm	0.00	0.01	0.04	0.04	0.06	0.02	0.03
Zoobenthos	>500 µm	0.00	na	0.00	na	na	na	0.00
Sedimentation		0.00	60.25	60.25	56.65	34.60	13.95	5.14
Pond bottom	>1000 µm	0.00	na	0.60	0.68	1.51	na	0.96
Sum		**0.00**	**(85.48)**	**95.43**	**78.71**	**44.99**	**(18.73)**	**8.27**

na: Zoobenthic samples did not contain sufficient sample material for analysis

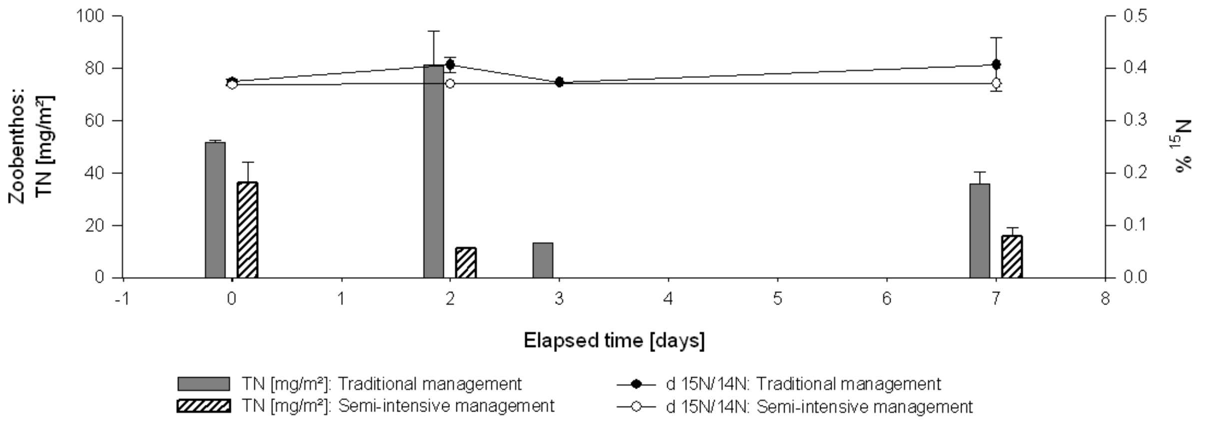

Figure 5.2: Total nitrogen abundance (TN, bars, left y-axis) and isotopic enrichment (At% of ^{15}N, lines, right y-axis) of macrobenthos organisms in the two pond managements over the elapsed time. No benthos was found in the sediment cores at day 3 in the semi-intensively managed pond.

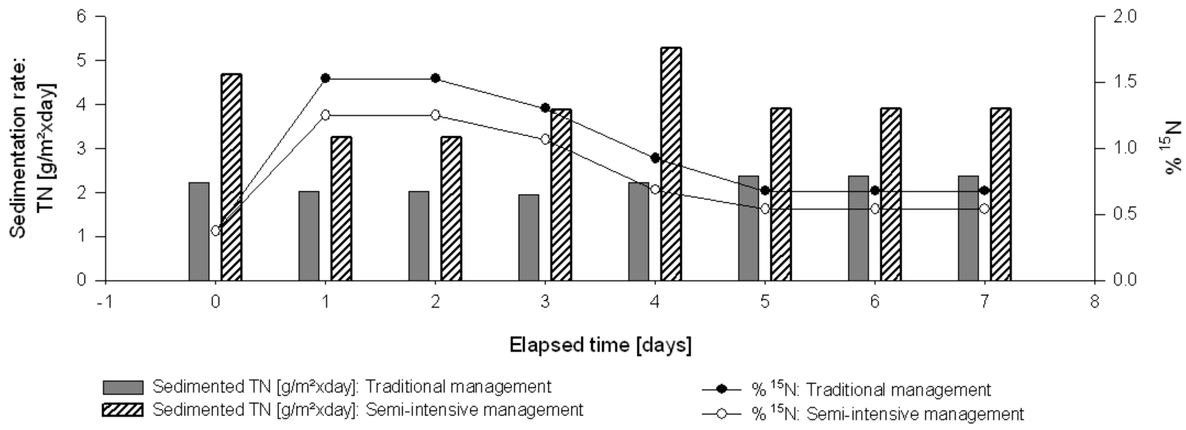

Figure 5.3: Sedimentation rate of total nitrogen (TN, bars, left y-axis) and isotopic enrichment (At% of ^{15}N, lines, right y-axis) of sediment matter in the two pond managements over the elapsed time.

In the pond under semi-intensive management, the tracer showed a more buffered assimilation pattern with lower uptake rates in the pelagic plankton fractions caused by the significantly higher biomass compared to the pond under traditional management. The turnover rates in the pelagic fractions were similar in both managements. In the fractions 1-15 µm, 15-60 µm and 60-200 µm, the maximal At% of ^{15}N concentrations were detected on day 3 with uptake rates of 1.0, 0.8 and 0.7 At% d^{-1}, respectively. The uptake rates were estimated to be 2386 mg m^{-2} d^{-1} nitrogen and 1.78 mg m^{-2} d^{-1} nitrogen for seston (1-15 µm) and the plankton fraction 15-60 µm in size, respectively. The meso-plankton fraction showed the maximum At% of ^{15}N on day four with uptake rate of 0.4 At% d^{-1}.

Zoobenthos estimates in the ponds fluctuated strongly over time due to the spatial distribution of zoobenthos in the pond bottom, though the mean abundance of zoobenthos was found to be higher under traditional pond management. However, no clear pattern of ^{15}N assimilation in response to the tracer application (Figure 5.2) was found for zoobenthos. Under semi-intensive management, no turnover rate was calculated because no clear maximum in enrichment was reached in this natural food resource during the period of the trial.

Under traditional pond management, the sedimentation rate of DM and TN were lower than under semi-intensive pond management (Figure 5.3, Table 5.4). Sediments under both types of pond management showed the highest At% of ^{15}N within the first and second day.

The percentages of applied tracer in the different pelagic plankton fractions, zoobenthos and sediments over the period of the experiment are summarized in the Table 5.5. Under both management schemes the highest share of assimilated tracer was found in seston (1-15 µm) which was the most abundant pelagic resources in both ponds. In the traditionally managed pond, 24.1% of the applied tracer was assimilated in this fraction on day one followed by an exponential decrease during the rest of the experiment. Under semi-intensive pond management, the maximal percentage of tracer in the seston fraction (1-15 µm) was reached on day two accounting for 33.8% of applied tracer.

The sum of all natural food resources larger than 15 µm in size assimilated less than 1% of total applied tracer at any time, while plankton in the range 15-60 µm assimilated a maximum of 0.24% (day one) under traditional and 0.67% (day two) under semi-intensive pond management.

Under both management schemes, high percentages of applied tracer were found to settle on the pond bottom accounting for 60%, 60% and 57% under semi-intensive management within days 1, 2 and 3, respectively, and for 50%, 50% and 38% under traditional management within days 1, 2 and 3, respectively. However, only up to 1.5% of the applied tracer accumulated within the 2 cm top layer of the pond bottom.

Under semi-intensive pond management, a greater amount of applied tracer was detected in the natural food than under the traditional pond management. With semi-intensive management, 95% of applied tracer was detected in the pelagic fractions and zoobenthos on the second day after application but under traditional management, a maximum of only 74% of applied tracer was detected on day one followed by a constant exponential loss. After 7 days, under both managements about 7% of the tracer was left in the natural food webs of the two ponds.

5.4 Discussion

The flow rate into the traditionally managed pond was in the same range as that reported for other ponds in the study area (Steinbronn 2009; Anyusheva *et al.* 2012). The period of monitoring should have been extended to more than 10 days, as the theoretical complete turnover of the pond volume was recorded to be 9.6 days under this inflow rate. However, the exponential reduction of tracer within the pelagic natural food web may be explained partly by out-flowing water transporting this fraction. The semi-intensively managed pond had no surface water inflow, but seepage of water may have occurred through the earthen dikes which have been reported to transport highly varying amounts of water between rice paddies and ponds in the study area especially through macro-pores in the dikes (Reinhardt *et al.* 2012). However, the high recovery rate of the tracer within the first days after application suggests a limited water lost through the dike but might have caused the low recovery rate of tracer after a week. According to the study of Reinhardt *et al.* (2012), vertical water flow and the loss of nutrients into the groundwater can be neglected, fixation on fractions not considered here and/or denitrification remain as possible reasons for the disappearance of the tracer.

Under both management schemes, seston (1-15 µm) and small micro-plankton (15-60 µm) represented the greatest amount of biomass in the water column. Similar results have been obtained for tropical polyculture systems, fertilized by manure and chemical fertilizer (Schroeder *et al.* 1990). The dominance of these pelagic resources is especially beneficial for the growth of silver carp and Nile tilapia which are capable of filtering and ingesting these small plankton fractions (Spataru 1977; Burke *et al.* 1986; Beveridge *et al.* 1989; Schroeder *et al.* 1990; Milstein 1992; Dempster *et al.* 1993, 1995; Turker *et al.* 2003; Milstein *et al.* 2006; Muendo *et al.* 2006; Zhang *et al.* 2006). For both pond managements studied, the growth performances of silver carp and Nile tilapia were reported to be dependent on the natural food resources since silver carp has been shown to grow significantly better under semi-intensive pond management than under traditional management (Pucher *et al.* 2013). The abundance of large micro-plankton and meso-plankton (60-200 µm and > 200 µm) were comparable under both types of management. This may have been caused by a high predatory pressure on these natural food resources by bighead carp which are reported to be filter feeders of zooplankton (Burke *et al.* 1986; Opuszynski and Shireman 1991; Milstein 1992; Cooke *et al.* 2009; Li *et al.* 2013), as are grass carp, common carp and Nile tilapia which are reported to additionally feed on zooplankton (George 1982; Rahman *et al.* 2008b). Similar ranges in the amounts of larger plankton in both ponds in spite of the much higher predator biomass in the semi-intensive system suggests that primary production is substantially higher in this latter system.

Under semi-intensive pond management, zoobenthos biomass was found to be lower which might have been caused by the higher stocking density as well as the higher biomass per area of common carp. Common carp is widely described as a bottom feeder that scavenges the pond bottom for zoobenthos (Jana and Sahu 1993; Milstein *et al.* 2002, 2006; Rahman *et al.* 2008a).

Most of the applied nitrogen tracer was quickly taken up by the seston fraction in both the ponds suggesting that the dissolved and bioavailable nitrogen were limited in both the ponds investigated. The high absolute At% of ^{15}N in the small fractions might have a certain error as these unexpected high values were out of the calibration range of the method. However, the traditionally managed pond showed higher uptake rates due to a lower abundance of plankton biomass as well as the generally lower nitrogen application from external sources within the traditional management compared to the semi-intensive management (Pucher *et al.* 2013). The estimated uptake of total nitrogen under traditional management was within the lower range in tropical ponds given by Hargreaves (1998) while the estimated uptake of nitrogen into the seston and small mirco-plankton under semi-intensive management exceeds the estimated maximum of the range of 1500 mg m^{-2} d^{-1} given by Hargreaves (1998). With increasing plankton size, the uptake rate dropped as the tracer was transferred into the higher trophic levels of the pelagic food web. The dynamics of uptake rates amongst different pelagic fractions in a subalpine lake were studied by Epstein *et al.* (2012) but the uptake rates were significantly lower than in the present study because the pelagic plankton was much less abundant and temperature lower. However, there were some similarities, for example, the turnover rates in both studies decreased with increasing size and trophic level of the planktonic fractions but were much higher in our study where the predatory pressure from stocked fish was much more intense.

In our study, sedimentation rates were high. The similarity of enrichments over time of settled material and seston (1-15 µm) suggests that a high proportion of the settled material originates from this plankton fraction. Similar patterns were found by Schroeder *et al.* (1991) and Lorenzen *et al.* (1997) who estimated phytoplankton to contribute more than 50% of settled material. Riise and Roos (1997) measured a re-activation of settled nitrogen in the form of ammonia of about 13.8 mmol m^{-2} d^{-1} in ponds in Thailand. In catfish ponds, Hargreaves (1997) estimated 25 to 33% of the ammonia dissolved in the water originates from re-dissolved nitrogen coming from sediments. These data show high nutrient re-activation from the settled material which is supported by the relatively low accumulation of tracer in the 2 cm top layer of pond bottom in comparison of the sedimented tracer amount. This speed of re-activation is a result either of zoobenthos, increasing bioturbation (Wag and Matisoff 1997), or the presence of omnivorous bottom feeders such common carp and mrigal. These fish species stir up and aerate the sediment during their scavenging activities and re-suspend and increase the bio-availability of settled nitrogen in pelagic habitats (Jana and Sahu 1993; Milstein *et al.* 2002; Rahman *et al.* 2008b). This stirring up of sediments not only increases turbidity (Chumchal *et al.* 2005) but also stimulates primary production (Chumchal and Drenner 2004; Glaholt and Vanni 2005) and the accumulation of nutrients in plankton (Rahman *et al.* 2008a). In the present study, the greater persistence of tracer in the pelagic natural food web, especially in the semi-intensively managed trial ponds, is probably due to the large numbers of bottom feeding fish continually stirring up the sediments.

With respect to pond management, these data are valuable for anyone seeking to increase the availability of added nitrogen and the efficiency of applied fertilizer for the production of biomass that can be used by fish. Nutrients in the static pond under semi-intensive pond management reached higher concentrations and persisted longer in the pelagic plankton resources. However, it may also be beneficial to inhibit the water flow through the earthen dikes by construction of concrete or other more impermeable dikes. The nitrogen dynamics within the pelagic plankton suggest that continual fertilization is more beneficial than intermittent fertilization at intervals of one to a few weeks as recommended by Das *et al.* (2011). As the smallest plankton fractions (1-15 µm and 15-60 µm) are the dominant natural food resources, higher stocking rates of silver carp and Nile tilapia might increase the utilization of nutrients assimilated by this natural food fraction. On the other hand, it might also be beneficial to reduce the stocking level of bighead carp because they are filter feeders on zooplankton which is present in relatively small amounts. Due to the high sedimentation rates in the ponds, we recommend that bottom feeding fish species such as the common carp should be stocked in higher densities to promote re-activation of settled nutrients when they scavenge and feed (Zambrano and Hinojosa 1999; Zambrano *et al.* 1999; Chumchal and Drenner 2004; Rahman *et al.* 2008a). In future research, special emphasis should be placed on studying the interaction of pond bottom and pelagic natural food resources, as there appears to be a high degree of interaction between sedimentation, re-suspension, re-activation and the assimilation of nutrients.

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5.5 References

Anderson RK, Parker PL, Lawrence A (1987) A 13C/12C tracer study of the utilization of presented feed by a commercially important shrimp *Penaeus vannamei* in a pond growout system. Journal of World Aquaculture Society 18:148-155

Anyusheva M, Lamers M, La N, Nguyen VV, Streck T (2012) Fate of pesticides in combined paddy rice–fish pond farming systems in Northern Vietnam. Journal of Environmental Quality 41:515-525

AOAC (1990) Official Methods of Analysis, 15th ed. Association of Official Analytical Chemists, Arlington, VA.

Avnimelech Y (2007) Feeding with microbial flocs by tilapia in minimal discharge bio-flocs technology ponds. Aquaculture 264:140-147

Avnimelech Y, Kochba M (2009) Evaluation of nitrogen uptake and excretion by tilapia in bio floc tanks, using ^{15}N tracing. Aquaculture 287:163-168

Beveridge MCM, Begum M, Frerichs GN, Millar S (1989) The ingestion of bacteria in suspension by the tilapia *Oreochromis niloticus*. Aquaculture 81:373-378

Bode A, Varela M, Prego R (2011) Continental and marine sources of organic matter and nitrogen for rías of Northern Galicia (Spain). Marine Ecology Progress Series 437:13-26

Bootsma HA, Hecky RE, Hesslein RH, Turner GF (1996) Food partitioning among Lake Malawi nearshore fishes as revealed by stable isotope analysis. Ecology 77:1286-1290

Borderelle A-L, Gerdeaux D, Giraudoux P, Verneaux V (2009) Influence of watershed's anthropogenic activities on fish nitrogen and carbon stable isotope ratios in nine French lakes. Knowledge and Management of Aquatic Ecosystems 392:1-13

Burford M, Thomson PJ, McIntosh RP, Bauman RH, Pearson DC (2004) The contribution of flocculated material to shrimp (*Litopenaeus vannamei*) nutrition in a high-intensity, zero-exchange system. Aquaculture 232:525-537

Burford MA, Preston NP, Glibert PM, Dennison WC (2002) Tracing the fate of ^{15}N -enriched feed in an intensive shrimp system. Aquaculture 206:199-216

Burke JS, Bayne DR, Rea H (1986) Impact of silver and bighead carp on plankton communities of channel catfish ponds. Aquaculture 55:59-68

Cam D, Rollet P-E, Mariotti A, Guillaume J (1992) Contribution relative de la productivité naturelle et de l'aliment compose dans la nutrition de *Penaeus japonicus* élevé en conditions semi-intensives. Aquatic Living Resources 4:174-180

Chumchal MM, Drenner RW (2004) Interrelationships between phosphorus loading and common carp in the regulation of phytoplankton biomass. Archiv für Hydrobiologie 161:147-158

Chumchal MM, Nowlin WH, Drenner RW (2005) Biomass-dependent effects of common carp on water quality in shallow ponds. Hydrobiologia 545:271-277

Cooke SE, Hill WR, Meyer KP (2009) Feeding at different plankton densities alters invasive bighead carp (*Hypophthalmichthys nobilis*) growth and zooplankton species composition. Hydrobiologia 625:185-193

Das PC, Jena J, Mishra B, Sarangi N (2011) Impact of varied frequency of nitrogen fertilization on the grow-out performance of *Labeo rohita* (Hamilton). Aquaculture Research 42:1859-1868

Dempster PW, Beveridge MCM, Baird DJ (1993) Herbivory in the tilapia *Oreochromis niloticus*: a comparison of feeding rates on phytoplankton and periphyton. Journal of Fish Biology 43:385-392

Dempster P, Baird DJ, Beveridge MCM (1995) Can fish survive by filter-feeding on microparticles? Energy balance in tilapia grazing on algal suspensions. Journal of Fish Biology 47:7-17

Epstein DM, Wurtsbaugh WA, Baker MA (2012) Nitrogen partitioning and transport through a subalpine lake measured with an isotope tracer. Limnology and Oceanography 57:1503-1516

Felsing M, Telfer T, Glencross B (2006) ^{15}N -enrichment of an aquaculture diet and tracing of cage culture waste in an estuarine environment. Journal of Applied Ichthyology 22:419-426

Focken U, Schlechtriem C, Abel HJ, Becker K (1999) Evaluation of the relative contribution of natural food and compound feed to fish growth based on $d^{13}C$ and $d^{15}N$ ratios. Isotopes in Environmental and Health Studies 35:296-297

Focken U (2007) Application of stable isotopes of carbon and nitrogen in aquaculture research. In: III International Conference Fishery, Belgrade, Serbia, 1-3 February, pp. 60-65

France RL (2012) Omnivory, vertical food-web structure and system productivity: stable isotope analysis of freshwater planktonic food webs. Freshwater Biology 57:787-794

Galván K, Fleeger JW, Peterson B, Drake D, Deegan LA, Johnson DS (2011) Natural abundance stable isotopes and dual isotope tracer additions help to resolve resources supporting a saltmarsh food web. Journal of Experimental Marine Biology and Ecology 410:1-11

George TT (1982) The Chinese grass carp, *Ctenopharyngodon idella*, its biology, introduction, control of aquatic macrophytes and breeding in the Sudan. Aquaculture 27:317-327

Glaholt Jr SP, Vanni MJ (2005) Ecological responses to simulated benthic-derived nutrient subsidies mediated by omnivorous fish. Freshwater Biology 50:1864-1881

Gondwe MJ, Guildford SJ, Hecky RE (2012) Tracing the flux of aquaculture-derived organic wastes in the southeast arm of Lake Malawi using carbon and nitrogen stable isotopes. Aquaculture 350-353:8-18

Gu B, Schelske CL, Hoyer MV (1996) Stable isotopes of carbon and nitrogen as indicators of diet and trophic structure of the fish community in a shallow hypereutrophic lake. Journal of Fish Biology 49:1233-1243

Hargreaves JA (1997) A simulation model of ammonia dynamics in commercial catfish ponds in the southeastern United States. Aquaculture Engineering 16:27-43

Hargreaves JA (1998) Nitrogen biogeochemistry of aquaculture ponds. Aquaculture 166:181-212

Herbeck LS, Unger D (2013) Pond aquaculture effluents traced along back-reef waters by standard water quality parameters, $\delta^{15}N$ in suspended matter and phytoplankton bioassays. Marine Ecology Progress Series 478:71-86

IAEA (2008) Atlas of isotope hydrology - Asia and the Pacific. International Atomic Energy Agency, Vienna, Austria, 138p.

Jana BB, Sahu SN (1993) Relative performance of three bottom grazing fishes (*Cyprius carpio*, *Cirrhinus mrigala*, *Heteropneustes fossilis*) in increasing the fertilizer value of phosphate rock. Aquaculture 115:19-29

Keough JR, Sierszen ME, Hagley CA (1996) Analysis of a Lake Superior coastal food web with stable isotope technique. Limnology and Oceanography 41:136-146

Kerner M, Ertl S, Spitzy A (2004) Trophic diversity within the planktonic food web of the Elbe Estuary determined on isolated individual species by 13C analysis. Journal of Plankton Research 26:1039-1048

Köthe J, Bitsch R (1992) Oxisolv plus microwave - a new way for sample pretreatment and sample preparation. Fresenius Journal of Analytical Chemistry 343:717-718

Larsen J, Roney JM (2013) Farmed Fish Production Overtakes Beef. Earth Policy Institute. http://grist.org/article/farmed-fish-production-overtakes-beef/, Accessed 11. July.

Li K, Xu Z, Liu Z, Gu B (2013) Stable isotope enrichment, dietary sources and trophic overlap between silver carp (*Hypophthalmichthys molitrix*) and bighead carp (*Aristichthys nobilis*). Aquaculture 402-403:8-12

Lochmann R, Phillips H (1996) Stable isotopic evaluation of the relative assimilation of natural and artificial foods by golden shiners *Notemigonus crysoleucas* in ponds. Journal of World Aquaculture Society 27:168-177

Lorenzen K, Struve J, Cowan VJ (1997) Impact of farming intensity and water management on nitrogen dynamics in intensive pond culture: a mathematical model applied to Thai commercial shrimp farms. Aquaculture Research 28:493–507

Luu LT (2001) The VAC system in Northern Vietnam. In: Integrated agriculture-aquaculture: A primer. FAO/IIRR/WorldFish Center, Fisheries Technical Paper 407, Rome, pp. 29-32

Merriam JL, McDowell WH, Tank JL, Wollheim WM, Crenshaw CL, Johnson SL (2002) Characterizing nitrogen dynamics, retention and transport in a tropical rainforest stream using an in situ ^{15}N addition. Freshwater Biology 47:143-160

Milstein A (1992) Ecological aspects of fish species interactions in polyculture ponds. Hydrobiologia 231:177-186

Milstein A, Wahab MA, Rahman MM (2002) Environmental effects of common carp *Cyprinus carpio* (L.) and mrigal *Cirrhinus mrigala* (Hamilton) as bottom feeders in major Indian carp polycultures. Aquaculture Research 33:1103-1117

Milstein A, Ahmed AF, Masud OA, Kadir A, Wahab MA (2006) Effects of the filter feeder silver carp and the bottom feeders mrigal and common carp on small indigenous fish species (SIS) and pond ecology. Aquaculture 258:439-451

Muendo PN, Milstein A, Dam AA van, Gamal E-N, Stoorvogel JJ, Verdegem MCJ (2006) Exploring the trophic structure in organically fertilized and feed-driven tilapia culture environments using multivariate analyses. Aquaculture Research 37:151-163

Naylor RL, Hardy RW, Bureau DP, Chiu A, Elliott M, Farrell AP, Forster I, Gatlin DM, Goldburg RJ, Hua K, Nichols PD (2009) Feeding aquaculture in an era of finite resources. PNAS 106:15103-15110

O´Brien JM, Hamilton SK, Podzikowski L, Ostrom N (2012) The fate of assimilated nitrogen in streams: an *in situ* benthic chamber study. Freshwater Biology 57:1113-1125

Opuszynski K (1972) Use of phytophagous fish to control aquatic plants. Aquaculture 1:61-74

Opuszynski K, Shireman JV (1991) Food passage time and daily ration of bighead carp, *Aristichthys nobilis*, kept in cages. Environmental Biology of Fishes 30:387-393

Parker PL, Anderson RK, Lawrence A (1989) A δ13C and $\delta^{15}N$ Tracer Study of Nutrition in Aquaculture: *Penaeus vannamei* in a Pond Growout System. In: Rundel PW, Ehleringer JR, Nagy KA (eds.) Stable Isotopes in Ecological Research. Ecological Studies 68, Springer, New York, Heidelberg, pp. 288-303

Primavera JH (1996) Stable carbon and nitrogen isotope ratios of penaeid juveniles and primary producers in a riverine mangrove in Guimaras, Philippines. Bulletin of Marine Science 58:675-683

Prowse GA (1971) Experimental criteria for studying grass carp feeding in relation to weed control. The Progressive Fish-Culturist 33:128-131

Pucher J, Steinbronn S, Mayrhofer R, Schad I, El-Matbouli M, Focken U (2013) Improved Sustainable Aquaculture Systems for Small-Scale Farmers in Northern Vietnam. In: Fröhlich HL, Schreinemachers P, Clemens G, Stahr K (eds.) Sustainable Land Use and Rural Development in Southeast Asia: Innovations and Policies for Mountainous Areas. Springer Verlag, Heidelberg, New York, Dordrecht, London, pp. 281-317

Rahman MM, Jo Q, Gong YG, Miller SA, Hossain MY (2008a) A comparative study of common carp (*Cyprinus carpio* L.) and calbasu (*Labeo calbasu* Hamilton) on bottom soil resuspension, water quality, nutrient accumulations, food intake and growth of fish in simulated rohu (*Labeo rohita* Hamilton) ponds. Aquaculture 285:78-83

Rahman MM, Nagelkerke LAJ, Verdegem MCJ, Wahab MA, Verreth JAJ (2008b) Relationships among water quality, food resources, fish diet and fish growth in polyculture ponds: A multivariate approach. Aquaculture 275:108-115

Redmond KJ, Magnesen T, Hansen PK, Strand Ã, Meier S (2010) Stable isotopes and fatty acids as tracers of the assimilation of salmon fish feed in blue mussels (*Mytilus edulis*). Aquaculture 298:202-210

Reinhardt N, Gut T, Lamers M, Streck T (2012) Water Regime in paddy rice systems in Vietnam: Importance of infiltration and bund flow. In: Stahr K, Fröhlich H, Clemens G, Schreinemachers P (eds.) International Scientific Conference "Sustainable Land Use and Rural Development in Moutain Areas". 16-18 April, University of Hohenheim, Stuttgart, Germany, pp. 221-222

Rezanka KM, Hershey AE (2003) Examining primary producer-consumer interactions in a Lake Superior tributary using ^{15}N -tracer, grazer-reduction, and nutrient-bioassay experiments. Journal of the North American Benthological Society 22:371-387

Riise JC, Roos N (1997) Benthic metabolism and the effects of bioturbation in a fertilised polyculture fish pond in Northeast Thailand. Aquaculture 150:45-62

Schroeder GL (1983a) Sources of fish and prawn growth in polyculture ponds as indicated by dC analysis. Aquaculture 35:29-42

Schroeder GL (1983b) Stable isotope ratios as naturally occuring tracers in the aquaculture food web. Aquaculture 30:203-210

Schroeder GL, Wohlfarth G, Alkon A, Halevy A, Krueger H (1990) The dominance of algal-based food webs in fish ponds receiving chemical fertilizers plus organic manures. Aquaculture 86:219-229

Schroeder GL, Alkon A, Laher M (1991) Nutrient flow in pond aquaculture systems. In: Brune DE, Tomasso JR (eds.) Aquaculture and Water Quality. World Aquaculture Society, Baton Rouge, LA, pp. 489–505

Somsiri T, Oanh DTH, Chinabut S, Phuong NT, Shariff M, Yusoff FM, Bartie K, Giacomini M, Robba M, Bertone S, Huys G, Teale A (2006) A simple device for sampling pond sediment. Aquaculture 258:650-654

Spataru P (1977) Gut content of silver carp - *Hypophthalmichthys molitrix* (Val.) - and some trophic relations to other fish species in a polyculture system. Aquaculture 11:137-146

Steinbronn S (2009) A case study: Fish production in the integrated farming system of the Black Thai in Yen Chau district (Son La province) in mountainous North-Western Vietnam - Current state and potential (PhD thesis). Institute for Animal Productions in the Tropic and Subtropics, Hohenheim Stuttgart, Germany, University of Hohenheim, 207p.

Tacon AGJ, De Silva SS (1997) Feed preparation and feed management strategies within semi-intensive fish farming systems in the tropics. Aquaculture 151:379-404

Turker H, Eversole AG, Brune DE (2003) Comparative Nile tilapia and silver carp filtration rates of Partitioned Aquaculture System phytoplankton. Aquaculture 220:449-457

Ventura M, Catalan J (2008) Incorporating life histories and diet quality in stable isotope interpretations of crustacean zooplankton. Freshwater Biology 53:1453-1469

Wag X, Matisoff G (1997) Solute transport in sediments by a large freshwater oligochaete, *Branchiura sowerbyi*. Environmental Science & Technology 31:1926-1933

Zah R, Burgherr P, Bernasconi S, Uehlinger U (2001) Stable isotope analysis of macroinvertebrates and their food sources in a glacier stream. Freshwater Biology 46:871-882

Zambrano L, Hinojosa D (1999) Direct and indirect effects of carp (*Cyprinus carpio* L.) on macrophyte and benthic communities in experimental shallow ponds in central Mexico. Hydrobiologia 408/409:131-138

Zambrano L, Perrow MR, Macías-García C, Aguirre-Hidalgo V (1999) Impact of introduced carp (*Cyprinus carpio*) in subtropical shallow ponds in Central Mexico. Journal of Aquatic Ecosystem Stress and Recovery 6:281-288

Zhang X, Xie P, Hao L, Guo N, Gong Y, Hu X, Chen J, Liang G (2006) Effects of the phytoplanktivorous silver carp (*Hypophthalmichthys molitrixon*) on plankton and the hepatotoxic microcystins in an enclosure experiment in a eutrophic lake, Lake Shichahai in Beijing. Aquaculture 257:173-186

6 Earthworm meal as fishmeal replacement in plant based feeds for common carp in semi-intensive aquaculture in rural Northern Vietnam

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Abstract

It was evaluated whether earthworm meal can fully replace fishmeal in supplemental feeds for common carp (*Cyprinus carpio*) that also feed on natural food resources in semi-intensive aquaculture. A net cage trial (32 nets) was carried out using three iso-nitrogenous feeds fed to common carp either at a level of 10 g $kg^{-0.8}$ metabolic body mass (5 fish per cage) or 20 g $kg^{-0.8}$ metabolic body mass (10 fish per cage). In feeds, fishmeal protein was replaced by 0%, 50% or 100% of protein from sun dried earthworms (*Perionyx excavatus*). At both stocking densities, control groups of fish fed only on natural food resources. The growth rate of fish increased with rising replacement of fishmeal by earthworm meal at both feeding rates. Large zooplankton were the predominant natural food resource. With increasing availability of large zooplankton, sun dried earthworm meal in plant-based supplemental feeds seemed better able to meet the nutritional requirements of common carp than fishmeal. Integration of earthworm production (vermiculture) into small-scale farms in developing countries may open the possibility for farmers in rural areas to engage in semi-intensive aquaculture by using earthworms as a feed ingredient in supplemental feeds for common carp.

6.1 Introduction

Nowadays, more than half of the fish being consumed by humans worldwide are produced by aquaculture (Naylor *et al.* 2009) and in 2012, aquaculture production overtook global beef production in quantity for the first time (Larsen and Roney 2013). This development in global fish supply is increasing the demand for feed resources, especially for high quality protein and high quality lipid feed resources such as fishmeal and fish oil (Naylor *et al.* 2009; Tacon and Metian 2009). The price of fishmeal has increased greatly within the past decade (Hardy 2010) due to the high demand which inhibits small-scale aquaculture enterprises in rural areas from increasing their fish production by using higher quality feed inputs. This, in turn, leads to the search for alternative highly nutritious feed ingredients in aquafeeds (Hardy 2010).

Rural small-scale aquaculture is very important for food security especially for poor people in developing countries in the tropics and subtropics (Tacon *et al.* 2010) and is often operated within integrated farming systems under traditional management schemes using low quality on-farm feed resources (e.g. northern Vietnam: Luu 2001; Dongmeza *et al.* 2009; Steinbronn 2009; Pucher *et al.* 2013, Pucher *et al*. 2014a). In this region, the introduction of semi-intensive pond aquaculture with omnivorous common carp as the main species in carp and tilapia polycultures led to higher fish production and financial net benefit than traditional aquaculture (Pucher *et al.* 2013). Semi-intensive aquaculture is based on enhanced natural food productivity within the pond combined with supplemental feeding of limiting nutrients (De Silva 1993).

In order to introduce semi-intensive pond management into rural areas, supplemental feeds must be developed based on locally available resources. For small-scale farms in northern Vietnam, Tuan (2010) developed such feeds for common carp (*Cyprinus carpio*), which are mainly based on ingredients that are locally available and produced but which still contain expensive, imported fishmeal. This makes the application of formulated feeds by farmers impossible as they are often not able or willing to buy them.

Earthworm meal has been investigated as a fishmeal replacement in several studies (Tacon *et al.* 1983; Stafford and Tacon 1984; Tuan 2010). Under laboratory conditions and without access to natural food resources, partial replacement of fishmeal protein by earthworm protein in full-feeds for common carp (*Cyprinus carpio*), Rohu (*Labeo rohita*) and Buenos Aires tetra (*Hemigrammus caudovittatus*) had a positive effect on growth performance (Tuan 2010; Paripuranam *et al.* 2011), while total replacement in feeds resulted in growth rates similar to those of fish on test diets that contained fishmeal (Tuan 2010). But it is unknown whether full replacement of fishmeal by on-farm produced earthworm meal may be beneficial for growth under pond conditions where fish also have natural food resources which are known to be of high nutritional value and contain high levels of protein and essential amino acids (Dabrowski and Rusiecki 1983; De Silva 1993; Ventura and Catalan 2010).

The aim of this study was to evaluate the suitability of earthworm meal as a fishmeal replacement in plant-based supplemental feeds for common carp under semi-intensive pond management when natural food resources were also available.

6.2 Materials and Methods

Over a period of three months, a net cage trial was conducted in Yen Chau district (Son La province) in mountainous northern Vietnam. In total, 32 net cages (1.5 x 1.5 x 2 m, mesh size of 1.5 cm) were installed in the pond of a small-scale farmer. The net cages were covered by mesh to stop the experimental fish from jumping out, to prevent natural predators (snakes, kingfishers, egrets) from getting in, and to deter theft by rod fishing which are all common problems in the research area (Steinbronn 2009). Two walkways were installed into the pond to facilitate feeding and sampling. Common carp for stocking (average body mass 31.0 g ± 6.8 g standard deviation (SD), n=240) were kept in the net cages for one week for acclimatization before the trial was started by feeding 10 g $kg^{-0.8}$ metabolic body mass.

Feeds and feeding rates

Three iso-nitrogenous and iso-lipidic feeds were formulated (Table 6.1, Table 6.2). The feeds were designed to contain 10% crude lipid (CL), 16% crude protein (CP) derived from plant ingredients, and 11% CP derived from animal resources either fishmeal or earthworm meal. In feeds 1, 2 and 3 fishmeal protein was replaced by 0%, 50% and 100% earthworm meal protein (*Perionyx excavatus*), respectively. As earthworm meal and fishmeal differed in CP content, rice straw meal was used as a high fibre ingredient to balance the animal derived protein levels without changing the nutritional values of the test feeds.

Eight treatments were applied in four replicates each. The three supplemental feeds were offered with low and high levels of supplement (10 and 20 g $kg^{-0.8}$ metabolic body mass, respectively). Fish receiving a low level of supplementary feed had greater access to natural food resources because of their low stocking density (five fish per net cage). Conversely fish fed a high level of supplemental feed were able to get relatively little natural food due to their higher stocking density (10 fish per net cage). At both stocking densities, there were control groups of fishes that were not fed any supplements in order to determine the nutritional value of the natural food resources alone. The feed amount of 20 g $kg^{-0.8}$ metabolic body mass was split and fed at 11 am and 5 pm but the feed amount of 10 g $kg^{-0.8}$ metabolic body mass was fed only once at 5 pm. Every 10 days, the fish were weighed and the feed amounts were adjusted according to the new weights.

Natural food resources

Natural food resources were monitored every 10 days. At three different places in the pond, two fractions of plankton (> 60 µm and > 200 µm) were sampled by means of pulling nets. For qualitative determination, the nets were vertically pulled up the water column. The zooplankton samples were filtered through a pre-dried and pre-weighed GF/C filter (Whatman) and dried at 60°C for determination of zooplankton dry matter (DM).

Every 10 days, top pond sediment layers (10 cm) were sampled in five replicates with a PVC corer of diameter 4.5 cm. The sediment samples were carefully washed through a 500 µm mesh. Zoobenthic organisms were segregated and stored in 5% formalin solution. Zoobenthic organisms were identified to family level, divided into body length classes and dry matter (DM) biomass estimated per pond area by using DM-length regressions from the literature (Miserendino 2001; Miyasaka *et al.* 2008).

Table 6.1: Chemical composition and energy content of single feed ingredients and final composition of trial feeds.

Feed ingredient	DM [% FM]	CA [% DM]	CP [% DM]	CL [% DM]	GE [MJ/kg DM]	Feed 1 [% DM]	Feed 2 [% DM]	Feed 3 [% DM]
Fishmeal	92.0	29.9	63.4	6.9	16.3	18	9	0
Earthworm meal	89.8	8.0	44.3	8.8	20.8	0	13	26
Heated Soy bean meal	94.6	5.0	40.0	19.4	22.6	32	32	32
Corn Meal	89.9	0.7	9.0	4.5	17.7	30	30	30
Rice bran	90.5	13.0	8.0	2.7	17.6	7	6	5
Cassava meal	90.4	2.0	1.2	0.5	16.8	6	6	4
Vitamin mix	90.0	0.0	0.0	0.0	0.0	1	1	1
Mineral mix	90.0	100.0	0.0	0.0	0.0	1	1	1
Rice straw	93.2	11.7	8.3	1.4	15.5	4	1.5	1
Sunflower oil	98.0	0.0	0.0	100.0	nd	1	0.5	0
Total						100	100	100

CA: crude ash; CL: crude lipid; CP: crude protein; DM: dry matter; FM: fresh matter; GE: gross energy

Table 6.2: Chemical and essential amino acid composition of trial feeds and requirements of juvenile common carp.

Component	Unit	Requirement *	Feed 1	Feed 2	Feed 3
DM	% of FM	na	90.8	90.0	88.4
CA	% of DM	na	10.9	8.5	6.5
CP	% of DM	32°	29.7	30.9	31.0
CL	% of DM	na	12.2	11.9	11.0
P	% of DM	0.70	0.32	0.25	0.17
TI	% of DM	na	0.29	0.24	0.23
GE	MJ/kg DM	13.4°	20.1	20.6	20.7
Thr	% of CP	4.7	3.7	3.7	4.0
Val	% of CP	4.4	4.0	4.2	4.3
Met	% of CP	2.2	1.5	1.5	1.5
Ile	% of CP	3.1	3.4	3.7	3.9
Leu	% of CP	4.4	6.9	7.0	7.6
Phe	% of CP	4.1	4.1	4.2	4.5
His	% of CP	1.6	2.1	4.2	2.3
Lys	% of CP	6.9	4.5	4.6	4.8
Arg	% of CP	5.3	6.0	5.6	5.9
Trp	% of CP	0.9	0.9	1.0	1.1

* (NRC 2011)

° digestible protein/energy

Arg: Arginine; CA: crude ash; CL: crude lipid; CP: crude protein; DM: dry matter; FM: fresh matter; GE: gross energy; His: Histidine; Ile: Isoleucine; Leu: Leucine; Lys: Lysine; Met: Methionine; P: phosphorus; Phe: Phenylalanine; Thr: Threonine; TI: Trypsin-Inhibitor; Trp: Tryptophan; Val: Valine.

Water quality monitoring

During a 10 day cycle, three water samples of 1.5 L were taken at three different sites of the pond at 25 cm water depth. The water samples were transported to the field laboratory immediately (10 minutes' drive). For determination of suspended particle concentration, sample water was sucked under weak vacuum through pre-dried (60°C) and pre-weighed GF/C filters (Whatman). Dry mass (105°C) and ash mass after combustion were used to determine the concentrations of total suspended solids and inorganic suspended solids.

Duplicate analyses were made of the filtrates from the suspended particle determination to measure dissolved total ammonia nitrogen (TAN), nitrite nitrogen (NO_2-N), nitrate nitrogen (NO_3-N), and ortho-phosphate phosphorus (PO_4-P) using the Spectroquant Test Kits (Cat. No. 14752, 14776, 09713, and 14848, respectively). Un-ionized ammonia nitrogen (UIA-N) concentrations were calculated according to Emerson *et al.* (1975).

Total nitrogen (TN) and total phosphorus (TP) were measured in unfiltered, mixtures of the three samples. In duplicate determinations, 20 mL of mixed pond water were completely oxidized by the addition of two microspoons of Oxisolv® (Merck) and were analysed for dissolved nitrate and ortho-phosphate (described above) being equivalent to TN and TP. Photometric analyses of dissolved TAN, NO_2-N, NO_3-N, PO_4-P, TN and TP were performed using a field photometer (Photolab S12; WTW).

Every 10 days, profiles of dissolved oxygen, pH and temperature were measured at two sites from the walk ways at sunrise, midday and sunset at 5, 25, 50, 75 and 100 cm water depth.

Measurements of dissolved oxygen, water temperature, and water pH were measured using a handheld pH/Oxi 340i Meter (WTW) equipped with a CellOx 325 oxygen sensor (WTW), and a SenTix 20 pH probe (WTW).

Chemical composition

From each net cage, fish were pooled, homogenized and freeze-dried. Samples of fish carcasses and feeds were analysed for DM and crude ash (CA) according to AOAC (1990). Total nitrogen (TN) and total carbon (TC) were determined using a C/N analyser (Vario MAX CN, Elementar Analysensysteme GmbH, Germany, N x 6.25). Crude lipid (CL) was determined by extraction with a Soxlet device, and gross energy (GE) with bomb calorimeter (IKA C 7000, Janke & Kunkel IKA-Analysentechnik, Germany) using a benzoic acid standard. TP was analysed by the colorimetric method of Gericke and Kurmies (1952) using a spectrophotometer (U-2000, Hitachi). The trypsin inhibitor activity was determined essentially according to Smith *et al.* (1980). The amino acid analysis of feeds was made by the State Institute for Agriculture Chemistry (Hohenheim, Germany) following EU standard methods 98/64/EG and 2000/45/EG.

Statistical analysis

Differences in proximate fish carcass composition were tested by one-factorial ANOVAs using STATISTICA 8 (StatSoft®, USA). Data were graphed by SigmaPlot 11.0.

6.3 Results

Water parameters of the trial pond over the experimental period are shown in Table 6.3. NO_2-N, TAN and PO_4-P were detected near the detection limit of the method used. Dissolved oxygen concentration was lowest in the early morning before sunrise (1.9 ± 0.5 mg L^{-1}).

Three potential natural food resources of common carp are shown in Table 6.4. Over the trial period, natural food availability showed temporary changes in all sampled fractions but zoobenthos showed the greatest variability with time. It had on average the lowest DM of all the natural food species in the pond and showed poor correlation with the growth of common carp. DM of zooplankton larger than 200 µm correlated best with the growth of common carp.

The abundance and availability of natural food resources in the pond were not sufficient for culturing common carp without supplemental feeding. Un-supplemented fish suffered a steady loss of body mass at both stocking densities. No fish died during the trial except for two in the unfed groups. The growth of fish under the different treatments is shown in Figure 6.1 as specific growth rates (SGR). In general, the SGRs were rather low, probably because the fish were stressed by the sight of predators (esp. snakes) and by farmers fishing in the ponds outside the cages. Similarly, small tilapia were observed to enter the net cages through the large mesh holes and compete with common carp for the supplemental feeds. On average, growth of common carp improved with increasing fishmeal replacement by earthworm meal under both feeding schemes (10 and 20 g $kg^{-0.8}$ metabolic body mass), but these were not significantly different.

The interactions of the test diets and the availability of zooplankton DM (> 200 µm in size) is depicted in Figure 6.2. The linear regressions show that when fish have access to natural food, they grow better when they are given a supplemental diet in which the animal protein comes from earthworm than when they are given one in which the animal protein source is fishmeal.

The feeding schemes differed significantly in their effects on the proximate body composition of the fish (see Table 6.5). Fish without supplemental feed had significantly lower DM, CL and GE but higher CA content than supplemented ones. Fish receiving double supplementation had even higher contents of CL and GE.

Table 6.3: Water quality parameters (mean ± SD) over the experimental period.

Parameter	**Unit**	**Mean ± SD**
NO_2-N	mg L^{-1}	0.04 ± 0.01
NO_3-N	mg L^{-1}	0.43 ± 0.15
TAN	mg L^{-1}	0.15 ± 0.07
UIA-N	mg L^{-1}	6.2 ± 1.8
TN	mg L^{-1}	1.81 ± 0.29
PO_4-P	mg L^{-1}	0.01 ± 0.01
TP	mg L^{-1}	0.16 ± 0.11
SDD	Cm	34.7 ± 2.0
pH		7.7 ± 0.1
Temperature	°C	31.9 ± 0.9
$O_2^{6\ am}$	mg L^{-1}	1.9 ± 0.5
$O_2^{12\ am}$	mg L^{-1}	6.3 ± 1.7
$O_2^{6\ pm}$	mg L^{-1}	7.9 ± 1.2
TSS	mg L^{-1}	32.4 ± 5.9
TSS_{org}	mg L^{-1}	14.6 ± 1.7
TSS_{inorg}	mg L^{-1}	17.8 ± 5.1

NH_3-N: un-ionized ammonia nitrogen; NO_2-N: nitrite nitrogen; NO_3-N: nitrate nitrogen; $O_2^{6\ am,\ 12\ am,\ 6\ pm}$: oxygen averaged over all measured depths at the respective day time; PO_4-P: ortho-phosphate phosphorus; SDD: Secchi disk depth; TAN: total ammonia nitrogen; TN: total nitrogen; TP: total phosphorus; TSS: total suspended solids; TSS_{org}: organic fraction of TSS; TSS_{inorg}: inorganic fraction of TSS

Table 6.4: Dry matter (DM) of different size groups of zooplankton and zoobenthos (mean± SD) per unit pond area as an estimate of the natural food available for stocked common carp.

	Size class	**DM per unit pond area [mg m^{-2}]**
Zooplankton	> 60 µm	772 ± 187
	> 200 µm	364 ± 88
Zoobenthos	> 0.5 cm	52 ± 53

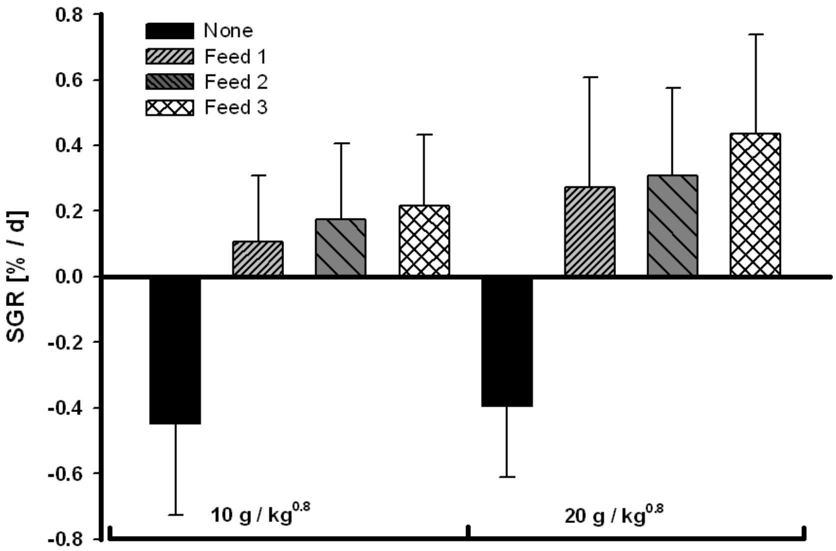

Figure 6.1: Specific growth rate (SGR; y-axis) of common carp receiving four feeds (None: no supplemental feeding and supplemental feeds 1, 2, and 3 in which fishmeal derived protein is replaced by 0%, 50%, and 100% earthworm meal protein, respectively) plotted against level of supplemental feeding (none, 10 g $kg^{-0.8}$ metabolic body mass, and 20 g $kg^{-0.8}$ metabolic body mass) and stocking density (5 and 10 fish per net cage).

SGR [% day^{-1}] = 100 * ((ln final body mass (g) – ln initial body mass (g)) / time (days))

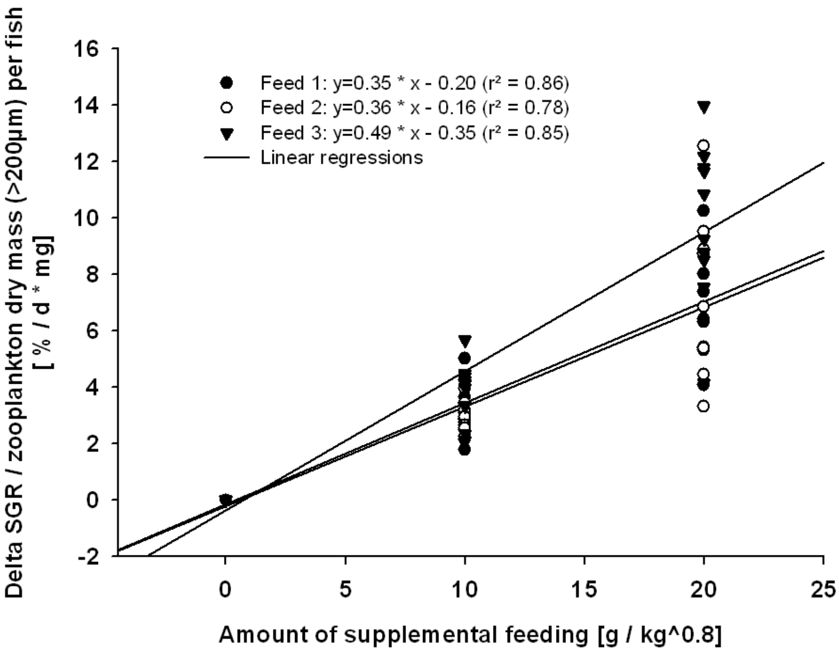

Figure 6.2: Delta SGR per zooplankton dry mass per fish (% d^{-1} mg^{-1}; y-axis) of common carp receiving three supplemental feeds (1, 2, and 3 in which fishmeal derived protein is replaced by 0%, 50%, and 100% earthworm meal protein, respectively) plotted against level of supplemental feeding (none, 10 g $kg^{-0.8}$ metabolic body mass, and 20 g $kg^{-0.8}$ metabolic body mass). Equations and r^2 given in the legend are describing the linear regression and the fitting accuracy of the respective feed.

Delta SGR = $SGR_{fed\ feed\ x} - SGR_{unfed}$

Table 6.5: Proximate body composition of trial fish fed the three test diets 1, 2 and 3 in which fishmeal protein was replaced by 0%, 50% and 100% earthworm meal protein, respectively. In four replicates each, these test diets were fed to groups of common carp at 0, 10 and 20 g $kg^{-0.8}$ metabolic body mass at stocking densities of five and ten fish per net cage.

Daily feeding	[g $kg^{-0.8}$]	0		10			20		
Stocking	[Fish/cage]	5	10	5	5	5	10	10	10
Feed		No	No	1	2	3	1	2	3
DM	% of FM	$13.6{\pm}1.7^{e}$	$17.4{\pm}1.0^{d}$	$23.5{\pm}0.1^{c}$	$24.7{\pm}1.4^{b}$	$24.2{\pm}0.5^{b}$	$28.8{\pm}2.8^{a,b}$	$27.6{\pm}4.7^{a,b}$	$28.8{\pm}1.2^{a}$
CA	% of DM	$30.1{\pm}5.2^{a}$	$24.2{\pm}3.5^{a}$	$15.3{\pm}1.1^{b}$	$14.9{\pm}1.3^{b}$	$14.5{\pm}1.0^{b}$	$11.8{\pm}0.9^{b}$	$11.6{\pm}0.6^{b}$	$10.8{\pm}0.5^{b}$
CP	% of DM	$67.1{\pm}5.3^{a,c,d}$	$73.9{\pm}2.6^{a}$	$71.1{\pm}1.2^{a}$	$71.5{\pm}2.8^{a,c}$	$69.3{\pm}2.1^{a}$	$62.8{\pm}1.6^{b,c}$	$61.0{\pm}2.0^{b,d}$	$60.1{\pm}1.6^{b,d}$
CL	% of DM	$0.9{\pm}0.3^{c}$	$1.3{\pm}1.0^{c}$	$9.8{\pm}1.5^{b}$	$11.4{\pm}3.5^{b}$	$12.6{\pm}2.2^{b}$	$23.4{\pm}2.7^{a}$	$25.2{\pm}2.9^{a}$	$24.6{\pm}1.7^{a}$
GE	MJ kg^{-1}	$15.3{\pm}1.4^{c}$	$16.9{\pm}1.3^{c}$	$21.5{\pm}0.6^{a}$	$21.7{\pm}0.6^{a}$	$22.0{\pm}0.4^{a,d}$	$24.4{\pm}0.7^{a,b}$	$24.8{\pm}1.1^{b,d}$	$24.8{\pm}0.2^{b,d}$

Mean values in the rows that do not share the same superscript(s) differ significantly in the pond management effect at $p \leq 0.05$.
CA: crude ash; CL: crude lipid; CP: crude protein; DM: dry matter; FM: fresh matter; GE: gross energy

6.4 Discussion

Earthworms as a feed resource

In the literature, earthworms are generally described as resources with high protein content and protein quality but these parameters have been shown to differ according to earthworm species and, to a lesser extent, the feed substrate (Tacon *et al.* 1983; Stafford and Tacon 1984; Sun *et al.* 1997; Changguo *et al.* 2006; Sogbesan *et al.* 2007; Dong *et al.* 2010; Tuan 2010). There are several reports that demonstrate the suitability of various earthworm species as components of aquafeeds while others suggest that single earthworm species can have negative effects on the growth and health of fish. In salmonids, low levels of fishmeal replacement by earthworm meal (*Dendrodrilus subrubicundus*, *Allolophora foetida*) produced enhanced growth performance (Akiyama *et al.* 1984; Stafford and Tacon 1984) but higher levels had increasingly negative effects (Stafford and Tacon 1984). In common carp without free access to abundant natural food resources, the replacement of fishmeal by dried earthworm meal (*Eudrilus eugeniae*) resulted in reduced growth (Nandeesha *et al.* 1988). Feeds in which there was incremental replacement of fishmeal by frozen *Perionyx excavatus* caused reduced growth in Marble goby (*Oyxerlotris marmorata*) and pangasius catfish (*Pangasius hypophthalmus*), but fish in a trial by Nhi *et al.* (2010) had manifestly higher growth rates when fed fresh earthworms rather than frozen ones. Tacon *et al.* (1983) reported several earthworm species (*Allolobophora foetida*, *Lumbricus terrestris*) as being suitable for feeds for rainbow trout. In contrast, frozen or freeze dried *Eisena foetida* was found to be unpalatable to trout due to haemolytic factors in the coelom fluid but the isolated protein of this earthworm species seems to be suitable as a food and feed resource (Medina *et al.* 2003; Kostecka and Paczka 2006). Coelomic fluid of earthworms was shown to play an important role in their immune reactions (Kauschke *et al.* 2007) and also to affect the immune reactions in other animals treated with coelomic fluid. The coelomic fluid of *Eisena foetida* had toxic effects in fishes and other vertebrates (Kobayashi *et al.* 2001; Ohta *et al.* 2003) but had less effect on invertebrate species (Kobayashi *et al.* 2001). Lysenin, a component of the coelomic fluid, was reported to produce toxic effects by binding to sphingomyelin (Yamaji *et al.* 1998; Kobayashi *et al.* 2001; Kobayashi *et al.* 2004), but heat-treatment of coelomic fluid caused a reduction in toxic effects suggesting lysenin and other haemolytic factors may be heat labile proteins (Tacon *et al.* 1983; Kauschke *et al.* 2007). Thus the preservation and processing methods used when some species of earthworms are incorporated into fish feed can greatly affect its palatability. Tacon *et al.* (1983) suggested pre-treatment of earthworm by removal of coelomic fluid, heat treatment in the course of drying, or blanching with hot water. However, toxic coelomic fluid was not detected in all earthworm species (Kauschke *et al.* 2007). *Perionyx excavatus* (used in this study) were found to negatively affect fish growth depending on the preservation methods used (Nhi *et al.* 2010) suggesting that there are anti-nutritional factors in this earthworm species too. In the present study, earthworm meal from *Perionyx excavatus* hardly elicited any negative effects on growth in common carp probably because the worms underwent a form of heat treatment during sun drying and the percentage of earthworm in the diets were low.

The protein quality of earthworm meal was reported to be comparable to that of fishmeal with high levels of essential amino acids for fish (Tacon *et al.* 1983; Tacon & Metian 2009; Tacon and Metian 2009; Dong *et al.* 2010; Tuan 2010; NRC 2011). As in full feeds, earthworm meal was suitable as a partial replacement for fishmeal with similar or beneficial effects on fish growth (described above). In this study growth increased as increasing levels of earthworm were used to replace fishmeal. Under semi-intensive pond management, there is a strong interaction between supplemental feed composition and the availability and nutritional quality of the natural food resources. Natural food resources differ in nutritional quality (De Silva 1993) although zooplankton and macro-zoobenthos, which are the main natural food sources of common carp (Kolar and Rahel 1993; Milstein *et al.* 2002; Rahman *et al.* 2008b), generally have a high protein content and high levels of essential amino acids (Dabrowski and Rusiecki 1983; De Silva 1993; Ventura and Catalan 2010). The availability and nutritional quality of natural food resources determines the nutritional quality of supplemental feeds needed to fully supply the requirements of cultured fish (Viola 1989; De Silva 1993). In the present study, the amounts of natural food resources available were not sufficient to support fish growth without supplemental feeding (negative growth of control groups). With increasing levels of supplemental feeding and reduction of natural food per fish (higher stocking density), the increasing effect of higher natural food availability on growth performance showed that protein was the limiting factor. This is not surprising as the test feeds were formulated below the protein requirements of common carp (NRC 2011) as suggested by De Silva (1993) for supplemental feeding at low stocking densities. Even so, the combination of earthworm meal and natural food seemed to meet the nutritional requirements of common carp more efficiently than fishmeal in the test feeds.

The SGRs of common carp correlated most closely with the abundance of zooplankton DM larger than 200 µm in size, which implies that large zooplankton are the most important natural food resources for juvenile common carp under these conditions. This feeding habit was also found by Pucher and Focken (2013) in other net cage trials designed to evaluate plant based supplemental feeds under local conditions in the research area. Rahman *et al.* (2008a) named large zooplankton as the most important natural food resources for common carp as soon as the supply of the preferred zoobenthos in the pond becomes limited. An increase in production of natural food should be aimed by changing the water management and fertilization management (Pucher *et al.* 2014a; Pucher *et al.* 2014b) to increase the financial benefit and growth of common carp fed on supplementary on plant-based feeds with earthworm as the animal protein resource. In the research area, the increase of natural food availability may be promoted by improving methods of fertilization as well as water flow management. For a detailed description of techniques for achieving these ends see Pucher *et al.* (2013; 2014a; 2014b).

Vermiculture as additional farming activity in integrated small-scale farms

As shown in this study, earthworms might be used by small-scale farmers in rural areas to produce supplemental feeds for omnivorous fish like common carp. This necessitates the introduction of earthworm production technologies (vermiculture) into rural areas of developing countries as an additional farming activity in integrated farming systems. Vermiculture accelerates nutrient cycling within the farms and generates additional financial benefit due to utilization of underutilized wastes and by-products of low quality (Sinha *et al.* 2002; Sogbesan *et al.* 2007) by producing a high qualitative feed resources for fish (or other animals) and highly fertile soil (called vermicompost) for gardening or pond fertilization (Ghosh 2004; Chakrabarty *et al.* 2009; Chakrabarty *et al.* 2010). In a case study performed in small-scale farms in northern Vietnam, Müller *et al.* (2012) calculated that vermiculture based on underutilized on-farm wastes (ruminant manure) produces 6 - 36 kg of earthworm DM per year and farm. Using this amount of earthworm meal in plant based supplemental feeds could produce 18-112 kg of additional common carp per farm, raising the financial yield by 12-75% per year compared to the traditional aquaculture applied by small-scale farmers in the research area (Steinbronn 2009; Pucher *et al.* 2013).

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6.5 References

Akiyama T, Murai T, Hirasawa Y, Nose T (1984) Supplementation of various meals to fish meal diet for salmon fry. Aquaculture 37:217-222

AOAC (1990) Official Methods of Analysis, 15th ed. Association of Official Analytical Chemists, Arlington, VA.

Chakrabarty D, Das SK, Das KM, Biswas PK (2009) Application of Vermitechnology in Aquaculture. Dynamic Soil, Dynamic Plant 3:41-44

Chakrabarty D, Das SK, Das MK, Bag MP (2010) Assessment of Vermicompost as Direct Application Manure in Fish Farming Ponds. Turkish Journal of Fisheries and Aquatic Sciences 10:47-52

Changguo X, Pingjiu Z, Genxing P, Duosheng Q, Qiuhua C (2006) Changes in diversity, protein content, and amino acid composition of earthworms from a paddy soil under different long-term fertilizations in the Tai Lake Region, China. Acta Ecologica Sinica 26:1667-1674

Dabrowski K, Rusiecki M (1983) Content of total and free amino acids in zooplanktonic food of fish larvae. Aquaculture 30:31-42

De Silva SS (1993) Supplemental feeding in semi-intensive aquaculture systems. In: New MB, Tacon AGJ, Csavas I (eds.) Farm-made aquafeeds. Proceedings of the FAO/AADCP Regional Expert Consultation on Farm-Made Aquafeeds, 14-18 December 1992. FAO-RAPA/AADCP, Bangkok, Thailand, 434p., pp. 24-60

Dong X-H, Guo Y-X, Ye J-D, Song W-D, Huang X-H, Wang H (2010) Apparent digestibility of selected feed ingredients in diets for juvenile hybrid tilapia, *Oreochromis niloticus* x *Oreochromis aureus*. Aquaculture Research 41:1356-1364

Dongmeza E, Steinbronn S, Francis G, Focken U, Becker K (2009) Investigations on the nutrient and antinutrient content of typical plants used as fish feed in small scale aquaculture in the moutainous regions of Northern Vietnam. Animal Feed Science and Technology 149:162-178

Emerson K, Russo RC, Lund RE, Thurston RV (1975) Aqueous ammonia equilibrium calculations: effect of pH and temperature. Journal of the Fisheries Research Board of Canada 32:2379-2383

Gericke S, Kurmies B (1952) Colometric determination of orthophosphorc acid using vanadate molybdate (German). Fresenius´ Journal of Analytical Chemistry 137:15-22

Ghosh C (2004) Integrated vermi-pisciculture - An alternative option for recycling of solid municipal waste in rural India. Bioresource Technology 93:71-75

Hardy RW (2010) Utilization of plant proteins in fish diets: Effects of global demand and supplies of fishmeal. Aquaculture Research 41:770-776

Kauschke E, Mohrig W, Cooper EL (2007) Coelomic fluid proteins as basic components of innate immunity in earthworms. European Journal of Soil Biology 43:110-115

Kobayashi H, Ohtomi M, Sekizawa Y, Ohta N (2001) Toxicity of coelomic fluid of the earthworm *Eisenia foetida* to vertebrates but not invertebrates: Probable role of sphingomyelin. Comparative Biochemistry and Physiology - C Toxicology and Pharmacology 128:401-411

Kobayashi H, Ohta N, Umeda M (2004) Biology of lysenin, a protein in the coelomic fluid of the earthworm *Eisenia foetida*. International Review of Cytology 236:45-99

Kolar CS, Rahel FJ (1993) Interaction of a biotic factor (predator presence) and an abiotic factor (low oxygen) as an influence on benthic invertebrate communities. Oecologia 95:210-219

Kostecka J, Paczka G (2006) Possible use of earthworm *Eisenia fetida* (Sav.) biomass for breeding aquarium fish. European Journal of Soil Biology 42:231-233

Larsen J, Roney JM (2013) Farmed Fish Production Overtakes Beef. Earth Policy Institute. http://grist.org/article/farmed-fish-production-overtakes-beef/, Accessed 11. July.

Luu LT (2001) The VAC system in Northern Vietnam. In: Integrated agricaulture-aquaculture: a primer. FAO/IIRR/WorldFish Center, Fisheries Technical Paper No 407, Rome, pp. 29-32

Medina AL, Cova JA, Vielma RA, Pujic P, Carlos MP, Torres JV (2003) Immunological and chemical analysis of proteins from *Eisenia foetida* earthworm. Food and Agricultural Immunology 15:255-263

Milstein A, Wahab MA, Rahman MM (2002) Environmental effects of common carp *Cyprinus carpio* (L.) and mrigal *Cirrhinus mrigala* (Hamilton) as bottom feeders in major Indian carp polycultures. Aquaculture Research 33:1103-1117

Miserendino ML (2001) Length-mass relationships for macroinvertebrates in freshwater environments of Patagonia (Argentina). Ecologia Austral 11:3-8

Miyasaka H, Genkai-Kato M, Miyake Y, Kishi D, Katano I, Doi H, Ohba S-Y, Kuhara N (2008) Relationships between length and weight of freshwater macroinvertebrates in Japan. Limnology 9:75-80

Müller JL, Pucher J, Tran TNT, Focken U, Kreuzer M (2012) The potential of vermiculture to produce on-farm feed resources for aquaculture in mountainous areas of North Vietnam. In: Proceedings of the International Scientific Conference "Sustainable Land Use and Rural

Development in Mountain Areas", University of Hohenheim, Stuttgart, Germany, 16-18 April 2012, pp. 113-114

Nandeesha MC, Srikanth GK, Basavaraja N, Keshavanath P, Varghese TJ, Bano K, Ray AK, Kale RD (1988) Influence of earthworm meal on the growth and flesh quality of common carp. Biological Wastes 26:189-198

Naylor RL, Hardy RW, Bureau DP, Chiu A, Elliott M, Farrell AP, Forster I, Gatlin DM, Goldburg RJ, Hua K, Nichols PD (2009) Feeding aquaculture in an era of finite resources. PNAS 106:15103-15110

Nhi NHY, Preston TR, Ogle B, Lundh T (2010) Effect of earthworms as replacement for trash fish and rice field prawns on growth and survival rate of marble goby (*Oxyeleotris marmoratus*) and Tra catfish (*Pangasius hypophthalmus*). Livestock Research for Rural Development 22:1-12

NRC (National Research Council) (2011) Nutrient Requirements of Fish and Shrimp. National Research Council of the National Academics, The National Academies Press, Washington D.C.

Ohta N, Aizu M, Kaneko T, Sato T, Kobayashi H (2003) Damage to the Gills, Skin and Other Tissues by Lysenin and the Coelomic Fluid of the Earthworm *Eisenia foetida* in Two Teleosts, *Tanichthys albonubes* and *Oreochromis mossambicus*. Journal of Experimental Zoology Part A: Comparative Experimental Biology 295:117-126

Paripuranam TD, Divya VV, Ulaganathan P, Balamurugan V, Umamaheswari S (2011) Replacing fish meal with earthworm and mushroom meals in practical diets of *Labeo rohita* and *Hemigrammus caudovittatus* fingerlings. Indian Journal of Animal Research 45:115-119

Pucher J, Focken U (2013) Intensification of common carp culture in rural areas of northern Vietnam by plant-based feeds with protein sources of different qualities. In: Poleksic, V. (ed), Proceedings of the 6th International Conference "Water & Fish", Belgrad-Zemun, Serbia, 12-14 June 2013. University of Belgrad, Faculty of Agriculture, pp. 44-49

Pucher J, Mayrhofer R, El-Matbouli M, Focken U (2014a) Effects of modified pond management on limnological parameters in small scale aquaculture ponds in mountainous Northern Vietnam. Aquaculture Research, (in press), pp. 1-15, doi:10.1111/are.12465

Pucher J, Mayrhofer R, El-Matbouli M, Focken U (2014b) 15N Tracer Application to evaluate Nitrogen Dynamics of Food Webs in two subtropical small-scale Aquaculture Ponds under different Managements. Isotopes in Environmental and Health Studies, (in press), pp. 1-14, doi:10.1080/10256016.2014.922963

Pucher J, Steinbronn S, Mayrhofer R, Schad I, El-Matbouli M, Focken U (2013) Improved Sustainable Aquaculture Systems for Small-Scale Farmers in Northern Vietnam. In: Fröhlich HL, Schreinemachers P, Clemens G, Stahr K (eds.) Sustainable Land Use and Rural Development in Southeast Asia: Innovations and Policies for Mountainous Areas. Springer Verlag, Heidelberg, New York, Dordrecht, London, pp. 281-317

Rahman MM, Jo Q, Gong YG, Miller SA, Hossain MY (2008a) A comparative study of common carp (*Cyprinus carpio* L.) and calbasu (*Labeo calbasu* Hamilton) on bottom soil resuspension, water quality, nutrient accumulations, food intake and growth of fish in simulated rohu (*Labeo rohita* Hamilton) ponds. Aquaculture 285:78-83

Rahman MM, Nagelkerke LAJ, Verdegem MCJ, Wahab MA, Verreth JAJ (2008b) Relationships among water quality, food resources, fish diet and fish growth in polyculture ponds: A multivariate approach. Aquaculture 275:108-115

Sinha RK, Herat S, Agarwal S, Asadi R, Carretero E (2002) Vermiculture and waste management: study of action of earthworms *Elsinia foetida*, *Eudrilus euginae* and *Perionyx excavatus* on biodegradation of some community wastes in India and Australia. The Environmentalist 22:261-268

Smith C, VanMegen W, Twaalfhoven L, Hitchcook C (1980) The determinations of trypsin inhibitor levels in foodstuffs. Journal of the Science of Food and Agriculture 31:341–350

Sogbesan AO, Ugwumba AAA, Madu CT (2007) Productivity potentials and nutritional values of semi-arid zone earthworm (*Hyperiodrilus euryaulos*; Clausen, 1967) cultured in organic wastes as fish meal supplement. Pakistan Journal of Biological Sciences 10:2992-2997

Stafford EA, Tacon AGJ (1984) Nutritive value of the earthworm, *Dendrodrilus subrubicundus*, grown on domestic sewage, in trout diets. Agricultural Wastes 9:249-266

Steinbronn S (2009) A case study: Fish production in the integrated farming system of the Black Thai in Yen Chau district (Son La province) in mountainous North-Western Vietnam - Current state and potential (PhD thesis). Institute for Animal Productions in the Tropic and Subtropics, Hohenheim Stuttgart, Germany, University of Hohenheim, 207p.

Sun Z, Liu X, Sun L, Song C (1997) Earthworm as a potential protein resource. Ecology of Food Nutrition 36:221-236

Tacon AGJ, Stafford EA, Edwards CA (1983) A preliminary investigation of the nutritive value of three terrestrial lumbricid worms for rainbow trout. Aquaculture 35:187-199

Tacon AGJ, Metian M (2009) Fishing for aquaculture: Non-food use of small pelagic forage fish-a global perspective. Reviews in Fisheries Science 17:305-317

Tacon AGJ, Metian M, Turchini GM, de Silva SS (2010) Responsible aquaculture and trophic level implications to global fish supply. Reviews in Fisheries Science 18:94-105

Tuan NN (2010) Development of supplemental diets for carp in Vietnamese upland ponds based on locally available resources (PhD thesis). Institute for Animal Productions in the Tropic and Subtropics, Hohenheim Stuttgart, Germany, University of Hohenheim, 159p.

Ventura M, Catalan J (2010) Variability in amino acid composition of alpine crustacean zooplankton and its relationship with nitrogen-15 fractionation. Journal of Plankton Research 32:1583-1597

Viola S (1989) Production of commercial feeds for warm water fish. In: Shilo M, Sarig S (eds.), Fish Culture in Warm Water Systems: Problems and Trends. CRC Press Inc., Boca Raton, Florida, pp. 143-162

Yamaji A, Sekizawa Y, Emoto K, Sakuraba H, Inoue K, Kobayashi H, Umeda M (1998) Lysenin, a Novel Sphingomyelin-specific Binding Protein. The Journal of Biological Chemistry 273:5300-5306

7 Improved sustainable aquaculture systems for small-scale farmers in northern Vietnam

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Abstract

Aquaculture is an important part of the farming system for ethnic Black Thai farmers in the uplands of Son La province, providing cash income and protein rich food for home consumption. The current aquaculture system, with grass carp as its main fish species, is a feed-based system, with leaf material from banana, maize and cassava as well as weeds and by-products from other farming activities, used as key inputs. As with all other feed-based aquaculture systems, this system depends on a constant flow of water in order to supply oxygen; however, the system is limited by feed and water availability and is threatened by a disease that affects only grass carp. This chapter provides a detailed description of the use of aquaculture within the Black Thai's farming system, an analysis of the feed resources currently used therein, as well as of those resources that may potentially be used. It also provides an analysis of grass carp diseases as well as a description of innovatory aquaculture practices geared towards replacing the disease susceptible grass carp with common carp, as the main species. These suggested modifications are based on the enhanced production of natural food in ponds and the application of supplemental feeds partly based upon on-farm resources. Finally, the potential for earthworms to be used as a high quality feed ingredient, one which can be produced on-farm from currently not used or under-utilized resources, is studied in terms of the production of supplemental feed for the common carp.

7.1 Role of aquaculture in Vietnam

Aquaculture has a long tradition in Vietnam, predominantly in the lowlands and coastal areas. Since the establishment of the General Fisheries Department in 1960, the development of aquaculture has been one of its focal activities. For the next two decades, the Department's activities were exclusively targeted at increasing fish production for domestic consumption, and were expanded to upland areas in conjunction with the expansion of paddy rice irrigation schemes in later years. Since 1980, the focus has shifted towards export production, with marine crustaceans initially the key product, and more recently Pangasius catfish (*Pangasianodon hypophthalmus* and *Pangasius bocourti*) (FAO 2006a). The direct and indirect governmental support provided to the aquaculture sector has resulted in the rapid growth of aquaculture production (Figure 7.1), and the different policies developed are reflected in the production data for aquaculture in terms of the domestic supply and consumption of fish and seafood (Table 7.1). While the production of marine crustaceans and Pangasius catfish is mainly for export, the aquaculture of carp and tilapia species bred in freshwater is highly important in the supply of animal protein to the Vietnamese population at the national level. On a regional scale, in remote areas such as the uplands, the food supply from aquaculture can only be assured based on regional production, as fish are highly perishable, and with a generally poor infrastructure the cost of transporting goods from the lowlands may exceed the production costs.

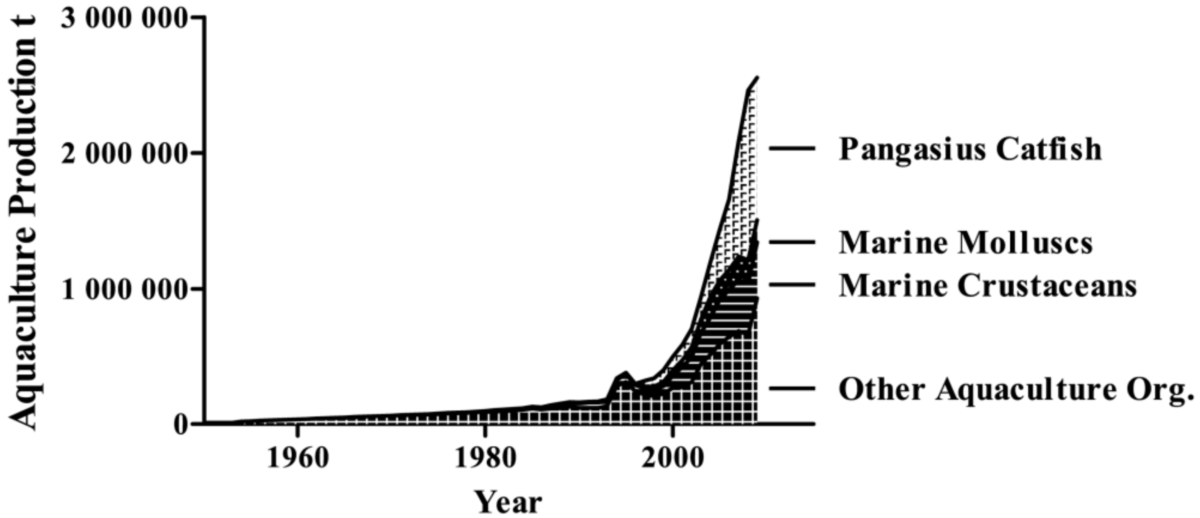

Figure 7.1: Aquaculture production in Vietnam- 1950 to 2009 (FAO 2011)

Table 7.1: Fish and seafood production and consumption in Vietnam- 1975 to 2005 (FAO 2012)

Year	Total population (million)	Fish and seafood (1000 t)				Protein supply per capita per day (g)		
		Production	Import	Export	Domestic supply	Total	From animals	From fish and seafood
1975	48.0	546	3	3	545	44.3	7.2	3.3
1980	53.3	559	2	3	557	45.9	7.1	3.0
1985	59.8	808	0	32	776	47.3	9.0	3.2
1990	66.2	939	0	57	881	49.2	9.9	3.5
1995	73.0	1465	29	131	1363	55.2	12.3	4.4
2000	78.7	2097	12	408	1701	60.9	15.8	5.5
2005	84.1	3367	547	1070	2844	71.1	22.0	7.4

FAO (2012) Food Balance Sheets

7.2 The integrated agriculture-aquaculture system used by Black Thai farmers in Yen Chau district

The integration of fishponds into the agriculture system is common among the ethnic Black Thai, a group settled predominantly in the mountainous regions of Son La province in northwestern Vietnam.

Within the overall framework of the Uplands Program, Steinbronn (2009) has described the locally integrated agriculture-aquaculture system used in Yen Chau district in Son La province, a system characterized by the polyculture of carp and tilapia fish species, with the main species being grass carp. The ponds used are integrated into the overall irrigation scheme as well as into the farming system, within which crop residues, leaves and weeds, as well as manure from large ruminants and pigs serve as feed and nutrient inputs.

Although the system has features usually associated with intensive systems, such as being feed-based and involving frequent water exchange (Edwards *et al.* 1988), annual fish yields are relatively low, at only 1.5 tons per ha per year, as compared to other integrated carp polyculture systems in northern Vietnam which have reported yields up to 6.7 tons per ha (Red River delta) (Luu *et al.* 2002). Nevertheless, Steinbronn (2009) showed that aquaculture production contributes significantly to food security, generates income and plays a significant role in farmers' livelihood strategies. The aim of her study was to provide a detailed and holistic understanding of the actual aquaculture system used and, in the second phase, to create tailor-made location-specific solutions that would have the potential to improve the livelihoods of farmers in an economically, socially and ecologically sustainable way. Data were collected between 2003 and 2006 based on interviews with fish farmers, village headmen and other stakeholders, as well as through an in-depth investigation of individually selected case study farms. Here, resource flows to and from the pond system were monitored quantitatively. The most important features of this aquaculture system are summarized in the following part of this section.

As stated in Chapter 1, more than half of the district's population is of Black Thai ethnicity; settled in highland valleys and along the banks of rivers or streams, where paddy rice is cultivated. Rice is the major food crop in this region and is predominantly used for subsistence, while maize and cassava are the main rain-fed crops - planted on hillsides as cash crops.

Bananas and occasionally cotton are planted in the upland fields, while fruits and vegetables are primarily produced in home gardens. The common livestock raised here include buffalos, cattle, goats, pigs and poultry. The mean farm size of the farmers interviewed for the study was around 1.7 ha, of which paddy fields accounted for approximately 11% and ponds 9%. Around 63% of the households in the study area owned one or more ponds and produced fish.

Although aquaculture production has a long history in northern Vietnam (Edwards *et al.* 1996), aquaculture activities in the study area are a relatively recent phenomenon, and about half the farmers interviewed stated they had dug their ponds within the previous two decades.

The average size of the ponds was found to be around 800 m2, most being earthen. Dykes were concreted only rarely, but surrounded by trees such as bamboo or fruit trees. Some pond embankments also served as vegetable production areas. Almost all the farmers had placed tree branches in their ponds to prevent angling or the use of nets by thieves, as theft is a widespread problem, especially in those ponds located far from farmers' houses.

Typically, ponds were constructed either in series or in parallel, and water flows through the ponds by means of gravity. The water in those ponds located in the valleys was usually supplied from the shared irrigation system. Activities carried out by individual farmers, such as the application of pesticides in paddy fields (Lamers *et al.* 2011) or even the practice of washing clothes in the canals (Alcaraz *et al.* 1993) may have had an influence on connected ponds in a negative way.

Fish are ecto-thermal animals, so water temperature strongly influences their growth and well-being. The ideal temperature range for fish culture is generally above 25°C for most warm-water Asian fish (Cagauan 2001) and feeding activity tends to decrease or stop at temperatures below 20°C (Ling 1977). Whereas the water temperatures in the study area are close to the optimum during the hot summer months, they are not satisfactory for fish growth in the dry, winter season (Diaz *et al.* 1998), especially in shallow ponds that do not have a source of fresh water during those periods (Dan and Little 2000). In addition to growth reduction, immune suppression characteristics have been revealed at the low water temperatures (Yang and Zuo 1997) occurring within the research area, and so the relatively cold winters in this area can limit fish production and even kill tropical species such as tilapia.

As water temperature increases, the dissolved oxygen (DO) content of water decreases due to the lower oxygen solubility, while at the same time the DO requirements of the fish increase. Oxygen represents the key limiting variable within the aquatic environment for fish growth, as the food intake of fish may be suppressed if their oxygen supply is limited (Ross 2000; Black 1998). In the study area, low DO levels are regularly observed at dawn, a time when fish are seen gulping at the pond surface, reflecting emergency respiration behavior.

Inflowing water carries DO into the system, but at the same time also carries sediments from bare and eroded upland fields. Especially during the hot, wet season, these sediments frequently color pond water red-brown, and during the study, the transparency of the water, as measured by the Secchi Disk Depth, was frequently found to be around 20cm or even lower. Water is considered turbid when it has a Secchi Disk Depth lower than 30cm (Sevilleja *et al.* 2001), and turbidity limits photosynthesis due to diminished sunlight penetration; thereby reducing the internal production of DO.

Fish in the study area are frequently stressed by low DO levels, and it is quite likely that the potential entry of pesticides and detergents, as well as sediments from the uplands, which may carry heavy metals (e.g., as a result of weathering of rocks and soils) into the pond system, may stress fish additionally. All these factors probably contribute to the relatively low fish yields in Yen Chau ponds.

Available water resources can become scarce, especially during the dry season. Usually, when paddy fields are irrigated and water is distributed among fields and ponds, the local irrigation authorities give priority to paddy fields, which in some cases leads to a complete halt in the supply of water to ponds. In combination with low rainfall, which was the case during the study phase, this leads to an enormous decrease in the water level, which negatively impacts on the fish. In severe cases farmers are forced to sell fish ahead of time.

In general, farmers practice multiple stocking. The main source of juvenile fish in the research area is a hatchery in Son La town, which was established by the government in the 1960s and was privatized in 2004. The most important fish species produced by the hatchery and reared by local farmers are grass carp, mud carp, mrigal, rohu, common carp, silver barb, the cichlid Nile tilapia and the filter feeders silver carp and bighead carp. During the study, farmers stocked between 4 and 8 different fish species at an average fish stocking density of 1 fish per m2. The continuous supply of young fish is often not guaranteed, thus farmers' stocking system often depends on the availability of fish rather than on long-term planning.

The local hatchery operators as well as farmers have observed a decrease in the quality of fish fingerlings over the last several decades, reporting low growth performance of the fish as well as a higher susceptibility of the fish to disease. There is some evidence that inappropriate management of the local hatcheries, such as the crossing breeding of fish without considering the degree of consanguinity, as well as the use of a generally restricted gene pool, has led to a genetic degeneration of fish stocks.

In terms of nutrients and resources, the polyculture ponds are well integrated into the overall farming system through manifold on-farm linkages between the fish, crops and livestock production activities. Figure 7.2 shows some important resource flows between the fish ponds and other production units on a typical farm. Usually, the leftovers from one production unit serve as inputs to other units, and both the production and the purchase of fish feed are rather unusual in this area. Paddy fields and fish ponds are closely linked to each other; for example, by a common irrigation system which leads water through paddy fields into ponds or vice versa. Farmers use weeds, aquatic plants and by-products from the paddy fields as fish feed, while the most important plant-derived feed inputs from the fields on the hillsides are leaves from banana, cassava and maize. Animal-derived pond inputs include manure from large ruminants or pigs. A detailed list of typical inputs to the fish ponds is given in the following section (8.3).

According to the classification of integrated agriculture-aquaculture systems provided by Prein (2002), the system in the study area is plant based since mainly aquatic or terrestrial macrophytes are used as feed. The herbivorous grass carp, which is able to process raw plant material, is the major species that feeds on the applied green fodder in the study area. However, not all of the plant materials currently applied have been found to be suitable as feed for the grass carp. Some of the leaves and grasses applied in the study area showed a limited feed value (Dongmeza *et al.* 2009) and several feeds even turned out to have a negative impact on fish growth (Tuan *et al.* 2007; Dongmeza 2009; Dongmeza *et al.* 2010). Further, the feed base for the non-grass carp species in this pond system is rather limited. These topics will also be discussed in the following section of this chapter (8.3).

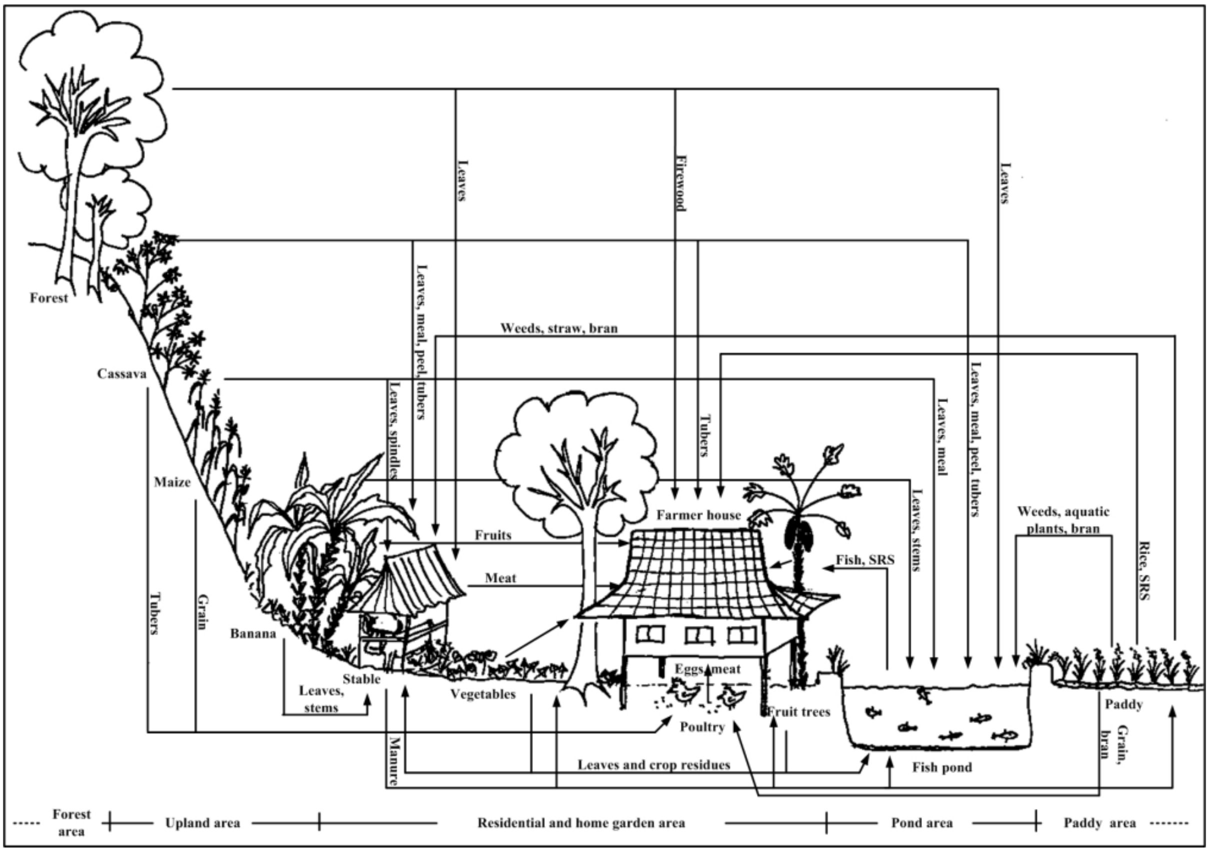

Figure 7.2: A topographical cross-section of the land in the study area and an example of nutrient flows between different farm activities (Steinbronn 2009)

Note: SRS = Self-recruiting species

Farmers' decisions regarding the use of a certain feed at a certain time of the year are often based on the availability of feed - such as the use of maize leaves during the growing period, the availability of labor - such as the use of rice bran during times of higher workloads, and current farming activities - including the use of weeds after hand-weeding of the crop fields has taken place. Fish diets vary between ponds, which can usually be explained by the ponds' locations. Ponds located next to the farmers' houses often receive more diverse feed inputs (e.g., kitchen waste or manure from nearby livestock pens) than those situated further away.

During our study, the average daily feed application was 196 kg of leaf material per hectare, which is a third of the feed amount used in intensive leaf-based aquaculture systems such as the Chinese mulberry dike-carp pond farming system described by Ruddle and Christensen (1993) or the Napier grass based tilapia culture described by Van Dam and colleagues (1993). However, the feed conversion rate of leaf material used within the Black Thai polyculture was found to be 8.8 (feed DM), which is comparable to the Chinese leaf-based aquaculture system (Ruddle and Christensen 1993), but is magnitudes higher than in aquaculture systems that use higher quality feeds. The high moisture content and the bulky nature of many of the applied feeds means they require large amounts of labour for their collection and transportation; for example, during the wet season, farmers spend around 2 hours per day just collecting and transporting fish fodder. However, in some cases, aquaculture activities can be combined with other farming tasks, such as the weeding of paddy fields which simultaneously produces feed for the fish.

Typically, farmers regularly catch aquatic products for their household consumption and then harvest larger amounts for sale at the end of the major rearing period, which usually lasts between 1 and 2 years. The produce is sold when the fish reach marketable size, when money is needed, when farmers have fixed harvest dates with the fish traders, or when water shortages or fish diseases force farmers to take action. In general, only big fish are caught, while small fish are kept in the ponds to allow further growth. During our study, average annual net production of the aquatic species, including 'self-recruiting' species (see below), was 1.54 ± 0.33 tons per ha, of which roughly two-thirds was sold. Besides the cultured species, farmers also harvest naturally entering self-recruiting species which can be fish or other aquatic organisms such as snails, mussels, crabs or shrimps. These self-recruiting species as well as the low-priced fish species such as tilapia, mud carp and silver barb, are typically consumed within the household, while the higher-priced grass carp is often marketed. On average, self-recruiting species made up approximately 3% of the total live biomass output of aquatic species during the study, whereas the grass carp accounted for over half (~ 52%). The high proportion of grass carp in this pond system is a result of its ability to use the available on-farm crop residues as major feed inputs, as well as its ability to grow rapidly. It can also be sold at relatively high prices. Unfortunately, frequently occurring grass carp diseases have led to high fish mortality rates in the region in recent years, and around 70% of fish farmers interviewed said they had had to cope with this problem in previous years, with some farmers reporting losses of their entire stocks. So far, the causative pathogen involved has not been identified. Furthermore, techniques applied by farmers in order to effectively prevent diseases or cure fish have so far failed. The grass carp disease is probably the most important restriction in this grass-carp-dominated aquaculture system, so within the framework of the Uplands Program, research aimed at diagnosing and preventing this disease, as well as treating it, has also been undertaken (see section 8.7).

Despite the loss of grass carp, it has been shown that fish production is still a lucrative business due to the low cost of pond inputs - since usually no off-farm inputs are required except fish seed, the low opportunity costs for labour and the relatively high fish prices. That fish is a high-valued commodity can be shown when comparing the average local price for fish of approximately 1.2 USD per kg (year 2005) with the average monthly per capita income of approximately 11.8 USD in Son La province (GSO 2004; 1 USD = 18,000 Vietnamese dong).

The interviewed farmers reported that their mean income per household (with an average of 5 members) had been almost 831 USD for the year 2004, of which approximately 12% was derived from sales of aquatic products. However, this percentage was calculated using data from those farmers who also did not sell any fish during the year under consideration.

In general, case study farmers produce fish for both income generation and home consumption. Farmers obtain a high share of their protein intake from aquatic products, and these products make an important contribution to farmers' nutrient intake and especially their supply of animal protein (Steinbronn 2009). It is widely acknowledged that fish and other living aquatic resources can constitute an important component of the human diet; they are considered to be nutrient-dense foods which provide protein, amino acids, fatty acids, minerals and vitamins (Prein and Ahmed 2000). While in the past fish production in the study area was predominantly for subsistence purposes, the system has now become increasingly market-orientated, with fish the third most important product for the farmers in terms of income generation after maize and cassava.

The demand for fish in the local market in Yen Chau cannot currently be completely satisfied by the local fish production alone. In the years 2003-2005, a road was upgraded to connect the northwestern mountains with the country's capital Hanoi. As a result, fish from the more intensive aquaculture areas in the lowlands, which were (and are) more economically developed than the mountainous region, began to flood the local markets. This development can be expected to continue, and it is likely farmers will be unable to compete in this market in the future. In order to produce fish in a sustainable way, the current system must be improved so that local fish production levels can be increased.

Vietnam is a very dynamic country in terms of its level of agricultural development, but its inhabitants will need to adapt quickly and flexibly in order to deal with frequently changing markets. Currently, aquaculture is only a minor component in the local farming system, but its importance might increase with greater farm specialization and market orientation, and it has been widely recognized that encouraging the further development of aquaculture production can contribute in a sustainable manner to food security and poverty alleviation in developing countries (Tacon 1997; Edwards 2000; Prein and Ahmed 2000).

7.3 Current and potential feed resources for the local aquaculture system

One of the factors mentioned in section 8.2as contributing to the low level of fish production in these ponds is the limited feed base, both for the grass carp and for the non-grass carp species. The current and potential feed resources for these species are discussed below, with the data presented based on interviews with local stakeholders and on an in-depth investigation into individual ponds during the period 2003 to 2006 in Yen Chau district (Steinbronn 2009).

The feed types currently used in the research area consist mainly of plants, either terrestrial or aquatic, collected from the wild or as the leftovers from cultivated crops. The typical feed types used are shown in Table 7.2. Important plant-derived inputs to ponds include cassava leaves, chopped cassava tubers, cassava peelings and fermentation residues. From banana plants, the leaves and young or soft parts of older (pseudo-) stems are also used, as are the leaves from maize and bamboo plants and occasionally from mulberry and vegetables. Additional inputs include the by-products of rice cultivation, such as rice bran and broken rice, as well as weeds and aquatic plants. The weed from paddy fields most commonly used for fish feed is barnyard grass (*Echinochloa crusgalli*). Animal-derived pond inputs include manure from large ruminants or pigs, of which buffalo dung is by far the most important.

Quantitatively the most important feed inputs (on a dry matter (DM) basis) used during the study period were fresh cassava, banana and maize leaves, weeds collected from the paddy fields and buffalo manure.

Table 7.2: Plant products used as fish feed (Steinbronn 2009)

Crop or plant type	Specification
Cultivated crops	
Cassava (*Manihot* sp.)	Leaves, tubers, peel, meal, fermentation residues
Banana (*Musa* sp.)	Leaves, chopped stems
Maize (*Zea* sp.)	Leaves, meal
Rice (*Oryza* sp.)	Bran, blades/straw, sorted grains, husks, fermentation residues
Bamboo (*Bambusa* sp.)	Leaves
Mulberry (*Morus* sp.)	Leaves
Fodder grass	Napier grass (*Pennisetum purpureum*)
Vegetables	Leaves and stems from e.g., water dropwort (*Oenanthe javanica*), sweet potato (*Ipomea batatas*) and water morning glory (*Ipomea reptans*)
Fruits	Fruits and leaves from e.g., figs (*Ficus glomerata*) and tamarind (*Tamarindus indica*)
Native plants	
Terrestrial plants	e.g., *Alternathera sessili, Chromolaena odorata, Commelina nudiflora, Cyperus imbricatus, Cyperus rotundus, Digitaria timorensis, Echinochloa crusgalli, Eclipta prostrata, Kyllinga monocephala, Sagitaria sagitifolia, Sporobolus indicus, Urochloa reptans* and *Wedelia calendulacea*
Aquatic plants	e.g., *Azolla imbricata, Lemna paucicostata, Pistia stratiotes* and *Salvinia natans*

Most of the above feed types cannot be consumed directly by humans; however, through their application in the ponds, they can be converted into high-value animal protein. Grass carp is believed to be the major species that feeds on the applied green fodder, accounting on average for 80% of the applied feed material used (fresh matter, FM) at the case study farms, as this species has to consume large quantities of plant material in order to obtain the nutrients required (Tan 1970; Cui *et al.* 1994; Cui *et al.* 1992). The gross relationship, in the case study ponds, between the amount of green fodder applied (FM) and the net gain in grass carp biomass (FM) showed that 1 kg of grass carp was produced when approximately 68 kg of grass and leaf material were applied to the pond.

Table 7.3 shows the gross chemical composition of some typical feeds used in the study area - data partly published by Dongmeza *et al.* (2009). Most of the feeds applied here have high moisture and fiber content and relatively low protein content. Dongmeza *et al.* (2009) found that the general quality of the applied feeds was rather poor; however, certain feeds such as cassava and mulberry leaves contained high crude protein contents (20% to 30%) (Dongmeza *et al.* 2009), though high protein content does not necessarily indicate a better quality diet, as demonstrated by authors such as Shireman *et al.* (1978) and Tan (1970). Low fiber content is also an important factor, and the digestibility coefficients for crude protein and gross energy; for example, decline significantly with increased fiber content of the plant feed (Hajra *et al.* 1987). As well as the gross energy and chemical composition, the presence of antinutrients may also influence fish growth (Francis *et al.* 2002; Francis *et al.* 2001a; Francis *et al.* 2001b). Banana leaves; for example, contain tannins and saponins, and cassava leaves contain cyanides, tannins and saponins, which show haemolytic activity (Dongmeza *et al.* 2009).

Table 7.3: Gross chemical composition of selected fish feed (Dongmeza *et al.* 2009; Steinbronn 2009)

Crop	Parts analyzed / specifications	DM % of FM	CP % of DM	EE % of DM	NDF % of DM	ADF % of DM	Lig % of DM	CA % of DM	GE MJ kg^{-1}
By-products and leaves from cultivated crops									
Cassava	Leaves without petioles (long-term variety)	28.1	23.4	8.7	29.5	17.8	6.7	7.6	21.0
Cassava	Leaves without petioles (short-term variety)	28.1	25.9	7.9	27.9	17.6	7.4	6.8	21.0
Cassava	Peel (long-term variety)	38.0	4.0	0.6	23.4	18.6	12.1	6.9	17.1
Cassava	Tubers (long-term variety)	78.6	1.4	0.3	7.5	2.0	0.5	2.5	16.4
Banana	Leaves without leaf veins	20.5	16.1	5.4	49.9	28.8	6.1	7.9	19.5
Maize	Leaves	36.7	13.0	2.6	56.1	34.9	6.6	11.0	17.2
Maize	Meal	86.9	10.9	6.5	13.6	3.7	0.3	1.4	20.9
Rice	Bran [1] (dry)	89.0	7.7	6.5	53.1	40.3	17.2	13.0	18.1
Bamboo	Leaves with soft stems	47.0	16.0	3.5	61.5	39.3	14.3	13.8	17.7
Mulberry	Leaves with soft stems	29.6	25.3	3.0	22.5	15.4	3.9	10.8	17.4
Napier grass	Blades of grass	19.8	16.3	2.9	60.5	36.8	6.6	13.6	16.0
Sweet potato	Leaves and soft stems	15.7	17.4	2.9	37.4	24.6	4.5	10.8	17.5
Native plants									
Barnyard grass	Blades of grass, contains also flowers	19.2	14.1	2.5	59.3	38.7	6.3	9.9	17.6
Mixed weeds[2]	Mix of weeds	14.6	15.6	2.3	62.4	35.7	9.5	13.3	16.9

ADF = Acid detergent fiber; CA = Crude ash; CP = Crude protein (N x 6.25); DM = Dry matter; EE = Ether extract; FM = Fresh matter; GE = Gross energy; Lig = Lignin (determined by solubilization of cellulose with sulphuric acid); MJ = Mega joule; NDF = Neutral detergent fiber

Values are means of duplicate analysis (n ≥ 2)

[1] Rice bran of low quality, partly containing rice hulls; [2] Mix of monocotyles and dicotyles usually collected from paddy fields and dykes (e.g., *Alternathera sessili*, *Commelina nudiflora*, *Cyperus rotundus*, *Digitaria timorensis*, *Eclipta prostata*, *Kyllinga monocephala*, *Sagitaria sagitifolia*, *Sporobolus indicus*,*Urochloa reptans* and *Wedelia calendulacea*)

So far, with the exception of a few items such as duckweed, little information has been published regarding the types of feed used for grass carp in the study area. Within the framework of the Uplands Program, feeding trials were carried out in aquaria and respirometry systems at the University of Hohenheim and in an aquarium system at the Hanoi University of Agriculture. In the experiments at the University of Hohenheim, grass carp were either fed a standard diet (control group), with the standard diet supplemented with dried cassava leaves or weed mixtures (Dongmeza 2009), with leaves from banana or bamboo (Dongmeza 2009), or with the leaves of maize or barnyard grass (Dongmeza *et al.* 2010). In all these trials, the leaves and weeds used, with the exception of the maize leaves, came from the farmers in Yen Chau. In a feeding trial at Hanoi University of Agriculture, grass carp fingerlings were fed solely on fresh banana leaves, barnyard grass or Napier grass (Tuan *et al.* 2007).

In the experiment carried out by Dongmeza (2009), grass carp that were supplemented with banana leaves had a significantly higher body weight gain compared to the control group fed the same amount of standard feed only. In contrast, the growth and feed conversion rate for grass carp supplemented with dried cassava leaves was generally lower in the cassava-supplemented fish as compared to the control group, even though these fish received leaves in addition to the same amount of standard diet as the control group and; thus, a larger amount of nutrients (Dongmeza 2009). In this case, the authors attributed the lower level of growth to the intake of antinutrients. Lower growth rates were also observed in grass carp supplemented with either bamboo leaves, barnyard grass or mixed weeds from the paddy fields, as compared to the respective control groups (Dongmeza 2009; Dongmeza *et al.* 2010). Groups of young grass carp (~ 20 g) fed solely on barnyard or Napier grass showed low fish growth rates and many of the fish died (Tuan *et al.* 2007). Tuan *et al.* (2007) concluded that these grasses are probably not able to support the growth of young grass carp when they are the sole source of food.

Although most of the feed supplied to the ponds in the study area is intended for grass carp, the observed growth rates were rather low, with an average specific growth rate of only 0.24%. While some of the feeds appear to exhibit good potential as supplementary feed, such as banana leaves and duckweed, others such as bamboo leaves, cassava leaves, barnyard grass and mixed weeds probably do not contribute significantly to the growth of fish.

While the feed items that have been discussed so far are most likely eaten almost exclusively by the herbivorous fish, the by-products of cassava, maize and rice (other than the leaves) are thought to play a major role as supplementary feed for the other fish species in the present system. In addition, most of these species are also known to feed on the natural food available in the ponds.

Quantitatively, the most important supplementary feed types in the case study ponds were rice bran and cassava peel. The rice bran was usually of low quality and had low crude protein content, and the peel from cassava tubers was found to be low in protein and fat and contained considerable amounts of fiber and lignin as well as cyanides (Dongmeza *et al.* 2009). Although tilapia, common carp, mud carp and mrigal are known to be able to make use of agricultural by-products (FAO 2006b), it is unlikely in the present case that either of these feeds contributed significantly to fish growth.

In the study area, the availability of livestock manure for pond use is limited by a low number of livestock and the competition with other use types such as paddy rice or gardening within the farm household. Manure from buffalo was by far the most commonly applied manure in the case study ponds and accounted on average for 94% of the total. The dry matter (19.2% of FM) and nitrogen (N)-content (1.4% of DM) of the buffalo manure used in the case study ponds corresponded to those reported by Edwards *et al.* (1994b; 1994a; 1996). Due to the low N-content of buffalo manure, it was necessary to apply high quantities to ponds in order to reach the recommended N-rate of 4 kg N ha^{-1} day^{-1} (Edwards *et al.* 1996; Edwards *et al.* 1994b). The daily loading rates in the case study were lower than 200 g N ha^{-1}, and thus far below these recommendations. In addition, the relatively high water exchange in the ponds limited the effect of fertilization.

Usually, higher fertilization is associated with an increase in plankton production (Nandeesha *et al.* 1984; Boyd 1982); however, the amount of plankton biomass in the case study ponds was generally low, which can be attributed to an inappropriate water management system with high flow rates, as there tends to be an inverse relationship between water flow rates and the abundance of plankton in the study area. On one side, the high flow rates in the study area caused a flushing-out of the phototrophic top water layer, whilst on the other side, they increased the turbidity and inflow of eroded particles, which limited the penetration of sunlight.

The current aquaculture system focuses on grass carp, but grass carp production has become a risky venture due to the occurrence of diseases that cause high fish mortality rates (see 8.2). As long as the diseases identified cannot be prevented or treated, it seems unwise to focus so much labor and other resources on this fish species. Further, according to the local fish traders, the local production of common carp, mud carp, mrigal and rohu is not sufficient to supply the demand of the Yen Chau market. Increasing the portion of common carp may therefore be a promising alternative for the farmers. Common carp generally fetch similarly high prices on the local market as grass carp, and may be reared by farmers themselves, which may save money and further help them to become more independent of the unreliable seed supplies provided by the hatcheries. Therefore, Steinbronn (2009) proposed a "non-grass carp-dominated system" with a species combination that favors common carp. Despite this, this proposed system still advocates that a certain number of grass carp should be stocked (e.g., 10%) in or-der to utilize the abundant leaf and grass material and to provide fish feces for fertilizing the pond. Here, small amounts of green plants of comparatively high nutritional quality should be used to supplement the feed. Furthermore, grass carp stocked at low densities have shown extremely high growth rates in polyculture ponds, as investigated by Sinha and Gupta (1975).

Under the proposed non-grass carp-dominated system, the feed base of the non-grass carp species would be improved through: a) an increase in the availability of natural food and use of a proper water management system, and b) an improved supplemental feed based on local products such as maize and cassava. Tuan (2010) tested different supplemental diets for common carp based on locally available resources such as meal from cassava, maize and soybean, as well as rice bran. In a feeding trial, common carp were fed either a control diet (with fishmeal being the major protein-source) or a diet in which 25%, 50% or 75% of fishmeal was replaced by these plant-derived resources. Even though fish feeding on the control diet showed the highest growth rates, the feed costs per fish produced were lowest in the diet in which 75% of the fishmeal was replaced by local products. This feed, in conjunction with a modified pond management system, was tested in field trials in collaboration with the Yen Chau farmers (Pucher *et al.* 2010a). The results of these trials are presented in section 8.5.

The use of earthworms as a fish feed ingredient has also been found to be a practicable alternative for farmers. Earthworms have high protein content (71% of DM) and can contribute to a significantly improved growth rate in common carp (Tuan 2010; Pucher *et al.* 2012b). The use of earthworms is discussed in section 8.8.

7.4 Design of an improved aquaculture system for small-scale farmers

Based on the above analysis of the problems and limitations experienced within the aquaculture system currently practiced by the Black Thai farmers in Yen Chau district, and the possibilities outlined, an improved system was designed. The goals of this system were to reduce the cultural dependency on the use of disease-prone grass carp, and to increase income generated from fish culture without reducing the contribution of the pond system to household protein supply. The only species that can catch and even exceed prices on the market similar to those obtained for grass carp is the common carp (Steinbronn 2009). The common carp is reared in many tropical, subtropical and temperate zone countries around the globe, predominantly in semi-intensive systems. In these systems, natural food is enhanced by fertilization and/or liming of the pond, and the natural food is supplemented by feeds typically lower in protein content than the physiological requirements of the fish (De Silva 1995), making the fish feed cheaper for balanced diets (Hepher and Pruginin 1981). Farmers in Yen Chau area have available a number of potential feed ingredients from other farming activities, such as rice bran and maize; however, in order to achieve efficient fish production levels with such a low-cost feed, natural food from the pond must supply sufficient protein and essential micro-nutrients. This can only be achieved in ponds with controlled water exchange, which is done to avoid the washing-out of nutrients supplied in the form of organic or inorganic fertilizers. Such nutrient-rich ponds also provide a suitable environment for tilapia and small, self recruiting fish, mollusks and crustacean species to grow, which then contribute to home consumption. Therefore, the suggested set of innovations are to control water flow through the fish pond by uncoupling the pond from the irrigation systems, to dig canals around the pond in order to catch run-off water from the adjacent upland areas, apply lime, organic (manure) and inorganic fertilizers, modify the species composition of the fish-pond - with fewer grass carp and more common carp, and apply supplemental feeds at least partly based on on-farm or locally available resources.

7.5 Implementation and evaluation of the modified pond management system

A modified, semi-intensive polyculture system centered on common carp was designed, based on an investigation carried out into the traditional aquaculture system used by the Black Thai farmers (Steinbronn 2009), a determination of the nutritional value of local resources such as fish feeds (Dongmeza 2009) and the formulation of cost efficient supplemental feeds for common carp, made mainly from locally available resources (Tuan 2010).

Implementation of the modified pond management system

The system was tested under field conditions in the Black Thai farmers' ponds in the research area (Pucher *et al.* 2010a; Pucher *et al.* 2010b). The main modifications made are listed in Table 7.4 and are described in the following section.

Table 7.4: Modifications made to the pond aquaculture system used for common carp, based on semi-intensive polyculture practices and corresponding levels of financial or labor inputs. Level of acceptance by farmers are also shown (Acharya *et al.* 2011 and own observations).

Activity	Reason	Additional Financial/ Labor Inputs	Level of Acceptance by Farmers
Drying pond	Basic hygiene	Medium	Low
Removing mud	Basic hygiene Minimize anoxia	High	Low
Installation of pipes	Water flow control	Low	High
Liming	Pond hygiene Reduction of turbidity	Medium	Medium
Circulating channel	Water flow control Reduction of turbidity	Medium	Medium
Greening dikes	Reduction of turbidity	Low	High
Common carp as main species	Low disease risks	Medium	Medium
Record book	Basic economic calculation	Low	Low
Dynamic water management	Increase of natural feed resources Minimize anoxia	Medium	Medium
Chemical fertilization	Increase of natural feed resources Minimize anoxia	Medium	Medium
Regular pellet feeding	Improved fish nutrition	High	Low

For reasons of pond hygiene, systematic aquaculture pond preparation practices had to be introduced. Steinbronn (2009) described the traditional Black Thai farmers´ aquaculture as a pond management system with irregular catch and stocking processes. Farmers caught low value fish for household consumption, wherein stocking times and harvesting of the ponds were dependent on the current workload and financial situation, and number of fingerlings provided by the traders. Complete harvests were performed rarely and only a few farmers drained all the water from their ponds. Consequently, farmers did not know the species' composition, stocking density or survival rate of the fish in their ponds. Both the gross accumulation of organic material and the sedimentation of particles derived from the eroded soils were frequently observed and reported by farmers in the research area. Under the modified pond management system, researchers recommended that farmers completely drain the ponds after each production cycle, plus remove accumulated sediments and indigestible feed fractions in order to reduce anaerobic bacterial activity (Sugita *et al.* 1992), as well as reduce oxygen consumption through decomposition (Barik *et al.* 2000), especially that caused by the intensive culture of grass carp. The ponds' bottoms then had to be dried out and quick-lime applied.

For the implementation of this semi-intensive pond aquaculture system, several structural modifications to the ponds were recommended. In the traditional ponds used in northern Vietnam, water from all neighboring water sources (channels, rice paddies and ponds) flows into the ponds without any control, either over the water which flows through openings specially created for the purpose, or over water flowing in passively through cracks and holes in the dikes (Reinhardt *et al.* 2012), or even as run-off from surrounding gardens, fields and forests. Tuan *et al.* (2010) reported a yearly run-off of 2.5% of precipitation for the period May

2009 to April 2010, and an average transport of 6.6 kg (minimum 2.1 kg to maximum 13.2 kg) eroded soil per m^2 per year for upland maize fields, with especially high erosion occurring in the spring time when there was very little soil cover and heavy rains occurred. For rice paddies in the research area, Schmitter *et al.* (2010; 2011) reported changes in soil fertility related to the distance of the water inflow in relation to paddy rice cascades, and explained these differences in soil fertility and corresponding grain yields as being due to sedimentation of the eroded particles from the uplands. While eroded particles have a yield increasing effect in rice paddies, Steinbronn (2009) reported that these particles also inhibit primary production in ponds by impairing the penetration of sunlight, even though in her study both the particles and the water that carried them brought in nutrients and organic matter. Lamers *et al.* (2011) reported that in their study both ground and surface water contained several pesticides that had been applied in the rice paddies and that, under existing practices in the research region, reached levels that posed serious environmental problems. As farmers tend to apply pesticides mostly in their rice paddies, negative impacts on fish, zooplankton, phytoplankton and zoobenthos tend to be most pronounced in water flowing from these areas, and can thus make fish consumption risky for human health.

In order to avoid the negative effects of eroded particles coming from surrounding upland fields and pesticides from neighboring paddy fields, our study ponds, using the modified management system, were isolated from external water flows. The dikes used needed to be strengthened against flooding caused by heavy rains (Schad *et al.* 2012), and cracks and macro pores in the dikes had to be sealed. Ponds were equipped with proper pipes for controlling inflows and outflows, with the outlet pipe situated at the deepest point in the pond so that the water could be completely drained for harvests and drying purposes. The constant water flow-through system was replaced by the periodic exchange of pond water, and channels were installed around the ponds to inhibit the inflow of run-off waters from surrounding upland fields. A persistent ground cover was established to stabilize the pond dikes and reduce the erosive effects of heavy rain. Such ground cover for upland fields and pond dikes not only minimizes erosion but also increases the feed base for grass carp and ruminants in the dry season, when green fodder is limited.

The aims of the water management scheme were to reduce the turbidity of the pond water, thus enhancing primary production, and to reduce the loss of nutrients caused by flushing out. Every two to four weeks, water was fed into the ponds to compensate for seepage, and to minimize turbulence in the pond water, accelerate the sedimentation of suspended particles and minimize the flushing out of nutrients. Only clear and clean water was fed into the pond; externally present water was not used during rains in order to inhibit the inflow of water containing eroded soil particles. Farmers apply pesticides on their fields in response to the weather, the occurrence of pests, based upon recommendations from the national extension service and on the advice of neighbors or family members. As a result, water was only used to fill ponds at times when no pesticides had been applied above the ponds.

Steinbronn (2009) reported poor growth among non-herbivore fish species under traditional pond management systems, and during net cage trials in the research area Yen Chau district, Pucher *et al.* (2011a, 2011b) also showed that traditional pond aquaculture practices were not appropriate for raising common carp due to a limited natural food base. In this study, pond productivity and natural food availability was shown to be not sufficient to raise common carp under the traditional fertilization techniques used and with ruminant manure used in combination with constant water flow-through. The common carp lost weight without supplemental feeding and grew only slowly under supplemental feeding – by 3% of their body mass each day. However, the fish grew better when chemical fertilizers were applied in stagnant water (Pucher *et al.* 2011a; Pucher *et al.* 2010a).

To enable primary and secondary production in water with reduced turbidity, ponds were fertilized using locally available resources. In certain regions of Son La province with good market access, Black Thai raise locally adapted pig breeds as livestock for income generation (Lemke *et al.* 2006), and Kumar and colleagues reported pig manure to be a better organic fertilizer than ruminant manure (2004a; 2005b; 2004b), while in our research area (Yen Chau district), Steinbronn (2009) reported pig manure to contain less crude protein than ruminant manure due to the poor feed base of pigs. As only a few households kept pigs, pig manure was applied in small quantities during trials in the research area, with organic fertilization performed through the application of cow and buffalo manure and through the stocking of grass carp (stocked at a density of 10 to 20 % of the carp polyculture). Several researchers' studies reported that stocking grass carp had a significant impact on the fertility of ponds by enhancing decomposition rates and directly releasing nutrients from the undigested leaf parts used as feed (Tripathi and Mishra 1986; Kumar *et al.* 2005b; Pomeroy *et al.* 2000). Urea and single superphosphate, which are available in the region and used by farmers in the upland fields, were also used in these studies, with organic and chemical fertilizers applied at an N/P ratio of 3:1. The amounts of fertilizer used were varied according to the on-farm availability of organic fertilizer, as well as current ammonia, total nitrogen and total phosphorus concentrations in the pond. The total amounts applied were calculated to provide 1.5ppm total nitrogen and 0.5ppm total phosphorus (Kumar *et al.* 2004a).

Under the traditional carp polyculture system of northern Vietnam, grass carp has been reported to be the main species (Steinbronn 2009; Kumar *et al.* 2005a; Kumar *et al.* 2005b; Tripathi and Mishra 1986); grown to augment the internal nutrient recycling processes of the ponds, and can be raised cost efficiently with the use of on-farm resources (Kumar *et al.* 2005b; Pomeroy *et al.* 2000).

Farmers in the research area reported the occurrence of a disease specific to grass carp after 1996 (Steinbronn 2009), one which led to mass mortalities in the region. In order to reduce the risks of farmers being impacted by the grass carp disease, grass carp was replaced almost entirely by common carp within the traditional carp polyculture system, and was then stocked only as a supplementary species. In this region, the common carp is considered a suitable principal species, because it is well accepted as food and fetches a good price on the local market, a price similar to that of grass carp. Farmers are familiar with its habits and ecological requirements and it is readily available from local traders as fingerlings for stocking, or can be reproduced naturally in farmers' ponds. Ponds were stocked at a density of 1.5 fish per m^2 as recommended by Rahman *et al.* (2008b), with common carp eventually accounting for around 50% of fish stocked in the area. Other carp species (such as silver carp, bighead carp, mud carp, grass carp) and also tilapia - all important as a supply of animal protein for domestic consumption, were stocked to similar levels.

Implementation of a modified feeding regime

The common carp is an omnivorous fish and prefers to scavenge pond bottoms in search of benthic organisms like oligochaetes, nematodes and insects, but is also capable of digesting zooplankton, plant derived diets and detritus (Rah-man *et al.* 2008a). Common carp in carp polyculture systems has been reported to increase the bio-availability of nitrogen and phosphorus through its scavenging activities, which increase bioturbation and recirculate sedimented nutrients (Rahman *et al.* 2008b; Jana and Sahu 1993; Milstein *et al.* 2002). This stimulates primary production (Chumchal and Drenner 2004) and the accumulation of nutrient in plankton (Rahman *et al.* 2008a), but also increases turbidity by recirculating sedimented material (Chumchal *et al.* 2005).

Due to the ability of common carp to utilize carbohydrates for their energy requirements (Kaushik 1995; Ufodike and Matty 1983) and to digest plant-derived proteins for their anabolism (Degani *et al.* 1997), only a small amount of animal derived protein is required in the diet of common carp to supply essential amino acids. Sulphur containing amino acids are most essential for fish, as the acids are not abundant in plant proteins and cannot be synthesized. In all animal diets, the most costly and limiting ingredient is animal derived protein, due to its high essential amino acids content. As a consequence, a challenge in the future will be to formulate feeds in which animal derived proteins are replaced by plant proteins or other sources of protein, and to increase the utilization of energy derived from lipids and carbohydrates in the feeds.

In intensive aquaculture, fish diets must be formulated to meet all requirements, as the feed provided is the sole source of energy and anabolic units for the fish. In semi-intensive fish culture systems on the other hand, the supplemental feed used should be formulated to increase the utilization of the natural food items by supplying limiting macro- and micro-nutrients. For common carp under semi-intensive feeding regimes, energy has been shown to be the first growth limiting factor (Pucher *et al.* 2011a, 2011b; De Silva 1995) and must be supplied by supplemental feed to reduce the utilization of animal derived proteins from natural food resources (zoobenthos and zooplankton) as an energy source. The second limiting factor is the quality of protein in the supplement feed.

Artificial fish feeds can be offered in different forms: dough, powder and sinking or floating pellets (Tacon and De Silva 1997). Dough and powders require less technical input and are cheap to prepare, but their use is often associated with high losses of nutrients via leaching and a general loss of feed due to ineffective feed uptake. In contrast, the preparation of sinking and floating pellets demands a higher technical input but enables more effective feed uptake by the fish and results in less nutrient leaching into pond water. The use of pellets also means that all the required nutrients are delivered at the same time, which is not possible using powders and dough where single feed ingredients are suspended in the water or float on the water's surface. In the study area of Yen Chau district, we tested sinking pellets as supplemental feed. Production of the pellets required a medium level of technical input and the pellets sank down to the bottom of the pond, which suited the feeding behavior of the target species (common carp) and partly prevented other species from consuming the supplemental feeds.

It is essential for resource-poor farmers in rural upland areas to use feeds for aquaculture that help give the highest level of profit. The most profitable feed formulation is not necessarily the one that leads to the highest growth rate, since more digestible ingredients are often more expensive. Given that fishmeal prices continue to rise in the rural markets of Vietnam, one study feed with an effective fish meal replacement (Tuan 2010) was introduced to cooperating farmers in the research area of Chieng Khoi commune and tested in pond trials. Abdelghany and Ahmad (2002) evaluated a daily supplemental feeding rate of 2.7% of total fish biomass in a polyculture stocked with more than 50% omnivorous fishes, and at a stocking density of 2.2 fish per m², which was considered optimal for fish and income production. In Chieng Khoi commune, Pucher *et al.* (2010a; 2010b) adjusted the amount of feed offered to 6% of the common carp biomass per day each month, under the assumption that growth of the common carp would be proportional to the growth of all the species present. Pre-tests showed that it was not possible to catch a representative number of all stocked species without significantly disturbing pond turbidity; therefore, the primary species, common carp (50% of all stocked species), was used as an estimate of the total fish biomass.

In Chieng Khoi commune, farmers were advised to split the daily feed into two portions; once in the morning and once in the evening (Rahman and Meyer 2009), but when the farmers were very busy they could only feed the fish once a day. Feeding in the evening only was thus recommended, because water oxygen levels in the morning tend to be low.

In our study in Chieng Khoi district, the method of application used for leaves as feed for grass carp was also modified. It had been observed that the farmers' normal practice was to feed cassava, banana and maize leaves complete with stems, branches and middle ribs, introducing large amounts of indigestible organic matter into the pond, and thus reducing the oxygen level by decomposition processes. To reduce the organic load experienced at the bottom of the ponds, farmers were advised to feed only those parts of the leaves known to be eaten by grass carp.

In order to improve the ability of farmers to calculate the investments and benefits of aquaculture as a farming activity, they were given a book in which they recorded all body masses, as well as the amounts and costs of inputs such as fingerlings, feeds and fertilizer, labor, time and water flows, plus outputs such as the number and mass of fish caught and other pond products. Dead fish or abnormal behavior among fish was also recorded. This practice gave farmers an overview of the input-output data, current stocking densities, species composition, and the survival and growth rates of different species, and so helped them to appreciate the financial benefits of aquaculture as a farming activity. Dung and Minh (2010) recommended that this kind of simple record book should be used in all farming activities, so that farmers can compare different land uses and farming activities and use it as an aid to their decision making.

Evaluation of implemented modifications in the pond management and feeding regime

Ponds under the traditional management system had a high variability in water quality (Pucher *et al.* 2010a; Steinbronn 2009) which was greatly influenced by the surrounding land use types and also water exchange. Heavy rain storms in particular rapidly changed the water quality, resulting in higher turbidity, and high ammonia levels sometimes above 1mg NH_4-N l^{-1} in the spring and autumn. In addition to introduced sediments, Steinbronn (2009) noted that there was a tendency for water inflow rates and plankton densities to be negatively correlated because ponds were designed with overflow as the only water outflow, meaning water was only ever removed from the top layer of the pond. However, this top layer is the most productive water layer of a pond as it contains most of the phototrophic plankton and is thereby the main source of oxygen and the base of pond productivity.

Depending on the source of water, the surrounding land use and soil types, ponds in the research area showed a high variability in terms of water quality, natural food availability and hence in the level of fish production and financial benefits to be gained from the aquaculture activities. Unsuitable water quality elements such as water temperature (Sifa *et al.* 2002; Cagauan 2001; Black 1998; Alcaraz *et al.* 1993; Chervinski 1982), water oxygen (Black 1998; Ross 2000) and water pH (Svobodova *et al.* 1993) levels were shown to have a negative effect on fish growth. Especially within extensive and semi-intensive aquaculture systems, fish growth is correlated with the availability and abundance of natural food resources (Rahman *et al.* 2008b; Muendo *et al.* 2006; Schroeder *et al.* 1990; Spataru *et al.* 1983; Kolar and Rahel 1993; Pucher *et al.* 2011b), and both water quality and the availability of natural food are primarily or secondarily affected by the suspended particle load. During the research in Yen Chau district, ponds with maize and cassava fields on yellow soils in the watershed above and fed by rain water, showed a distinctly higher suspended solids load than ponds located on the rice paddy plateaus and fed by reservoir water. Under the modified pond management system, a reduction in turbidity was obtained, leading to higher primary production activity levels and with higher oxygen production peaks during the day (Pucher *et al.* 2010a). Oxygen limitations occurred in the morning, similarly to those observed under the traditional pond management system. This suggested better primary production activities, resulting in higher oxygen production during the day but also higher oxygen depletion at night. Nevertheless, higher primary production levels stimulated by fertilization and reduced water flow-through resulted in a greater abundance of natural food resources and provided more support for growth, especially of the filter-feeder fish species such as silver carp, bighead carp and tilapia. Also, grass carp under the modified pond management system showed a higher growth rate than under the traditional system, indicating a better feed base of natural food due to lower stocking densities (Sinha and Gupta 1975) and consumption of pelleted supplemental feed which was intended to feed common carp. All stocked species under the semi-intensive pond management system, such as the common carp, grass carp, silver carp, bighead carp and tilapia, showed significantly higher specific growth rates, of 2.6 ± 0.2, 2.4 ± 0.3, 2.5 ± 0.1, 2.9 ± 0.2, and 3.4 ± 0.6 respectively, and when compared to those under the traditional management system, which were1.8 ± 0.1, 1.9 ± 0.7, 1.7 ± 0.0, 2.0 ± 0.0, and 2.5 ± 0.4 respectively (Pucher *et al.* 2010b). Net production (kg/1000m²) over the seven months of the trial under the traditional pond management system was 87.4 ± 43.6, with 36.8 ± 18.8 for grass carp, 4.3 ± 5.5 for common carp, 16.7 ± 3.8 for silver carp, 8.3 ± 4.4 for bighead carp and 16.1 ± 15.4 for tilapia (Pucher *et al.* 2010b; Pucher *et al.* 2010a). Net production (kg/1000m²) under the semi-intensive management system was227.5 ± 41.6, with 45.5 ± 11.8 for grass carp, 35.8 ± 12.7 for common carp, 60.8 ± 2.8 for silver carp, 39.6 ± 13.0 for bighead carp and 35.7 ± 21.9 for tilapia. All fish species, except grass carp and tilapia, had significantly higher production levels under the semi-intensive than the traditional pond management system. The level of production for the common carp was relatively low under the semi-intensive management system, which could be explained by a low recovery rate for the fish of around 38%. Under the traditional pond management system, the recovery rate for the common carp was around 20%, and; therefore, even lower. As under the semi-intensive pond management system, the average specific growth rate for the common carp was 2.4%, while under the traditional pond management system it was significantly lower at 1.9%. This may have been caused by high inter-specific and intra-specific competition for the supplemental feed pellets and natural food resources, especially for the macro zoobenthos. It may also have been caused by a reduction in the genetic variability of the fish hatchery populations due to in-breeding, and this was mentioned by farmers and has been described as typical for rural hatchery brood stock populations (Kohlmann *et al.* 2003; Eknath and Doyle 1985) resulting in a low growth potential or a suppression of the immune defense system. In Son La province, most fish seed is produced either in the Son La city hatchery or transported from hatcheries in Bac Ninh province (approximately 300 km away from the research area). In contrast to reports from local farmers in Son La province (Steinbronn 2009; Thai *et al.* 2006), Thai *et al.* (2006) found that the common carp population at the Son La city hatchery exhibits greater genetic diversity than the three experimental lines at the Research Institute for Aquaculture No. 1 (RIA 1), or in the wild populations of Vietnamese common carp. Thai *et al.* (2007, 2006) attributed this finding to the successful dissemination of imported genetically improved stocks. The findings of Thai *et al.* (2007; 2006) indicated that the poor growth of common carp reported by farmers in Son La province was caused by a poor feed base and low water quality.

Despite the low growth rate among common carp in the field trials, the modified pond management system did lead to significantly higher fish production levels overall (on average 4.5 times higher) and less variable financial net benefits from aquaculture activities when compared to farming activities (Pucher *et al.* 2010a). This was a result of the use of higher quality resources as feed inputs and of the introduction of measures to increase primary production. Financial net benefit accounted for 367 ± 133 USD per 1000 m² of pond under modified management for a year, and 83 ± 356 USD per 1000 m² of pond under traditional management for a year (based on local prices from 2009; 1 USD = 18,000 VND, leaf material was not taken into account). Both fish production levels and financial net benefit showed a very high variability under the traditional pond management system, caused by different environmental land use conditions surrounding the ponds (Pucher *et al.* 2010a; Steinbronn2009). Under the modified pond management system, the uncoupling of the pond from the surrounding land use activities through the minimizing of water inflows into the pond, resulted in a lower variability in terms of both fish production levels and financial net benefits across ponds.

The acceptability of the overall modified pond management scheme in the research area, as well as the improvements implemented by the Black Thai trial farmers are discussed in Section 8.6. For the farmers, there are several areas of uncertainty associated with the use of the modified pond management which might impact their level of acceptance of such a system, such as the need for constant investment in higher quality feeds, especially the expensive, high protein feed ingredients like fish meal and heated soybean meal. There is some evidence supporting the use of earthworms found in on-farm waste in the research area (Müller *et al.* 2012), and these could be used as a feed ingredient in order to reduce the costs of providing supplementary feed (Pucher *et al.* 2012b), and; therefore, increase the net benefit, reduce the constant financial input and reduce the risks associated with the adoption of the modified pond management system. These further innovations are discussed in more detail in Section 8.8.

In over-fertilized ponds there is an increased risk of algae blooms and die-offs resulting in complete oxygen depletion, and as a consequence, the risk of losing any financial investments in fish stocks is higher than under the traditional management system. Furthermore, by using common carp as the main species in the modified system, there is the risk of these fish being affected by diseases such as the Koi Carp Virus CyHV-3, which was recently identified in the brood stock of the Son La hatchery (Richard Mayrhofer, personal communication).

To minimize the risks involved in the adoption of a modified pond management system, further research, either off-farm or through farmers using participatory methods, should be conducted, especially with regard to fertilization schemes adapted to local soil conditions and water sources. Further research is also required on alternative feed ingredients of a high protein content, the details of which will be discussed in Section 8.8.

7.6 Farmers' perceptions of the proposed innovations in the aquacultural system

The previous section presented a potential solution using a modification of the existing pond and feed management practices, a modification which showed some promising results - with a significant potential to improve the household incomes derived from aquaculture. Building on these insights, this section follows upon how farmers perceived the proposed set of innovations, in order to provide important information in support of further expansion of such practices to the wider farming community.

Traditionally, information about improvements in farming activities in the research area has been exchanged horizontally between friends, neighbors and within the family (Acharya *et al.* 2011; Schad *et al.* 2011). This was confirmed by farmers who said that the various state extension services (see also Chapter 11) were rarely reliable sources of information regarding new technologies, nor did they provide much support how these technologies could be applied to the specific mountain environment. Informal information channels- like the ones mentioned above - are regarded as more valuable sources of information as they provide more applicable knowledge. More recently, TV and private shops have become supplemental information sources, especially on the subject of new technologies. These sources; however, were criticized by farmers for not often providing information applicable to the uplands context, as they are rarely adapted to the local environmental conditions in such areas. For example, these information sources do not take into account the state of the local markets for inputs and outputs, the education levels of the farmers, nor the availability of resources such as the fingerlings of new species, high quality feed ingredients, lime or agricultural machinery suitable for the local farmers' fields.

When introducing new technologies into rural areas, any modifications must be based on existing indigenous knowledge (Hoffmann *et al.* 2009); furthermore, locally available resources should be used wherever possible and access to essential resources must be assured to minimize discouraging failures by innovative farmers who have adopted a particular technology.

In the aquaculture subproject of the Uplands Program, several modifications to the traditional aquaculture system were developed and tested, with Table 7.4 showing the level of acceptance of farmers during the action-research pilot trials for the single modifications. The majority of modifications were made in response to the problems and needs mentioned specifically by farmers (Steinbronn 2009), and this approach is broadly in line with the philosophy of participatory innovation development (Rai and Shrestha 2006).

Modifications were kept simple and were based on locally available knowledge and physical resources (as was outlined in Section 8.5). All developed modifications were tested on-farm in farmers' ponds under the supervision of the farmers themselves, and all trials were conducted in pairs of neighboring ponds receiving water from different water sources. Pilot farmers were selected based on the water sources of the ponds and the willingness of both the farmers and their neighbors to collaborate.

Summarizing the trial setup described in Section 8.5, the following key strategies were combined and tested by farmers as a package for systematic innovation:

1. Reducing turbidity in ponds through the control of water flows and fertilization, thereby increasing both oxygen production and natural feed resources
2. Lowering the risk of mass mortalities from grass carp disease by improving basic pond hygiene
3. Introducing semi-intensive polyculture of the common carp with supplemental feeding, and
4. Keeping daily records of pond inputs and outputs to enable an economic analysis of pond aquaculture and to estimate basic feed conversions.

A farmer's willingness to adopt an innovation or modification is, typically, inversely correlated to the financial or labor inputs required (Reardon 1995), and also depends on the level of understanding of the farmer, on his or her trust in the profitability of the innovation and on the innovation being introduced at an appropriate time. Rogers (2003) identified still more factors that determine whether an innovation will be fostered or whether the probability of its adoption will be limited. This section focuses on the criteria which were found to be the most decisive in this context.

Farmers considered low cost modifications aimed at improving their control over water inflows and lowering the turbidity of the pond as a means of reducing the risk of fish diseases occurring. These modifications were widely implemented, for farmers had observed that the mortality and morbidity rates among grass carp were linked to turbidity in the ponds, especially after heavy rain. However, these modifications were not understood as measures that would increase primary production levels. Though farmers consider green water as being better for fish culture than turbid pond water, most farmers do not know that greener water is linked to higher natural food resources and higher oxygen production levels. Farmers have traditionally applied organic fertilizer, such as buffalo manure, which is seen as a direct feed source for the fish rather than a means of supporting the development of green water. In addition, a basic knowledge of biology and nutritional requirements of the cultured fish species is very sparse; farmers usually do not know the specific feed resources available for the different stocked fishes under their traditional polyculture system, except in the case of fish such as grass carp, which feed directly on the materials applied by the farmers themselves. Natural food resources such as phytoplankton, zooplankton and zoobenthos, and their importance for each specific fish species, are generally not understood. Consequently, investment in the chemical fertilization of ponds is not seen by farmers as a means of providing internal food for the fish and is, therefore, not accepted.

Owing to the limited financial savings to be found among most Black Thai households, regular expenditure on farm resources with a market value, such as chemical fertilizers or ingredients for the preparation of pellets for daily feeding activities, is generally only adopted by the better educated and wealthier farmers, whose incomes are more stable than those of the typical Black Thai households, and who are, moreover, willing to invest in innovations that concentrate on aquaculture.

Another important factor limiting the adoption of the proposed modification to the system is the almost total absence of record-keeping, for farmers rarely keep track of basic labor times and expenditures, nor of (non-)marketed outputs, in order to calculate overall financial benefits. These deficiencies in book-keeping make it impossible for farmers to compare losses against gains for a single farm activity, or to carry out overall cost/benefit calculations.

Furthermore, the ability of farmers to adopt new technologies and adapt them to local circumstances is limited by a lack of both basic biological and economic understanding.

In the pilot study, application of the entire set of proposed modifications was shown to multiply the financial net return from pond aquaculture when compared with the traditional system (Pucher *et al.* 2010a), thereby, turning local aquaculture into a semi-intensive and more valid business. However, these modifications were seen as a rather radical innovation for Black Thai farmers (Acharya *et al.* 2011), because they require a constant investment of time and financial resources, which does not fit well into the seasonal pattern of work and budget of a typical Black Thai household. During the three years of collaboration, farmers had the chance to acquire knowledge on aquaculture through practically daily contact with researchers, as well as during workshops held on fish biology and general aquaculture. But it was only the trial farmers who chose aquaculture as one of their key farming activities that showed an increase in knowledge and disseminated it to their peers, whilst, on average, the collaborating farmers did not learn significantly more than the non-collaborating farmers (Acharya 2011; Hoffmann *et al.* 2009).

This is remarkable given the fact that in general it was observed that many Black Thai farmers showed great interest in making improvements to their aquaculture practices, though these were generally the few better-off and higher educated farmers, those who might also be considered “local innovators”. The interest of these farmers was especially attracted to the feed production machines and the technologies used for introducing the intensive production of high-priced aquaculture species, as shown and advertised on TV and applied by lowland fish farmers. Several better-off farmers showed great willingness to invest in the improved aquaculture by, for example, digging new ponds, integrating earthworm culture into their farming system and/or by buying feed processing machines. In the past, farmers adapted rapidly to changes in local markets and adopted ‘cutting edge’ technologies aimed at intensifying single farming activities (e.g., hybrid maize varieties, paddy rice and the use of pesticides). These innovations were promoted by governmental agencies and farmers changed their farming systems towards less integrated and sustainable farming ventures, adopting farming activities more likely to give higher levels of food security and greater financial benefits (Friederichsen 2008). Without governmental programs in place, the better educated and financially well-situated farmers might be expected to further invest in aquaculture and become the precursors of innovation. It is hoped that the introduction of new machines into rural markets may promote this development.

Recommendations for future innovation dissemination

In summary, better-off and more educated farmers in the study area are more likely to introduce improvements and invest in aquaculture in the future, and as a consequence, the introduction of aquaculture innovations must be accompanied by adequate education programs, those well adapted to the local context and aimed at farmers who are interested in changing their aquaculture practices into more beneficial farming activities. As well as educating the ordinary aquaculture farmer, such initiatives would increase the knowledge base of local innovators, who could then adopt an even stronger role as exemplary models for other farmers. Ongoing soil degradation might increase the willingness of farmers to change their current farming systems and may guide them towards greater investment in aquaculture as an alternative and relatively land independent farming activity. Catastrophes such as plagues, floods and droughts may also increase the willing-ness of farmers to invest in new farming ventures in the future.

In order to promote local aquaculture and enable its development in the medium term, an adequate supply of fingerlings of the cultured species, raised under modern and hygienic management systems, must be assured and trading access given to essential resources and products. Once an innovation is adapted to local conditions and widely introduced into a rural area, a strong information and extension effort has to be made to reach out to all farmers. If poor farmers are to gain from these innovations, new institutional arrangements such as 'share-cropping' or 'producer-associations' have to be developed, adopted and directed towards more concerted action in aquaculture.

7.7 Disease management

For more than ten years, grass carp populations in almost all the northern provinces of Vietnam have suffered from high mortality rates of up to 100%, due to unknown pathogens and/or exogenic factors. In Vietnam, the diseases show a seasonal pattern, occurring mainly at the beginning (March to April) and end (October to November) of the wet season, the worst being Red Spot Disease (RSD) which leads to hemorrhagic changes and the appearance of ulcers on the skin of the diseased fish (Van *et al.* 2002).

Between 2008 and 2011, the Uplands Program research area in Yen Chau district was visited six times as part of an epidemiological study and to collect samples. In total, 197 fish were sampled, including 76 grass carp with clinical signs, 50 control grass carp without symptoms, plus 37 diseased, as well as 34 apparently healthy control fish from other species.

Beside the main symptom of hemorrhagic changes and ulcers on the skin, other clinical symptoms such as a darkening or bleaching of the skin, a loss of scales, hemorrhagic intestines, necrosis of the gills, exophthalmia or enophthalmia, and erratic swimming behavior, were found in some of the diseased fish without red spots.

Parasitological examinations employed during the dissection of the sampled fish revealed moderate infestations of ectoparasites. Monogenean trematodes like *Dactylogyrus* sp. and *Gyrodactylus* sp., ciliata including *Trichodina* sp. and *Ichthyophthirius* sp., flagellate such as *Ichthyobodo* sp. and *Lernaea* sp., and a copepod crustacean were detected. Most of the parasites found on the grass carp do not kill healthy fish under good environmental conditions, though *Ichthyophthirius* sp. can lead to high mortality rates among grass carp and other fish species (Uzbülek and Yildiz 2002; Elser 1955; Wurtsbaugh and Alfaro 1988), but this was detected in only three sampled grass carp at a moderate level of infestation. Therefore, the high mortality rates among grass carp in the research area were not primarily attributable to parasites; however, the presence of external parasites could be considered a predisposing factor for the entry of secondary invading bacteria due to micro lesions or injuries to the skin and gills (Liu and Lu 2004; Xu *et al.* 2007; Busch *et al.* 2003; Berry *et al.* 1991).

Different bacterial species were isolated from the diseased grass carp using conventional bacteriological techniques and were identified morphologically and bio-chemically as motile *Aeromonads*, *Flavobacterium columnare*, *Vibrio* sp. and *Pseudomonas* sp. (Mayrhofer *et al.* 2011). These bacteria are well known facultative fish pathogens, able to cause the characteristic RSD symptoms.

Epidemiological investigations in the research area revealed pre-disposing and stress factors such as rapid changes in water temperature, high turbidity levels and low oxygen concentrations in the water (Steinbronn 2009); handling stress, malnutrition (Tuan *et al.* 2007), pollutants such as pesticides (Steinbronn *et al.* 2005) and the presence of detergents, all of which tend to suppress the immune systems of the fish and facilitate bacterial infections.

Virological investigations were performed using different fibroblastoid and epithelial cell lines, but no specific viruses related to grass carp mortality were found. The same results were also obtained by testing the samples against fish viruses related to Red Spot Disease, such as Grass Carp Hemorrhagic Virus (GCHV) (Qiya *et al.* 2003) and Rhabdovirus (Ahne 1975) utilizing universal and specific primers with polymerase chain reaction assays.

Furthermore, the presence of *Aphanomyces invadans*, an oomycete that is known to produce hemorrhagic changes in the skin and high losses in several fish species (OIE 2009), was not detected by Polymerase chain reaction (PCR) in any of the sampled fish.

Conclusion

From the results here it can be concluded that the grass carp disease in Yen Chau district in the north of Vietnam is a multifactorial disease which seems to be caused primarily by bacterial agents. It is known that most of the opportunistic bacteria, like that isolated from the fish samples, are able to affect fish only under poor environmental conditions such as high temperatures, a lack of oxygen, water turbidity and other factors that cause stress. Commonly used pesticides washed into the water can depress the immune system of the fish, leading to a decreased ability to fight disease (Shea and Berry 1984). In the research area, Anyusheva *et al.* (2012) measured the levels of pesticides in the water of ponds and rice fields considered toxic for common carp and Daphnia magna. It was found that pesticides were often transported from paddies into fish ponds shortly after pesticides had been applied on the rice paddies, due to high flow rates specific to the research area.

Due to the fact that isolated bacteria are ubiquitous opportunistic pathogens, it is recommended that the environmental conditions the fish live in should be improved, because stressors pre-dispose fish to suffer from bacterial borne diseases (Snieszko 1974; Ahmed and Rab 1995).

As a control measure, the conditions under which most fish are cultured and reared in the research area must be improved, and on this some general advice can be given in order to reduce the mortality rates found among grass carp. The regular exchange of water, as well as fish between the ponds, must be reduced or stopped completely to avoid the possibility of carrying over pathogens either via the water flow or via the fish. Stopping the water inflow will also reduce the turbidity of the water, and in cases where ponds are surrounded by steep slopes, it is advised that ditches should be dug around them to avoid sediments, pesticides and fertilizers being washed in after heavy rains. Removing mud from the bottom of the ponds, which contains large amounts of decomposing organic material and bacteria that contribute to oxygen depletion, would also be beneficial - increasing oxygen concentrations in the water. The use of washing powder in streams that enter the ponds should also be avoided and, while pesticides are being applied, the channels that connect paddy fields and ponds should be closed. To avoid the oral intake of pesticides, weeds from treated paddy fields should not be used as fish feed (Pucher *et al.* 2012a). In a comparative trial by Pucher *et al.* (2010a), improved management practices had a positive effect on the recapture rate of grass carp, even though no disease occurrence was observed over the seven months of the trial. Under the traditional pond management regime, 42 ± 24% of the stocked grass carp could not be recaptured, while under the modified management regime this figure was 26 ± 21%. However, it is not known whether the better survival rate under the modified pond management system in this study could be attributed to better water quality, a better feed base or a lower stocking density for the grass carp.

7.8 Outlook for further innovations in upland aquaculture and conclusion

In Chapter 8 we have described the local traditional pond management system used by the Black Thai households in Yen Chau district, and the importance of pond aquaculture for the group's food security and income generation. Given the limitations of this traditional aquaculture practice, a modified pond management system was designed, introduced into the research area and tested under local field conditions during action-research pond trials. During the trial, local fish production levels increased due to a change in the water regime and the use of higher quality inputs. Higher financial inputs into feed resources, as funded by proceeds from the harvest, led to greater net benefits being gained from aquaculture. However, the level of acceptability of this set of innovations to the local farmers was limited due to the need for constant financial inputs to be made (such as feed ingredients and fertilizers), as well as the additional knowledge required on feed preparations, feed ingredients storage, and feed and fertilizer handing. Better market access, both for trading pond products and for buying pond inputs, was also highlighted as a requirement. Aquaculture was named by only a few farmers as their main farming activity and most practiced aquaculture as a financial low-input farming activity and preferred to financially invest in other land uses, such as maize, paddy rice and cassava. To overcome the financial constraints in adopting aquaculture innovations, farmers may need financial assistance from micro-credit facilities or farmer cooperatives, plus they may need additional information via extension systems or fish farmer schools regarding good aquaculture practices in relation to marginal upland areas.

Further adjustments to the modified pond management system, those introduced to suit farmers' requirements, may also be needed. Firstly, the level of financial inputs required to introduce the new system must be reduced. During animal production activities, feed is generally known as the main expense, especially in the case of high protein feed ingredients, and the high protein feed ingredients used in these trials were heated soybean meal and fishmeal. However, to date, the availability of these ingredients has been limited in the research area, as it has across the marginal uplands, so there has been little demand. Even though Vietnamese markets are known to react rapidly to changing conditions, the demand for these resources will probably remain low in the future, because these feed ingredients are relatively costly in the local market and globally. Therefore, farmers are unlikely to invest in this fishmeal based fish feed, even though it would be to their net benefit. In the meantime, it is more than likely that competition for fishmeal will increase over time, both in Vietnam and worldwide, and prices will thus rise still further as fishmeal becomes a limited resource. To avoid fishmeal expenses, the use of earthworms is a promising alternative. The study here showed that an average household of Black Thai farmers in the research area produces enough organic waste to consider earthworms as a replacement for fishmeal in terms of supplemental feed (Müller *et al.* 2012). Earthworm meal was shown to be able to partly replace fishmeal in the diets of common carp under laboratory conditions (Tuan and Focken 2009), while under pond conditions, no significant difference was found between the performance of fish diets containing fishmeal or those containing earthworm meal, due to the simultaneous consumption of pond products such as zooplankton and zoobenthos (Pucher *et al.* 2012b). Further research is needed; however, in order to consider making technical modifications to the vermiculture techniques used, such as improving the worm harvest, developing optimal techniques for the use of worms in aquafeeds, and carrying out investigations into the alternative on-farm organic materials that could be used.

Fertilization using organic and inorganic fertilizer in static water requires the use of an optimal fertilizing scheme to help reduce the risk of nutrient accumulation in the sediments and of algae mass blooms and algae mass die-offs occurring. A precautionary fertilizing scheme adapted to local soil, climate and water conditions is needed for diluting pond water in cases of emergency, especially in areas with limited water resources.

Another possible innovation relates to the supply of common carp for stocking the fish ponds. Currently, common carp partly originate from hatcheries and partly from spontaneous reproduction within the fish ponds. Spontaneous reproduction of common carp and tilapia is unpredictable in terms of timing and out-comes; therefore, farmers have little advance information on their fish stocks and fish may be rather small at the end of the annual growing season, resulting in high mortality rates during the cold months. Some farmers could produce common carp fry on their own, by adapting the system of spawning ponds (Dubisch ponds) practiced in Europe for more than a century. This would allow the local production of common carp fry and fingerlings, and thereby, help farmers to stock their ponds according to their own demands; not governed by the erratic supply provided by the traders. By supplying farmers with good, virus-free brood stock, such decentralized fry production techniques could help to avoid the spread of CyHV that occurs when using the provincial hatcheries (see Section 8.5).

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7.9 References

Abdelghany AE, Ahmad MH (2002) Effects of feeding rates on growth and production of Nile tilapia, common carp and silver carp polycultured in fertilized ponds. Aquaculture Research 33:415-423

Acharya L (2011) Innovation history of fish pond reforms in Chieng Khoi district, Son La province, Vietnam (unpublished). University of Hohenheim, Stuttgart, Germany

Acharya L, Pucher J, Schad I, Focken U, Hoffmann V (2011) Intensifying fish pond business - An interdisciplinary innovation study on information needs of Black Thai farmers in Chieng Khoi commune, Vietnam. Tropentag 2011 "Development on the margin", Bonn, Germany, 5-7 October 2011

Ahmed M, Rab MA (1995) Factors affecting outbrakes of epizootic ulcerative syndrome in farmed fish in Bangladesh. Journal of Fish Diseases 18:263-271

Ahne W (1975) Arhabdovirus isolated from grass carp (*Ctenopharyngodon idella*). Archives of Virology 48:181-185

Alcaraz G, Rosas C, Espina S (1993) Effect of detergent on the response to temperature and growth of grass carp, *Ctenopharyngodon idella*. Bulletin of Environmental Contamination and Toxicology 50:659-664

Anyusheva M, Lamers M, La N, Nguyen VV, Streck T (2012) Fate of pesticides in combined paddy rice–fish pond farming systems in Northern Vietnam. Journal of Environmental Quality 41(2):515–525.

Barik SK, Mishra S, Ayyappan S (2000) Decomposition patterns of unprocessed and processed lignocellulosics in a freshwater fish pond. Aquatic Ecology 34:185-204

Berry CR, Babey GJ, Shrader T (1991) Effect of *Lernaea cyprinacea* (Crustacea: Copepoda) on stocked rainbow trout (*Oncorhynchus mykiss*). Journal of Wildlife Diseases 27:206-213

Black KD (1998) The environmental interactions associated with fish culture. In: Black KD, Pickering AD (eds) Biology of farmed fish. Sheffield Academic Press, Canada, pp 284-325

Boyd CE (1982) Water quality management for pond fish culture. In: Developments of aquaculture and fisheries science. 9th edn. Elsevier, Amsterdam, Oxford, New York, p 318

Busch S, Dalsgaard I, Buchmann K (2003) Concomitant exposure of rainbow trout fry to *Gyrodactylus derjavini* and *Flavobacterium psychrophilum*: Effects on infection and mortality of host. Veterinary Parasitology 117(1-2):117-122

Cagauan AG (2001) Water quality management for freshwater fish culture. In: Utilizing different aquatic resources for livelihoods in Asia, a resource book. International Institute of Rural Reconstruction, International Development Research Centre, Food and Agriculture Organization of the United Nations, Network for Aquaculture Centers in Asia-Pacific and International Center for Living Aquatic Resources Management, Cavite, Philippines, pp 294-299

Chervinski J (1982) Environmental Physiology of Tilapias. In: Pullin RSV, Lowe-McConnell RH (eds.) The biology and culture of tilapias. ICLARM Conference Proceedings 7, 432 p. International Center for Living Aquatic Resources Management, Manila, Philippines, pp 119-128

Chumchal MM, Drenner RW (2004) Interrelationships between phosphorus loading and common carp in the regulation of phytoplankton biomass. Archiv für Hydrobiologie 161:147-158

Chumchal MM, Nowlin WH, Drenner RW (2005) Biomass-dependent effects of common carp on water quality in shallow ponds. Hydrobiologia 545:271-277

Cui Y, Liu X, Wang S, Chen S (1992) Growth and energy budget in young grass carp, *Ctenopharyngodon idella* Val., fed plant and animal diets. Journal of Fish Biology 41:231-238

Cui Y, Chen S, Wang S (1994) Effect of ration size on the growth and energy budget of the grass carp, *Ctenopharyngodon idella* Val. Aquaculture 123:95-107

Dan NC, Little DC (2000) Overwintering performance of Nile tilapia *Oreochromis niloticus* (L.) broodfish and seed at ambient temperatures in Northern Vietnam. Aquaculture Research 31:485-493

De Silva SS (1995) Supplemental feeding in semi-intensive aquaculture systems. In: New MB, Tacon AGJ, Csavas I (eds) Farm-made aquafeed. FAO Fisheries Technical Paper 343. FAO, Rome, pp 24-60

Degani G, Yehuda Y, Viola S, Degani G (1997) The digestibility of nutrient sources for common carp, *Cyprinus carpio* Linnaeus. Aquaculture Research 28:575-580

Diaz F, Espina S, Rodriguez C, Soto F (1998) Preferred temperature of grass carp, *Ctenopharyngodon idella* (Valenciennes), and brema carp, *Megalobrama amblycephala* (Yih), (Pisces, Cyprinidae) in horizontal and vertical gradients. Aquaculture Research 29:643-648

Dongmeza E (2009) Studies on the nutritional quality of plant materials used as fish feed in Northern Vietnam. Dissertation, University of Hohenheim, Stuttgart, Germany

Dongmeza E, Steinbronn S, Francis G, Focken U, Becker K (2009) Investigations on the nutrient and antinutrient content of typical plants used as fish feed in small scale aquaculture in the moutainous regions of Northern Vietnam. Animal Feed Science and Technology 149:162-178

Dongmeza E, Francis G, Steinbronn S, Focken U, Becker K (2010) Investigation on the digestibility and metabolizability of the major nutrients and energy of maize leaves and barnyard grass in grass carp (*Ctenopharyngodon idella*). Aquaculture Nutrition 16(3):313-326

Dung PTM, Minh LN (2010) Improved household financial literacy as a way to sustainability – initial impacts from book-keeping model in My Duc and potential expansion. International Symposium "Sustainable Land Use and Rural Development in Mountainous Regions of Southeast Asia", Hanoi, Vietnam, 21-23 July 2010

Edwards P (2000) Aquaculture, poverty impacts and livelihoods, vol 56. ODI Natural Resource Perspectives, London, UK

Edwards P, Pullin RSV, Gartner JA (1988) Research and education for the development of integrated crop-livestock-fish farming systems in the tropics. ICLARM Studies and Reviews 16. International Center for Living Aquatic Resources Management, Manila, Philippines

Edwards P, Kaewpaitoon K, Little DC, Siripandh N (1994a) An assessment of the role of buffalo manure for pond culture of tilapia. II. Field trial. Aquaculture 126:97-106

Edwards P, Pacharaprakiti C, Yomjinda M (1994b) An Assessment of the role of buffalo manure for pond culture of tilapia. I. On-station experiment. Aquaculture 126:83-95

Edwards P, Demaine H, Innes-Taylor N, Turongruang D (1996) Sustainable aquaculture for small-scale farmers: need for a balanced model. Outlook on Agriculture 25:19-26

Eknath AE, Doyle RW (1985) Indirect selection for growth and life-history traits in Indian carp aquaculture. 1. Effects of broodstock management. Aquaculture 49:73-84

Elser HJ (1955) An epizootic of ichthyophthiriasis among fishes in a large reservoir. Progressive Fish Culturist 17(3):132-133

FAO (2006a) National Aquaculture Sector Overview. Viet Nam. National Aquaculture Sector Overview Fact Sheets. Text by Nguyen, T.P. & Truong, H.M. In: FAO Fisheries and Aquaculture Department [online]. Rome.

http://www.fao.org/fishery/countrysector/naso_vietnam/en. Accessed 2 February 2012

FAO (2006b) Search aquaculture fact sheets: cultured aquatic species. http://www.fao.org/fishery/culturedspecies/search/en. Accessed 30th of August 2006

FAO (2011) Aquaculture production 1950 – 2009. (Data set for Fishstat Plus) Fisheries Department, Fishery Data and Statistics Unit.

FAO (2012) Food balance sheets. Vietnam. http://faostat.fao.org/site/368/default.aspx#ancor. Accessed 22 Jan 2012

Francis G, Kerem Z, Makkar HPS, Becker K (2002) The biological action of saponins in animal systems – a review. British Journal of Nutrition 88(6):587-605

Francis G, Makkar HPS, Becker K (2001a) Antinutritional factors present in plant-derived alternate fish feed ingredients and their effects in fish. Aquaculture 199:197-227

Francis G, Makkar HPS, Becker K (2001b) Dietary supplementation with a *Quillaja* saponin mixture improves growth performance and metabolic efficiency in common carp (*Cyprinus carpio* L.). Aquaculture 203:311-320

Friederichsen JR (2008) Opening up knowledge production through participatory research? Agricultural research for Vietnam's Northern uplands. Peter Lang, Frankfurt a.M.

GSO (2004) Result of the survey on household living standards 2002. Statistical Publishing House Hanoi, Vietnam

Hajra A, Tripathi SD, Nath D, Chatterjee G, Karmakar HC (1987) Comparative digestibility of dietary plant fibre in grass carp, *Ctenopharyngodon idella* (Val.). Proceedings of the Na-tional Academy of Science India 57:231-236

Hepher B, Pruginin Y (1981) Commercial Fish Farming: With Special Reference to Fish Culture in Israel. Wiley-Interscience, John Wiley & Sons, New York

Hoffmann V, Gerster-Bentaya M, Christinck A, Lemma M (2009) Rural Extension, Volume 1: Basic Issues and Concepts. Margraf Publishers, Weikersheim, Germany

Jana BB, Sahu SN (1993) Relative performance of three bottom grazing fishes (*Cyprius carpio*, *Cirrhinus mrigala*, *Heteropneustes fossilis*) in increasing the fertilizer value of phosphate rock. Aquaculture 115:19-29

Kaushik SJ (1995) Nutrient requirements, supply and utilization in the context of carp culture. Aquaculture 129:225-241

Kohlmann K, Gross R, Murakaeva A, Kersten P (2003) Genetic variability and structure of common carp (*Cyprinus carpio*) populations throughout the distribution range inferred from allozyme, microsatellite and mitochondrial DNA markers. Aquatic Living Resources 16:421-431

Kolar CS, Rahel FJ (1993) Interaction of a biotic factor (predator presence) and an abiotic factor (low oxygen) as an influence on benthic invertebrate communities. Oecologia 95:210-219

Kumar MS, Burgess SN, Luu LT (2004a) Review of nutrient management in freshwater polyculture. Journal of Applied Aquaculture 16:17-44

Kumar MS, Luu LT, Ha MV, Dieu NQ (2004b) The nutrient profile in organic fertilizers: Biological response to nitrogen and phosphorus management in tanks. Journal of Applied Aquaculture 16:45-60

Kumar MS, Binh TT, Burgess SN, Luu LT (2005a) Evaluation of optimal species ratio to maximize fish polyculture production. Journal of Applied Aquaculture 17(1):35-49

Kumar MS, Binh TT, Luu LT, Clarke SM (2005b) Evaluation of fish production using organic and inorganic fertilizer: Application to grass carp polyculture. Journal of Applied Aquaculture 17:19-34

Lamers M, Anyusheva M, La N, Nguyen VV, Streck T (2011) Pesticide pollution in surface- and groundwater by paddy rice cultivation: A case study from Northern Vietnam (short communication). Clean – Soil, Air, Water 39: 356-361.

Lemke U, Kaufmann B, Thuy LT, Emrich K, Valle Zárate A (2006) Evaluation of smallholder pig production systems in North Vietnam: Pig production management and pig performances. Livestock Science 105:229-243

Ling SW (1977) Aquaculture in Southeast Asia, a historical overview. University of Washington Press, Seattle and London

Liu YJ, Lu CP (2004) Role of Ichthyophthirius multifiliis in the infection of Aeromonas hydrophila. Journal of Veterinary Medicine Series B: Infectious Diseases and Veterinary Public Health 51:222-224

Luu LT, Trang PV, Cuong NX, Demaine H, Edwards P, Pant J (2002) Promotion of small-scale pond aquaculture in the Red River Delta, Vietnam. In: Edwards P, Little DC, Demaine H (eds) Rural Aquaculture. CABI Publishing Oxon, UK and New York, USA, pp. 55-75

Mayrhofer R, Soliman H, Pucher J, Focken U, El-Matbouli M (2011) Grass carp mortalities in Northern Vietnam - Investigations from 2008-2010. 15th International Conference on Diseases of Fish and Shellfish, Split, Croatia, 12-15 September 2011. European Association of Fish Pathologists

Milstein A, Wahab MA, Rahman MM (2002) Environmental effects of common carp *Cyprinus carpio* (L.) and mrigal *Cirrhinus mrigala* (Hamilton) as bottom feeders in major Indian carp polycultures. Aquaculture Research 33:1103-1117

Muendo PN, Milstein A, Dam AAv, Gamal E-N, Stoorvogel JJ, Verdegem MCJ (2006) Exploring the trophic structure in organically fertilized and feed-driven tilapia culture environments using multivariate analyses. Aquaculture Research 37:151-163

Müller JL, Pucher J, Tran TNT, Focken U, Kreuzer M (2012) The potential of vermiculture to produce on-farm feed resources for aquaculture in mountainous areas of North Vietnam. International Scientific Conference "Sustainable Land Use and Rural Development in Mountain Areas", University of Hohenheim, Stuttgart, Germany, 16-18 April 2012

Nandeesha MC, Keshavanath P, Dinesh KR (1984) Effect of three organic manures on plankton production in fish ponds. Environment and Ecology 2(4):311-317

OIE (2009) Epizootic ulcerative syndrome. In: OIE (ed) Aquatic Manual. 6th Edition. Office Internationale des Epizooties, Paris, p Chapter 2.3.2

Pomeroy KE, Shannon JP, Blinn DW (2000) Leaf breakdown in a regulated river: Colorado River, Arizona, USA. Hydrobiologia 434:193-199

Prein M (2002) Integration of aquaculture into crop-animal systems in Asia. Agricultural Systems 71:127-146

Prein M, Ahmed M (2000) Integration of aquaculture into smallholder farming systems for improved food security and household nutrition. Food and Nutrition Bulletin 21:466-471

Pucher J, Mayrhofer R, El-Matbouli M, Focken U (2010a) Improvements in pond management and application of low-cost fish feed increase fish production and raise the benefit of small scale aquaculture systems in Yen Chau. International Symposium "Sustainable Land Use and Rural Development in Mountainous Regions of Southeast Asia", Hanoi, Vietnam, 21-23 July 2010

Pucher J, Mayrhofer R, Hung TQ, El-Matbouli M, Focken U (2010b) Growth functions of fish species cultured in small scale upland aquaculture systems under traditional and modified pond management schemes in Yen Chau, Northern Viet Nam. Tropentag 2010 "World Food System – A Contribution from Europe", Zurich, Switzerland, 14-16 September 2010

Pucher J, Mayrhofer R, El-Matbouli M, Focken U (2011a) Effect of supplemental feeding practice in the culture of common carp in dependency on natural food availability. 9th Asian Fisheries and Aquaculture Forum, Shanghai, China, 21-25 April 2011

Pucher J, Mayrhofer R, El-Matbouli M, Focken U (2011b) Interaction of natural food and supplemental feeding for common carp in semi-intensively managed ponds in the marginal uplands of Son La province, Northern Vietnam. Tropentag 2011 "Development on the margin", Bonn, Germany, 5-7 October 2011

Pucher J, Gut T, Mayrhofer R, Lamers M, Streck T, El-Matbouli M, Focken U (2012a) Hydrophobic pesticides on feed material for grass carp: Toxicology and accumulation in fish. International Scientific Conference "Sustainable Land Use and Rural Development in Mountain Areas", University of Hohenheim, Stuttgart, Germany, 16-18 April 2012

Pucher J, Tuan NN, Yen TTH, Mayrhofer R, El-Matbouli M, Focken U (2012b) Earthworm meal as alternative animal protein source for full and supplemental feeds for Common Carp (*Cyprinus carpio* L.). International Scientific Conference "Sustainable Land Use and Rural Development in Mountain Areas", University of Hohenheim, Stuttgart, Germany, 16-18 April 2012

Qiya Z, Hongmei R, Zhenqiu L, Jianfang G, Jing Z (2003) Detection of grass carp hemorrhage virus (GCHV) from Vietnam and comparison with GCHV strain from China. High technology letters 9:7-13

Rahman MM, Meyer CG (2009) Effects of food type on diel behaviours of common carp *Cyprinus carpio* in simulated aquaculture pond conditions. Journal of Fish Biology 74:2269-2278

Rahman MM, Jo Q, Gong YG, Miller SA, Hossain MY (2008a) A comparative study of common carp (*Cyprinus carpio* L.) and calbasu (*Labeo calbasu* Hamilton) on bottom soil resuspension, water quality, nutrient accumulations, food intake and growth of fish in simulated rohu (*Labeo rohita* Hamilton) ponds. Aquaculture 285:78-83

Rahman MM, Nagelkerke LAJ, Verdegem MCJ, Wahab MA, Verreth JAJ (2008b) Relationships among water quality, food resources, fish diet and fish growth in polyculture ponds: A multivariate approach. Aquaculture 275:108-115

Rai S, Shrestha P (2006) Guidelines to participatory innovation development. PROLINNOVA Nepal Programme, Kathmandu

Reardon T (1995) Sustainability issue for agricultural research strategies in semi-arid tropics: Focus on the Sahel. Agriculture Systems 48:345-359

Reinhardt N, Gut T, Lamers M, Streck T (2012) Water Regime in paddy rice systems in Vietnam: Importance of infiltration and bund flow. In: Stahr K, Fröhlich H, Clemens G, Schreinemachers P (eds.) International Scientific Conference "Sustainable Land Use and Rural Development in Mountain Areas", University of Hohenheim, Stuttgart, Germany, 16-18 April 2012.

Rogers E (2003) Diffusion of Innovations.5th Edition edn. Free Press, New York

Ross LG (2000) Environmental physiology and energetics. In: Beveridge MCM, McAndrew BJ (eds) Tilapias: biology and exploitation. Kluwer Academic Publishers, Great Britain, pp 89-128

Ruddle K, Christensen V (1993) An energy flow model of the mulberry dike-carp pond farming system of the Zhujiang Delta, Guangdong Province, China. In: Christensen V, Pauly De (eds) Trophic models of aquatic ecosystems. ICLARM Conference Proceedings 26. International Center for Living Aquatic Resources Management, Manila, p 390

Schad I, Roessler R, Neef A, Zarate AV, Hoffmann V (2011) Group-based learning in an authoritarian setting? Novel extension approaches in Vietnam's Northern uplands. Journal of Agricultural Education and Extension 17:85-98

Schad I, Schmitter P, Saint-Macary C, Neef A, Lamers M, Nguyen L, Hilger T, Hoffmann V (2012) Why do people not learn from flood disasters? Evidence from Vietnam's North-western mountains. Natural Hazards 62:221-241

Schmitter P, Dercon G, Hilger T, Ha TTL, Thanh NH, Lam N, Vien TD, Cadisch G (2010) Sediment induced soil spatial variation in paddy fields of Northwest Vietnam. Geoderma 155:298-307

Schmitter P, Dercon G, Hilger T, Hertel M, Treffner J, Lam N, Vien TD, Cadisch G (2011) Linking spatio-temporal variation of crop response with sediment deposition along paddy rice terraces. Agriculture, Ecosystems and Environment 140:34-45

Schroeder GL, Wohlfarth G, Alkon A, Halevy A, Krueger H (1990) The dominance of algal-based food webs in fish ponds receiving chemical fertilizers plus organic manures. Aquaculture 86:219-229

Sevilleja R, Torres J, Sollows J, Little D (2001) Using animal wastes in fishponds. In: Integrated agriculture-aquaculture, a primer. Fisheries Technical Paper No. 407. FAO, Rome, Italy

Shea TB, Berry ES (1984) Suppression of interferon synthesis by the pesticide carbaryl as a mechanism for Enhancement of Goldfish Virus-2 Replication. Applied and Environmental Microbiology 47:250-252

Shireman JV, Colle DE, Rottmann RW (1978) Growth of grass carp fed natural and prepared diets under intensive culture. Journal of Fish Biology 12:457-463

Sifa L, Chenhong L, Dey M, Gagalac F, Dunham R (2002) Cold tolerance of three strains of Nile tilapia, *Oreochromis niloticus*, in China. Aquaculture 213(1-4):123-129

Sinha VRP, Gupta MV (1975) On the growth of grass carp, *Ctenopharyngodon idella* Val. in composite fish culture at Kalyani, west Bengal (India). Aquaculture 5:283-290

Snieszko SF (1974) The effects of environmental stress on outbreaks of infectious diseases of fishes. Journal of Fish Biology 6:197-208

Spataru P, Wohlfarth GW, Hulata G (1983) Studies in the natural food of different fish species in intensively manured polyculture ponds. Aquaculture 35:283-298

Steinbronn S (2009) A case study: Fish production in the integrated farming system of the Black Thai in Yen Chau district (Son La province) in mountainous North-Western Vietnam - Current state and potential. Dissertation, University of Hohenheim, Stuttgart, Germany

Steinbronn S, Geiss C, Fangmeier A, Tuan NN, Focken U, Becker K (2005) The use of pesticides in paddy rice and possible impacts on fish farming in Yen Chau / Son La province / Northern Vietnam. Deutscher Tropentag 2005 “The Global Food & Product Chain - Dynamics, Innovations, Conflicts, Strategies”, Hohenheim, Stuttgart, Germany, 11-13 October 2005

Sugita H, Takayama M, Ohkoshi T, Deguchi Y (1992) Occurrence of microaerophilic bacteria in water and sediment of grass carp culture pond. Aquaculture 103:135-140

Svobodova Z, Lloyd R, Machova J, Vykusova B (1993) Water quality and fish health. FAO, Rome, Italy

Tacon AGJ (1997) Contribution to food fish supplies. In: Department FF (ed) Review of the State of World Aquaculture, FAO Fisheries Circular No. 886. Rome, Italy. http://www.fao.org/docrep/003/w7499e/w7499e00.htm

Tacon AGJ, De Silva SS (1997) Feed preparation and feed management strategies within semi-intensive fish farming systems in the tropics. Aquaculture 151:379-404

Tan YT (1970) Composition and nutritive value of some grasses, plants and aquatic weeds tested as diets. Journal of Fish Biology 2:253-257

Thai BT, Pham TA, Austin CM (2006) Genetic diversity of common carp in Vietnam using direct sequencing and SSCP analysis of the mitochondrial DNA control region. Aquaculture 258:228–240

Thai BT, Burridge CP, Austin CM (2007) Genetic diversity of common carp (*Cyprinus carpio* L.) in Vietnam using four microsatellite loci. Aquaculture 269:174-184

Tripathi SD, Mishra DN (1986) Synergistic approach in carp polyculture with grass carp as a major component. Aquaculture 54:157-160

Tuan NN (2010) Development of supplemental diets for carp in Vietnamese upland ponds based on locally available resources. Dissertation, University of Hohenheim, Stuttgart, Germany

Tuan NN, Focken U (2009) Earthworm powder as potential protein source in diets for common carp (*Cyprinus carpio* L.). Tropentag 2009 "Biophysical and Socio-economic Frame Conditions for the Sustainable Management of Natural Resources". Hamburg, Germany, 5-8 October 2009

Tuan NN, Steinbronn S, Dongmeza E, Dung B, Focken U, Becker K (2007) Growth and feed conversion of the grass carp (*Ctenopharyngodon idella*) fed on fresh plant material under laboratory conditions in Viet Nam (Poster). Tropentag 2007 "Resource use efficiency and diversity in agroecosystems", Witzenhausen, Germany, 9 -11 October 2007

Tuan VD, Thach NV, Phuong HV, Hilger T, Keil A, Clemens G, Zeller M, Stahr K, Lam NT, Cadisch G (2010) Fostering rural development and environmental sustainability through integrated soil and water conservation systems in the uplands of Northern Vietnam.

International Symposium "Sustainable Land Use and Rural Development in Mountainous Regions of Southeast Asia", Hanoi, Vietnam, 21-23 July 2010

Ufodike EBC, Matty AJ (1983) Growth response and nutrient digestibility in mirror carp (*Cyprinus carpio*) fed different levels of cassava and rice. Aquaculture 31:41-50

Uzbülek MK, Yildiz HY (2002) A report on spontaneous diseases in the culture of grass carp (*Ctenopharyngodon idella* Val. 1844), Turkey. Turkish Journal of Veterinary and Animal Sciences 26:407-410

Van Dam AA, Chikafumbwa FJKT, Jamu DM, Costa-Pierce BA (1993) Trophic interactions in a napier grass (*Pennisetum purpureum*)-fed aquaculture pond in Malawi. In: Christensen V, Pauly D (eds.) Trophic models of aquatic ecosystems. ICLARM Conference Proceedings 26. International Center for Living Aquatic Resources Management, Manila, p 390

Van PT, Khoa LV, Lua DT, Van KV, Ha NT (2002) The impacts of red spot disease on small-scale aquaculture in Northern Vietnam. In: Arthur JR, Phillips MJ, Subasinghe RP, Reantaso MB, MacRae IH (eds) Primary aquatic animal health care in rural, small-scale, aquaculture development. Fisheries Technical Paper No. 406. FAO, Rome, pp 165-176

Wurtsbaugh WA, Alfaro R (1988) A mass mortality of fishes in Lake Titicaca (Peru-Bolivia) associated with the parasite *Ichthyophthirius multifiliis*. Transactions of the American Fisheries Society 117:213-217

Xu DH, Shoemaker CA, Klesius PH (2007) Evaluation of the link between gyrodactylosis and streptococcosis of Nile tilapia, *Oreochromis niloticus* (L.). Journal of Fish Diseases 30:233-238

Yang X, Zuo W (1997) Relationship between water temperature and immune response of grass carp (*Ctenopharyngodon idellus* C. et V.). Asian Fisheries Science 10:169-177

8 General discussion

8.1 Applied methodologies

The research was carried out in a remote area (Yen Chau district, Son La province, Northern Vietnam) and the collaborating small-scale farerms belonged mainly to the Black thai ethnic minority. In such remote regions not all people are able to speak Vietnamese which may lead to misunderstandings in the work with them not only due to lingual miscommunication but also due to cultural differences (Vien 2003). Therefore, a proper cultural understanding and close contact to the local collaborators is essential both for the scientists as well as for their translators. During our studies, students and former students from the Hanoi University of Agriculture acted as both translators and field assistants. These students played a major role in the success of the research as they had to integrate into the culture of the ethnic minorities and create trust and a willingness to collaborate with the researchers. The role of these assistants in field research should be evaluated in future. Only in this way, the close collaboration over the three years of this study was able to generate valuable data on the suitability of modifications on pond management under the environmental and cultural circumstances of the remote upland regions.

In the study area, no proper laboratory was available and access to electricity and the internet was very erratic. Therefore, simple and robust methods and equipment were chosen for these studies to assure the quality and usability of the data. Access to high quality standards for calibrations (e.g. pH electrode, oxygen electrode, redox potential electrode) was very limited and they had to be imported from Germany. All analytical drying, freeze-drying and weighing was performed in suitable laboratories in Hanoi. Following analyses (proximate composition and isotopic analysis) were performed at the University of Hohenheim. Pestizide analysis were performed at the Research Center for Environmental Technology and Sustainable Development of the Hanoi University of Science.

For pellet feed production, ingredients were locally purchased by farmers, except for fishmeal. The fishmeal available in the uplands is of poor quality, is not traded in sufficient quantity and therefore needed to be purchased from feed companies in Hanoi. Simple mincing machines were used to produce the pellets following solar drying of the material. During rainy periods, drying was done using electrical heaters.

In Chapter 2, it was concluded that 1) Fish fed material contaminated with pesticides for a limited period of time (10 days, resembling farmers´ pesticide application practices) suffered measurable negative health effects and their feed uptake was reduced and 2) Pesticides taken up by fish via contaminated feeding material during a pesticide application campaign accumulate in fish flesh and thereby pose a potential threat to human health. To evaluate the real extent of this threat, future studies on the effects of orally applied pesticides on fish health and production should focus on actual scenarios in several ponds including the seasonal and spatial variations that occur over the complete production cycle. Further, these investigations might be linked to data on pesticide contaminated fish in markets as performed by Hoai *et al.* (2011).

Chapters 3 and 4 describe a comparison of two management systems, traditional pond management and modified pond management. Several limitations of the local traditional pond management had previously been identified (Dongmeza 2009; Dongmeza *et al.* 2009; Steinbronn 2009; Dongmeza *et al.* 2010). In an attempt to counteract these limitations and improve pond yields a set of changes were made to the traditional pond management and the modified regime was tested against the traditional one under field conditions. These changes included alterations in water management, the use of higher quality fertilization, application of basic pond hygiene practices, changes in species composition, and the application of supplemental pellet for omnivorous fish species. This set of changes was tested for its effect on limnological parameters, the abundance of natural food resources, growth performance of fish, nutrient utilization efficiency and the net benefit in comparison to the traditional pond management. An alternative to testing the effect of this ´package` of changes would have been to conduct a series of experiments, each one of which would have measured the effect of only one change in management. We rejected this approach as unfeasible given the limited resources available and because several effects of single management changes have already been described in the literature (e.g. Wohlfarth and Schroeder 1979; Schroeder *et al.* 1990; Milstein *et al.* 1991; Zoccarato *et al.* 1995; Tacon and De Silva 1997; Condie and Webster 2002; Chumchal and Drenner 2004; Glaholt Jr and Vanni 2005; Kumar *et al.* 2005b) and several changes make sense only in combination, e.g improved fertilization and control of water flow. It was also felt that other single effects could probably be better evaluated under more controlled conditions such as in laboratories, mesocosms or experimental ponds. In the experiment presented here (Chapter 3 and 4) the changes were combined so as to give a set of changes that would interact to improve local fish production. This holistic approach was tested in ponds for scientific reasons and as a model that could be observed by by farmers wishing to improve their production system. The use of simple methods for the budgeting of pond in- and outputs (daily recording and weighing) were both, easly applicable to farmers, which makes these data more reliable and accurate, and more comprehensible to the farmers. The measuring of the inflow rates was performed by buckets. Continuous measurement via pressure sensors or water clocks were applied in single ponds (unpublished data). But the plus in information was felt not to be in balance with the addititional investement in time and equipement to apply continuous inflow rate measurements.

Chapter 5 reports the results of a tracer experiment to study the nitrogen (N) dynamic in the natural food webs of the two pond managements over a period of one week. A search of the literature failed to provide any guidelines as to appropriate dosage levels of tracer in such a relatively small-scale and short-term experiment so we had to base the amounts applied on those that had been used in studies performed in larger lakes, rivers and aquaculture systems (Burford *et al.* 2002; Merriam *et al.* 2002; Epstein *et al.* 2012). Unfortunately, it turned out that these amounts of marker were well suitable to study long-term efffects on higher trophic levels, but were too high to accurately determine short-term effects in small plankton. The uptake rates and recovery rates in the smallest fractions of plankton within the first few days after application were much higher than expected and the resulting high absolute values of atom percent (At%) of ^{15}N in these small fractions could not be measured accurately because they were outside the calibration range of the method. Furthermore, the amounts of these fractions obtainable from the two 50 L samples of pond water were too little to allow a replication of the analysis.

However, the potential errors associated with these extremely high absolute values do not detract from the significance of the comparisons that can be made between the N dynamics under the different pond managements nor between the measured uptake and turnover rates of the plankton fractions. Although the amounts of ^{15}N applied for the week-long tracing experiment in the natural food webs in pond proved to be too high, this high amount of tracer applied enabled the measurement of ^{15}N at levels that could be accurately measured and provided useful information on N flow into fish body mass for a period of one month (unpublished data).

In the net-cage feeding experiment (Chapter 6), the fish were cultured under pond conditions that are typical for the study area. The fish performed rather poorly, probably because of the environment within the net-cages. It might be worthwhile to repeat this experiment under more controlled conditions in an experimental facility without the disturbing activities (e.g. fishing, bathing buffalos, and predation) that occurred in the study pond. In this study area, it might also be a good idea to perform a scaled-up version of the experiment in several ponds belonging to local farmers to compare the effects on fish growth of supplemental test feeds containing different levels of fishmeal and earthworm meal.

8.2 Semi-intensification of pond aquaculture in mountainous Northern Vietnam

The transition of grass carp-dominated traditional pond aquaculture towards a common carp-dominated semi-intensive pond aquaculture using locally available higher qualitative fertilizer and feed resources than traditionally used and a restricted water inflow scheme did improve water quality, oxygen supply, natural food availability, fish growth, fish yield and financial net benefit (Chapter 3, 4 and 5). In particular, the filter feeding fish species, silver carp and bighead carp, showed significantly higher growth rates and higher yield under modified pond management. Common carp had a significantly higher production and grass carp had significantly higher growth rates (Chapter 4). The proximate composition of fish under semi-intensive pond management suggests that these fish enjoyed an improved nutritional status compared to fish under traditional management (Chapter 4). Generally, the efficiencies with which feeds and fertilizers were transformed into fish biomass were higher under modified pond management (Chapter 4). The change from water flow-through under traditional pond management towards a more static water regime reduced the impact of surrounding land use on ponds and may turn aquaculture under semi-intensive pond management into a more predictable and profitable farming activity with reduced risks of high fluctuations in water quality and natural food availability.

Water management and turbidity

In Northern Vietnam, ponds are well integrated into the rice paddy systems and use the same water distribution systems such as natural rivers, creeks or man-made channel systems that receive water from lakes, rivers and reservoirs. Ponds and paddy rice fields are often connected to each other and receive water at similar rates. In the trial ponds under traditional pond management described here (Chapter 3 and 5), flow rates were similar to those reported by two previous studies in the research area with flow rates of 120±40 L m^{-2} d^{-1} (Anyusheva *et al.* 2012) and 34-202 L m^{-2} d^{-1} (Steinbronn 2009). In ponds, the continuous water flow-through was reported to increase nutrient loss from the ponds through reduced retention times (Demir and Kirkagac 2005; Nhan *et al.* 2008a, b). The water flowing into the paddies and ponds was reported to transport eroded particles and nutrients into the ponds and paddy fields while the inflowing water varied in concentrations of total suspended solids and nutrient loads as these parameters are highly dependent on the season, severity of erosion on fields upstream and weather conditions, such as intensity of rain (Schmitter *et al.* 2010a; Schmitter *et al.* 2012). Although the introduction of eroded particles and suspended nutrients had been shown to have a fertilizing effect in rice paddies (Schmitter *et al.* 2010a, b), the high total suspended solid concentration of inflowing water in traditionally managed ponds caused average water transparencies of 30 cm secchi disc depth (Steinbronn 2009; Chapter 3), which is considered to be turbid (Sevilleja *et al.* 2001). The low transparency of pond water under traditional management reduced light penetration and had deleterious effects on physico-chemical water parameters (Chapter 3). Turbid ponds absorbed solar radiation in the top water layers of the ponds resulting in considerable stratification during the day. This effect has been shown to occur particularly in small and shallow turbid ponds (Condie and Webster 2002). The strong stratification under traditional pond management was not disrupted by turbulence caused by constantly inflowing water.

Under semi-intensive pond management tested here, changes in water management reduced both the introduction of eroded particles and the loss of nutrients. Therefore, measures to control surface water inflow were implemented. Channels were dug round the ponds to prevent ingress of runoff water from the surrounding fields and thus reduce the introduction of eroded particles. These changes in water management resulted in a reduction of turbidity (Secchi disc depth of on average 97 cm under modified compared to 28 cm under traditional management), reduced concentration of inorganic suspended particle load and allowed a stronger phototrophic top layer to develop in the ponds (Chapter 3).

However, seepage of water was not inhibited under either pond management strategies. This could have led to nutrient leakage by horizontal water flow through the dikes or, to a lesser extent, by vertical flow into the ground water as has been shown to occur in some situations in rice paddies (Reinhardt *et al.* 2012). Proper sealing and strengthening of dikes may help to control these losses. Some farmers in the study area had already invested time and resources into strengthening the dikes with the primary aim of increasing the stability of dikes during floods which had been occurring with increasing frequency in the uplands (Schad *et al.* 2012). However, the effects of lining the dikes have not yet been studied with respect to their effects on limnology and economy.

Water quality

In the study area, the water quality of traditionally managed ponds was affected by the surrounding land use, which altered the rate of input both of nutrients and of eroded particles by inflowing water. Even though substantial amounts of N were added to the ponds via feeds and fertilizers under traditional pond management, the concentrations of dissolved N compounds and total N (TN) in the pond water did not differ from those in the water flowing in from the reservoir and channels (Fröhlich *et al.* 2013). Also the higher input of nutrients via fertilizer under modified pond management in static ponds did not result in higher levels of dissolved N compounds in ponds (Chapter 3). This suggests (a) high uptake rates of dissolved N by biological assimilation and/or (b) loss by deposition of nutrient-rich particles in sediments and/or (c) leakage by horizontal water flow through the dikes or vertical flow into groundwater. High uptake rates of dissolved N compounds in pond water and sedimentation of nutrients were shown to occur (Chapter 5) under both pond managements. These processes led to low levels of fish-toxic ammonia, nitrite and nitrate under both management schemes (Chapter 3, 5 and 6) that were in suitable concentrations for fish culture (Dabrowska and Sikora 1986; Dabrowska and Wlasow 1986; Abbas 2006). Not only N but also phosphorus (P) compounds were found in low levels under both pond managements, but ratios of dissolved N compounds to dissolved ortho-phosphate and TN to total phosphorus (TP) in pond water were both higher than the TN/TP ratio of the fertilizer that was added to the ponds. This implies that N was removed at a lower rate than P. This result was expected because P is known to quickly deposit in pond bottom sediments (Correll 1998) which makes it a major limiting nutrient in aquatic environments for primary production (consequently also for oxygen production) in ponds (Elser *et al.* 1990). Thus, this is a crucial factor for fish production (Black 1998; Ross 2000). In tropical aquaculture, this situation is exacerbated because the high water temperatures decrease oxygen solubility in water while oxygen requirements of pond organisms increase due to their higher levels of activity.

In the studies presented here (Chapter 2, 3, 5 and 6), oxygen levels were shown to be low in the morning, which was also reported to be a serious problem for the local traditional pond aquaculture (Steinbronn 2009). The modifications made to the traditional pond managements resulted in higher oxygen levels and higher net oxygen production along with lower turbidity, better light penetration, a deeper phototrophic water layer and higher abundances especially of small-sized phytoplankton (Chapter 3 and 5).

Fertilization and feeding

In tropical pond polycultures, the distinction between fertilizers and feeds applied to the ponds is not clear-cut since manure can serve as direct feed for fish as reported by farmers (Steinbronn 2009) and uneaten feeds and feces of fish serve as fertilizer for natural food resources (e.g. phytoplankton, zooplankton, zoobenthos) for fish species in the polyculture. In the present studies, manure from buffaloes and cows served as the main source of organic fertilization as these livestock were commonly reared in the research area. Households in the study area rarely keep pigs as livestock and consequently, pig manure was rarely applied by farmers. The quality and quantity of organic fertilizer used by the farmers were similar to those reported by other studies in Northern Vietnam (Luu 2001; Kumar *et al.* 2004a, b; Kumar *et al.* 2005b; Steinbronn 2009). Under traditional pond management, substantial fractions of nutrients were applied to the ponds through feeds rather than as fertilizers, as 20-35% of the dry matter of the feed resources was not consumed by fish and does therefore decay on the pond bottom and ultimately serve as fertilizers.

Under modified pond management, the supplemental application of chemical fertilizers substantially increased the input of N and P from fertilizers. These nutrients were applied to the ponds at approximately similar rates via feeds and fertilizers. Under traditional pond management, the main inputs of N and P were applied in the form of feeds (leaves and by-products). However, the total inputs of N and P from both feeds and fertilizers were identical in the two tested management schemes (Chapter 3). Under neither management did the applied organic and/or inorganic fertilization raise the N input to the level of 4 kg ha^{-1} day^{-1} N as recommended for tropical semi-intensive aquaculture (Edwards *et al.* 1994, 1996). In future, therefore, the amount of N applied as fertilizer could be increased but the extent to which this could be done would be limited, mainly by the level of dissolved N compounds (esp. ammonia and nitrite) which might be toxic at higher levels. The concentration of N compounds dissolved in water was found to be very low under both pond managements (Chapter 3, 5 and 7) because both the uptake rate into the natural food web and sedimentation rate of N were found to be very high (Chapter 5). Only about 8% of the applied N amount was measured after one week in the natural food resources (e.g. phytoplankton, zooplankton) and in the top layers of sediments (Chapter 5). Consequently, a more frequent application of N fertilizer might be beneficial.

In traditional aquaculture in Northern Vietnam, plant-derived feed resources such as leaves of maize, cassava, bamboo, banana and grasses, weeds and other farm by-products are applied to the ponds. These feeds are directly utilizable only by grass carp, which is widely reported to feed on macrophytes after it reaches a body length of 25-30 mm (Opuszynski 1972; De Silva and Weerakoon 1981). Feed intake of grass carp is known to be dependent on kind of feed, fish size and water temperature (Opuszynski 1972; Cai and Curtis 1990; Osborne and Riddle 1999). The leaves and by-products used as feeds were similar in proximate composition to values found in previous studies in the research area (Dongmeza *et al.* 2009; Steinbronn 2009). These feed resources were reported to be of low nutritional quality with a food conversion ratio (FCR) of about 7.7 ± 2.3 on a DM basis (Steinbronn 2009), and are only palatable to grass carp, not to other fish species cultured in the polyculture. Under traditional pond management, the plant materials led to growth rates of grass carp that were within the range of growth rates reported in other studies of grass carp fed on these feeds. Cassava leaves fed to grass carp resulted in specific growth rates of 0.5% day^{-1} (Tan 1970) and Napier grass resulted in specific growth rates of 1.0 to 2.7% day^{-1} (Tan 1970; Venkatesh and Shetty 1978; Shrestha and Yadav 1998). Most of the applied plant feed resources were considered as unsuitable by Dongmeza *et al.* (2009) due to high levels of anti-nutrients such as cyanide, phytic acid and tannins, which were shown to have adverse effects on growth and/or feed utilization (Ng and Wee 1989; Becker and Makkar 1999; Francis *et al.* 2001). Some of the applied plant materials even resulted in a loss in weight in grass carp (Dongmeza *et al.* 2010). Generally, the applied plant feed resources had high fibre contents that far exceeded the suggested maximum 15% crude fibre content of feeds for grass carp (Liao *et al.* 1980; Mao *et al.* 1985). High fibre contents in feeds are known to reduce the digestibility of nutrients and energy (Steffens 1989; Anderson *et al.* 1991; NRC 2011). These plant feeds are not only of low nutritional quality but are fed in such a form that only about 65-80% is consumed by grass carp (Chapter 4). The unconsumed fractions remain in the pond and may act, after decomposition, as fertilizers. These decomposition processes are known to use large amounts of oxygen (Klanjšček *et al.* 2012) which is the cause that these resources are fertilizers of low quality.

Not only do the plant resources gathered from fields and used as feeds have a poor nutritional quality, but we (Chapter 2) demonstrated that there is a risk that these resources may be contaminated by pesticides used on crops and, when subsequently eaten by grass carp, may negatively affect both their feed uptake and health. In Vietnam, pesticides are reported to be applied at much higher levels than recommended or needed (Normile 2013). In the research area, several pesticides (like fenitrothion, imidacloprid, fenobucarb) were found in surface and ground waters at levels that pose environmental risks for aquatic organisms (Lamers *et al.* 2011).

Under modified pond management, the replacement of the macro-herbivorous and disease-prone grass carp by the omnivorous common carp necessitates a change of feeding management that reduces the input of leaf material but increases the level of feeds with higher nutritional value. In the study area, a plant-based supplemental feed was introduced. This pelleted feed was mainly based on locally available feed resources but contained about 10% of fishmeal which is not locally available but was, however, necessary in order to supply essential, especially sulphur-containing, amino acids. Such feeds are composed to supply in particular energy to the diet of the common carp but lack sufficient amounts of protein and amino acids (NRC 2011). In semi-intensive aquaculture, the function of the supplemental feed is to supply nutritional compounds that are limited in the natural food resources of the pond (De Silva 1993). As natural food resources of the common carp (zooplankton and zoobenthos) contain higher levels of crude protein than that required by common carp, digestible energy is the first limiting factor in semi-intensive aquaculture and needs to be supplied by the supplemental feeds so that the natural food resources can be efficiently utilized (Viola 1989). Under semi-intensive management, the high lipid content of the common carp at the harvest time indicates an oversupply of energy resources in the supplemental feeds towards the end of the trials (Chapter 4). With increasing fish biomass during the production period (no fish were harvested in the meantime) the demand for feed increased continously. In this situation, the content of crude protein in supplemental feeds may need to be increased as the natural food resources that are provided by the pond are limited (De Silva 1993). A dynamic optimization of the feeding management is required as the fish stock in the ponds rises either by increasing the availability of protein from natural food resources through higher fertilization or by increasing the level of crude protein in the pelleted feeds as suggested by De Silva (1993).

In the present study, reseachers (Chapter 3 and 4) provided daily supplemental feed at 3% of the estimated total fish biomass as this has been shown to be the most effective feeding level in a fish polyculture dominated by omnivorous fish (Abdelghany and Ahmad 2002). For the calculation of feeding rates in the semi-intensively managed ponds, it was assumed that common carp make up to 50% of the total fish biomass. This initial assumption turned out to be wrong as the relative growth of other stocked species was much higher, which resulted in the fish being provided with an increasingly lower pellet feed supply over time from an initial level of 3% down to about 1% of total fish biomass. This may explain the poor relative growth rate of common carp since they were the most dependent on the pelleted feed. The poor growth of common carp may also be explained by interspecific competition for the pelleted feeds with tilapia and grass carp. These species increased their growth significantly under semi-intensive pond management which suggests that they were freely consuming the supplemental pelleted feed. To increase the production of fish in general and common carp in particular, a consistent daily feeding rate of 3% of total fish biomass is recommended. Modified feeding methods (e.g. using feeding tables) might be chosen to deter fish species other than common carp from feeding on the applied pellets. As the fish increase in weight it is recommended that the total feed offered should be raised and that the crude protein level in the feeds should be increased or else more fertilizer should be applied to the ponds to promote natural food productivity.

Traditionally, pond aquaculture is a farming activity characterised by low financial input. The frequent application of pelleted feed is little accepted by farmers as this requires a constant financial input (Chapter 7) even though under semi-intensive pond management the financial net benefit can be many times that under traditional management (Chapter 4). As protein is the most expensive nutritional compound in feeds and as animal-derived protein feed ingredients of a suitable quality and quantity (e.g. fishmeal) are not available in the rural areas, alternative animal feed resources might encourage farmers to use this technology. Müller *et al.* (2012) tested locally the potential for using organic farm by-products and wastes for the production of earthworms. Local small-scale farmers showed great interest in this technology (vermiculture). Generally, earthworms contain high levels of high quality protein but actual values depend on the earthworm species and their feed substrate (Tacon *et al.* 1983; Stafford and Tacon 1984; Sun *et al.* 1997; Changguo *et al.* 2006; Sogbesan *et al.* 2007; Dong *et al.* 2010; Tuan 2010). Certain earthworm species have been reported to contain haemolytic factors in the coelom fluid that negatively affect the health of fish (Kobayashi *et al.* 2001; Medina *et al.* 2003; Ohta *et al.* 2003). Lysenin was also reported as a toxic compound in coelomic fluid (Kobayashi *et al.* 2001; Kobayashi *et al.* 2004). However, haemolytic factors and lysenin seem to be heat labile (Tacon *et al.* 1983; Kauschke *et al.* 2007). Thus certain preservation and processing methods applied to earthworms before being fed to fish can greatly affect their palatability (Nhi *et al.* 2010). Pre-treatment of earthworm by removal of coelomic fluid, heating as a method of drying, or blanching with hot water was recommended by Tacon *et al.* (1983). Nhi *et al.* (2010) showed previously frozen *Perionyx excavatus* to have a negative effect on fish growth. Using the same earthworm species, we (Chapter 6) found hardly any negative effects of sundried meal on growth in common carp presumably because the worms had been heated during sun drying. Under semi-intensive pond management, earthworm were used at increasing replacements of fishmeal in several supplemental feeds, which resulted in an improved growth. A combination of earthworm meal in feeds and natural food was found to meet the nutritional requirements of common carp more efficiently than fishmeal in the test feeds (Chapter 6). In experiments by Pucher and Focken (2013) with plant-based supplemental feed the growth of common carp improved as the availability of natural food increased. An increase in pond productivity to elevate natural food availability should be aimed at in future to increase the financial benefit and growth of common carp fed on supplemental plant-based feeds.

Natural food resources

Under both types of pond management, the seston (1-15 µm particle size) and the smallest fractions of phyto- and zooplankton (15-60 µm) made the highest contribution to the suspended natural food resources (Chapter 3 and 5). Similar findings were reported for tropical polyculture aquaculture that was fertilized by manure and chemical fertilizer (Schroeder *et al.* 1990). These small plankton fractions were shown to quickly take up dissolved N compounds. The turn-over and sedimentation rate of N were also rapid and therefore it was suggested that a high proportion of the settled material originates from plankton pf 1-15 µm in size (Chapter 5). Schroeder *et al.* (1991) and Lorenzen *et al.* (1997) estimated phytoplankton to contribute more than 50% of settled material. However, nutrients seem to be re-activated from the pond bottom and act as a nutrient source for suspended plankton (Chapter 5) as has been shown in other tropical aquaculture systems (Hargreaves 1997; Riise and Roos 1997).

The change in water management and the application of chemical fertilizer under semi-intensive pond management significantly increased the biomass of seston and smaller sized phytoplankton in the phototrophic water layer compared to traditional pond management. The higher phytoplankton abundance as well as biomass served as an enhanced natural food resource for the stocked silver carp that filter-feeds on phytoplankton (Spataru 1977; Schroeder *et al.* 1990) and resulted in significantly higher growth rates and higher yields of silver carp under semi-intensive compared to traditional pond management. An even higher fish yield and an increased utilization of nutrients might be achieved by using higher stocking rates of silver carp and Nile tilapia that are able to feed on the small-sized plankton as other fish species are not able to utilize this natural food resource.

Even though the higher phytoplankton biomass under semi-intensive pond management serves as a potential food base for higher levels of zooplankton, larger (> 500 µm) zooplankton increased neither in number nor in biomass. The low abundance of larger zooplankton under both pond managements suggests a high predatory pressure of the filter feeder bighead carp and the omnivorous feeders tilapia and common carp that are known to feed on larger zooplankton (Burke *et al.* 1986; Milstein 1992; Rahman *et al.* 2008b; Cooke *et al.* 2009; Pucher and Focken 2013). In a bottom-up and top-down control mechanism as described by Burke *et al.* (1986), the high predatory pressure of fish in the studies presented here resulted in a zooplankton composition that is dominated by rotatoria and other smaller plankton. Larger zooplankton was shown to serve as the most important natural food resource for common carp in the study area and the growth of common carp was shown to increase under supplemental feeding with an increase in the abundance of larger zooplankton (Pucher and Focken 2013; Chapter 6). As common carp is one of the fish species with a higher commercial value in the study area (Chapter 4), it might also be of financial benefit to reduce the stocking level of low-valued bighead to reduce the predatory pressure on larger zooplankton and thereby increase the abundance of these species. Large zooplankton was named as the most important natural food resource for common carp as soon as the preferred zoobenthos in a pond becomes limited (Rahman *et al.* 2008a).

Zoobenthos biomass was found to be lower under semi-intensive than under traditional pond management (Chapter 3 and 5). This might have been caused by the higher stocking density of common carp which is widely described as a bottom feeder that scavenges the pond bottom for zoobenthos (e.g. Jana and Sahu 1993; Muendo *et al.* 2006). The scavenging activity of common carp might further explain why there were no differences in the composition of the pond sediments even though fertilizer and feed inputs differed in quality between the two types of management. This feeding habit of common carp is known to increase the bio-availability of N and P as it aerates sediments and re-suspends deposited nutrients (Jana and Sahu 1993; Milstein *et al.* 2002). Due to the high inputs of total carbon via organic fertilizers and low quality leaf material under both management schemes, sediment redox potential was with on average 10 mV below the optimal range of 100-200 mV (Hussenot and Martin 1995). This indicates an undersupply of oxygen and negatively affected the productivity of zoobenthos (Kolar and Rahel 1993), which in turn reduced the natural food resources of bottom feeders such as common carp. Ponds under both types of management had low oxygen levels below 3 mg L^{-1} at dawn (Chapter 3) which were caused by respiration and decomposition of organic matter in the sediments (Barik *et al.* 2000).

Fish health management

Low retrieval rates of common carp (20-38%) and grass carp (41-67%) were found under both types of pond management (Chapter 4). These low retrieval rates lowered the profitability of pond aquaculture because these two species are the dominating ones in both management regimes and have the highest market value.

Grass carp is known to be a delicate fish species which is susceptible to diseases that lead to mass mortalities especially in Northern Vietnam (Edwards 2000; Van *et al.* 2002; Steinbronn 2009; Chapter 7). Even though, no mass mortalities of grass carp were observed during the study (Chapter 4), the low retrieval rate of grass carp suggests a high unobserved mortality that may have been due to a multifactorial disease caused by a combination of opportunistic bacteria, unsuitable environmental conditions and malnutrition (Chapter 3, 4 and 7).

In the research area, farmers rarely observed common carp suffering from diseases. However, only mortalities of many common carp would have been noticed by farmers. They do not know the numbers of stocked common carp as ponds are rarely drained completely and common carp is reproducing naturally in ponds (Steinbronn 2009). However, the low retrieval rate of common carp under both pond managements suggests that mortalities were high but hidden from the observations of farmers. In 2011, the Koi Herpes Virus (CyHV-3) was identified in the province´s largest hatchery in Son La city (Chapter 7) where the fingerlings for this trial were purchased from. This virus is a species-specific agent that causes mass mortalities of common carp all over the world but which had not been identified in Vietnam before 2011. The occurrence of this virus might become a threat to small-scale farmers in rural areas such as Northern Vietnam, as fish seed and fingerlings are produced and traded by hatcheries and nurseries under insufficient hygienic conditions and broodstock management. Training of managers of hatcheries and nurseries in modern management strategies could well be needed to assure an adequate supply of higher quality fingerlings of cultured species and to counteract the risk of spreading newly emerging diseases from bacteria, viruses, parasites and fungi in rural areas.

Benefit for income generation and food security

For small-scale farmers, the financial net benefit of the traditional pond aquaculture varied greatly (Steinbronn 2009; Chapter 4 and 7). The modifications towards a semi-intensive pond management resulted in higher net-benefits in all ponds but required higher expenditures on pond modifications, fish feed and fertilization. These higher expenses were equivalent to 20% of the annual income of a local household at the national poverty line. It is more likely that better-off and more educated farmers would be willing and able to invest is such changes. However, pond management changes towards semi-intensive pond aquaculture are making the production of fish a more predictable and more beneficial farming activity in terms of financial return and fish yield than traditional pond management.

The production of stocked fish and self-recruiting species (i.e. small indigenous fish, crabs, shrimps, snails) that are mainly used for home consumption (Steinbronn 2009) was many times higher under semi-intensive pond management than under traditional pond management (Chapter 4). This results in a greater supply of animal protein from the pond to the respective households and increases both household food security and cash income.

8.3 Technology transfer

In the study area, farmers adapted rapidly to changes in local markets and adopted key technologies to intensify single farming activities (e.g., hybrid maize varieties, paddy rice culture and the use of pesticides). These innovations were promoted by governmental agencies as essential to give higher levels of food security and greater financial benefits (Friederichsen 2008). However, new technologies in aquaculture have not yet been adequately transferred by the government extension services to the uplands. More recently, TV and private shops have started to provide supplemental information on new technologies. However, these providers have often been criticized by farmers for not providing enough information applicable to the uplands. More specifically, insufficient account is taken of the ability of the local markets to handle inputs and outputs, the education levels of the farmers and the availability of necessary resources (e.g. fingerlings of new species, high quality feed ingredients, lime, and suitable agricultural machinery). It has been observed that many Black Thai farmers take great interest in getting to know about improvements to their aquaculture practices but rarely go as far as investing any money or labour in them (Acharya *et al.* 2011). Upland farmers have shown special interest in feed-processing machines and technologies for intensive production of high-price aquaculture species that feed on higher trophic levels and that are cultured by lowland fish farmers. The upland farmers learned about these species from TV. However they often do not take into account that it is impossible to culture these species under local conditions and without having suitable feeds, fingerlings, aeration and other prerequisites.

In this research project, the majority of modifications were made in response to the problems and needs mentioned by local upland farmers (Steinbronn 2009). In line with the concept of participatory innovation development (Edwards 2000; Rai and Shrestha 2006; Hoffmann *et al.* 2009), the modifications were kept simple and were mainly based on locally available physical resources (as suggested by Udo *et al.* 2011) and on existing local knowledge of aquaculture. In the pilot studies, the application of the entire set of proposed modifications towards the development of a semi-intensive aquaculture practice was shown to increase fish production and to improve the net financial return from pond aquaculture compared with the traditional system and thereby transforming local aquaculture into a profitable business while improving food security (Chapter 4). However, the entire set of modifications was perceived by the Black Thai farmers as a rather radical innovation (Acharya *et al.* 2011), especially as it requires a constant investment of time and financial resources in order to provide feeds and chemical fertilizers, etc.

As Reardon (1995) demonstrated for other rural small-scale farmers, farmers in the study area quickly adopted single, low cost and minimal labour intense innovations from the set of available modifications (water control measures, reduction of turbidity), which they understood as helpful. Farmers were especially lacking in knowledge of the biological processes occurring in their ponds (e.g. oxygen production), or of natural food resources (e.g. phytoplankton, zooplankton, zoobenthos) or of the feeding habits of most of the fish species that were present in their ponds but which were not actively fed by them. Consequently, investments in modifications for these kinds of improvements were not well accepted, because they were not understood by the farmers to be beneficial. Therefore, future training in the application of innovations also necessitates the transfer of biological background knowledge.

The factors in the set of modifications that farmers were least likely to invest in, were shown to be regular expenditures on resources that had a monetary value, such as chemical fertilizers or ingredients for the preparation of pellets for daily feeding. This type of continual investment does not fit well into the seasonal pattern of expenditures of a typical Black Thai household. Especially poorer farmers were shown to be more risk-averse (Saint-Macary *et al.* 2013) and were therefore wary of investing in these innovations. Only better-off farmers, who had more stable incomes (through having a shop or undertaking more intensive, market oriented farming activities) and who had generally higher education, showed great willingness to invest in improved aquaculture by digging new ponds, investing in feed ingredients and their storage, integrating earthworm culture into their farming system or buying feed-processing machines. During this project, farmers always had the possibility to acquire knowledge of aquaculture technologies through their daily contact with researchers, or during the many workshops that formed an important part of the farmers' school that was set up by the researchers to provide information on fish biology and general aquaculture technologies. Only farmers who chose aquaculture as one of their key farming activities gained much knowledge from these courses and used it to change their pond management (Acharya 2011). These "adapters" might act as future "local innovators" and spread the technologies via the traditional horizontal technology transfer scheme typical of small-scale farmers (Acharya *et al.* 2011; Schad *et al.* 2011). With suitable governmental programs in place to promote better pond management, feed preparation and ingredient storage, the better-educated and more prosperous farmers are expected to further invest in aquaculture and form the vanguard of innovation. It is hoped that the introduction of new machines into rural markets may promote this development. To help poorer, more vulnerable farmers also to benefit from these innovations, new institutional arrangements such as share-cropping, producer-associations, fish-farmers cooperatives and group-learning courses might be developed, adopted and directed towards more concerted action in aquaculture (Edwards 2000; Minh 2010; Schad *et al.* 2011). These activities must be accompanied by adequate education programs that include the biologic background of innovations and must be adapted to the local context, as stated by Edwards (2000). Further, farmers should be trained in record-keeping as part of the technology transfer package as they rarely keep track of basic inputs of resources, labour and expenditure, nor of (non-)marketed outputs. The use of record-keeping allows farmers to compare losses against gains for single farm activities and to carry out overall cost/benefit calculations so that they can adjust their management strategies (Dung and Minh 2010).

As regular expenditure on feeds and fertilizer proved to be the major stumbling block preventing farmers from adapting their management strategies, ways of reducing these costs might result in a broader acceptance of these innovations. For example, the culture of earthworms (vermiculture) might be suitable for implementation in small-scale farms in rural areas for the production of high quality feed ingredients (Müller *et al.* 2012) that can substitute for fishmeal in supplemental pellet feeds (Chapter 7). Vermiculture uses underutilised organic wastes and by-products of low quality (Sinha *et al.* 2002; Sogbesan *et al.* 2007) to produce earthworms as well as a highly fertile soil (called vermicompost) which can be used for gardening or pond fertilisation (Ghosh 2004; Chakrabarty *et al.* 2009; Chakrabarty *et al.* 2010).

Potentially, vermicompost, applied to ponds, might reduce the use of chemical fertiliser and thereby reduce costs for the farmers.

Increased fish production at household level is likely to improve food security and/or income generation and may also improve the supply of fish at a regional level. In our research area the amount of fish produced locally could not meet the demand (see price development of fish species, Chapter 4). In 2004 and 2005, 60-65% of the fish harvests in local ponds were used for home consumption, being equivalent to 7.6 kg per household per month (Steinbronn 2009). In countries such as Vietnam, the fish consumption of rural fish-producing farm households was much higher than non-fish producing farm households (Dey *et al.* 2000). About 80% of the Vietnamese population like to eat fish (Lem *et al.* 2004) and fish is in many regions an animal-derived food as important as meat (Mishra and Ray 2009). Since about 25% of the population in North-West Vietnam is undernourished (Mishra and Ray 2009), it is very likely that increases in local fish production will also improve food security.

8.4 Recommendations

Recommendations to farmers:

- Application of basic pond hygiene such as liming and occasionally drying of the pond to reduce the potential risk of disease and anoxic sediment layers
- Water flow control measures are recommended to reduce nutrient loss and to reduce the introduction of eroded particles (reduction of turbidity) by the installation of pipes to control the water in- and outflow, installation of channels around ponds as well as greening of dikes
- Sealing of dikes to reduce the loss of nutrients through horizontal water flow
- Continuous supplemental fertilisation with higher quality resources (e.g. chemical fertilisers) to increase the primary and secondary productivity in the pond and thereby increase the natural food base for polyculture as well as the oxygen supply within the pond
- Reduced stocking of bighead carp to increase the natural food base for other higher trophic level species with higher market value
- Reduced stocking of the disease-prone grass carp and increased stocking of common carp
- Supplemental feeding of pellets based on locally available resources
- More careful selection of plant feeds applied to the pond including avoiding the addition of unconsumable parts of the plants
- Including vermiculture in the pond management changes to reduce the costs of animal-derived feed ingredients and fertilisation
- Record-book keeping to make it possible for farmers to compare losses against gains for single farm activities, and to carry out overall cost/benefit calculations of single management changes

- Formation of fish farmers cooperatives to organise courses on how to adjust of pond management to the local circumstances
- Formation of fish farmer cooperatives to reduce the level of investments needed by individual farmers for feed ingredients, feed-preparation machinery and feed ingredients storage and to provide a strong base for collective bargaining to maintain the selling price of fish and to minimise the cost of pond inputs.

Institutional recommendations:

- Introduction of aquaculture innovations must be accompanied by adequate education programs that are adapted to the local conditions and that include information on the biological rationale behind innovations
- Training programs should be aimed at farmers who are interested in changing their aquaculture practices into more beneficial farming activities
- Efforts should be made to help poor farmers to benefit from these innovations. New institutional arrangements such as ‘share-cropping’ or ‘producer-associations’ have to be developed, adopted and directed towards more concerted action in aquaculture
- Managers of hatcheries and nurseries must be better trained to assure an adequate supply of higher quality fingerlings of cultured species and to counteract the spread of new emergent diseases caused by bacteria, viruses, parasites and fungi.

8.5 Conclusion and outlook

The change in pond management from a grass carp dominated, traditional pond aquaculture towards a common carp dominated semi-intensive pond aquaculture was accomplished by using mainly locally available higher quality fertiliser and feed resources and by restricting the water flow-through. These changes improved the water quality, oxygen supply, turbidity and availability of natural food. The fraction of pelagic plankton communities in the ponds containing the smallest organisms were particularly favoured and responded to the changes in water and fertilizing management and responded with large increases in their populations. However, the low availability of dissolved N and P compounds and the persistence of inserted N in the natural food web for about a week after its application, suggests that the potential exists for further improvements in natural food availability by continually applying fertilisers or at least at more frequent (e.g. every few days) intervals. The schedules of application of specific fertilisers might have to vary spatially and seasonally due to differences in environmental factors such as water availability, turbidity or temperature, and requires further research. The change from flow-through systems towards static water systems reduced the impact of surrounding land use on ponds and indicated that conditions within ponds can be made more predictable with lower fluctuations in water quality and availability of natural food.

These improved culture conditions, due to changes in water management and the use of higher quality inputs, resulted in increased fish production. In particular, the filter feeding fish species such as silver carp benefited from the higher availability of small-sized plankton under modified pond management. Bighead carp, a filter feeder on larger plankton, benefited less as the abundance of its natural food resources was less affected by the new management schemes. For farmers it might, therefore, be beneficial to reduce the abundance of bighead carp and increase the stocking density of silver carp as both fish species fetch the same price on local markets. This could result in higher production of silver carp and higher availability of larger zooplankton due to reduced predatory pressure from bighead carp. Larger abundances of zooplankton would support the growth of other fish species with higher market value, such as common carp. The results showed that although zoobenthos, the favourite natural food of the scavenging, omnivorous common carp, is limited and needs to be promoted in availability. The high rates of sedimentation of nutrients and their removal from the natural food web might be reduced by the feeding habits of this fish, which tend to re-activate settled nutrients and thus increase their utilisation within the pond. In future research, special emphasis should be put on the interaction of pond bottom and pelagic natural food resources, as there appears to be a high degree of interaction between sedimentation, re-suspension, re-activation and assimilation of nutrients.

Under modified pond management, the common carp replaced the disease-prone grass carp as the main species in the polyculture. This required the introduction of pelleted feeds to supplement the natural food resources. Even so, the increase in common carp production was below that expected, which may have been because the fish were not given enough supplemental feed and/or because too little natural food was accessible. Strong interspecies competition might also have limited their growth. It is recommended that the feed base for common carp should be broadened either by higher supplemental feeding, by increasing natural food resources (see above) or by developing feeds or feeding regimes that inhibit other species from feeding on the pellets intended for common carp (e.g. by the introduction of feeding tables or by attracting subsidiary species with other feeds more suited to their feeding behaviour). Even though no mortalities in common carp were observed by farmers, the retrieval rate was lower than expected. This may have been the result of hidden mortality caused by the Koi Herpes Virus which has been identified in the research area. This disease only affects common carp but might have been transferred from pond to pond by other fish species that acted as carriers. Currently, common carp stock originates partly from hatcheries and partly from spontaneous reproduction within the fishponds. Some farmers could specifically produce fry of common carp in their own ponds by adapting the technology of spawning ponds (Dubisch ponds) which has been practiced in Europe for more than a century. This would allow the local production of common carp fry and fingerlings, thereby reducing the risk of rapidly spreading viruses in the region. By supplying farmers with good, virus-free brood stock, such decentralised fry production techniques could help to avoid the spread of Koi Herpes Virus and similar diseases that occurs when the provincial hatcheries are the main sources of fingerlings. An alternative strategy would be to ensure that the provincial hatcheries are well equipped and their personnel well-trained in modern methods and techniques.

In the experiments performed for this thesis, farmers observed no mass mortality of the disease-prone grass carp even though the retrieval rate was low. However, the growth performance of grass carp did increase under modified management. This improvement might have been caused by better culture conditions (better water quality, higher dissolved oxygen) but might also have been due to an improved feed base, lower stocking densities or the supplemental feeding of pellets. However, it was shown that plant material from pesticide-treated fields should be used as feeds for grass carp with caution because pesticide residues reduce feed intake and adversely affect fish health.

In general, changing towards a semi-intensive pond management that used higher quality feeds and fertilisers with reduced water flow and lower turbidity in ponds to produce an omnivorous main fish species was shown to increase not only fish yields but also nutrient efficiencies and economic net-benefit. This set of pond management modifications enables adaptable farmers to diversify their farms' outputs by using farm products for the production of higher-value fish. This strategy helps to increase income and the ability of farmers to cope with sudden financial shocks as well as improving the nutritional status of small farming households. The modifications in pond management have the potential to raise aquaculture production especially in mountainous watersheds that experience strong erosion from uplands as is often the case in Northern Vietnam.

However, the level of acceptability of tested changes in pond management to the local farmers was limited due to the need for continual financial inputs (such as the purchase of feed ingredients and fertilisers), as well as the necessity of farmers to know about feed preparation, storage of feed ingredients, and handling feed and fertilisers. Better market access, both for trading pond products and for buying pond inputs were also important requirements. There was also a strong tendency amongst farmers to accept only those management changes that involved low financial inputs which would have the effect of minimizing any potential improvements in fish production. Therefore, these changes may not be applied over the long term or expended to a larger region. To overcome the financial constraints to the adoption of innovations, farmers may need assistance from micro-credit facilities or farmer cooperatives, plus they may also need additional information from extension systems or fish farmer schools regarding good aquaculture practices in relation to marginal upland areas. Farmers would also greatly benefit from an improved knowledge of financial management, feed/fertiliser ingredients, and the technology of feed mincers / pelleters. The financial environment could be further improved by reducing costs to make all the proposed pond management modifications more acceptable. In this regard, earthworm was shown to be suitable as a replacement for expensive fishmeal in supplemental feeds. However further research is needed to optimise this technology including finding ways to improve the worm harvest, developing optimal techniques for the use of worms in aquafeeds, and carrying out investigations into alternative on-farm organic materials that could be used as feedstock. Additionally, vermisoil from the production of earthworm might be used as a fertiliser applied directly to the pond. Both the use of earthworm as fishmeal replacement in supplemental feeds and the use of vermisoil as fertiliser, reduce the costs of implementing the pond management changes and thereby might increase their acceptability. Investigating the acceptability of a combination of the pond management changes and vermiculture based on locally available organic wastes was not within the remit of this thesis and would require further investigation. However, it could already be shown that there is a potential to increase fish production by small-scale farmers in the upland area by implementing pond management changes and thereby contribute to income generation and local food security.

8.6 References

Abbas HH (2006) Acute toxicity of ammonia to common carp fingerlings (*Cyprinus carpio*) at different pH levels. Pakistan Journal of Biological Sciences 9:2215-2221

Abdelghany AE, Ahmad MH (2002) Effects of feeding rates on growth and production of Nile tilapia, common carp and silver carp polycultured in fertilized ponds. Aquaculture Research 33:415-423

Acharya L (2011) Innovation history of fish pond reforms in Chieng Khoi district, Son La province, Vietnam (Master thesis). Institute for Social Science of the Agricultural Sector, Stuttgart, Hohenheim, University of Hohenheim, 72p.

Acharya L, Pucher J, Schad I, Focken U, Hoffmann V (2011) Intensifying fish pond business - An interdisciplinary innovation study on information needs of Black Thai farmers in Chieng Khoi commune, Vietnam. In: Becker M, Kreye C, Ripken C, Tielkes E (eds.) Tropentag 2011 "Development on the margin", Bonn, Germany, 5 - 7 October. DITSL GmbH, Witzenhausen, pp. 124

Anderson J, Capper BS, Bromage NR (1991) Measurement and prediction of digestible energy values in feedstuffs for the herbivorous fish tilapia (*Oreochromis niloticus* Linn.). British Journal of Nutrition 66:37–48

Anyusheva M, Lamers M, La N, Nguyen VV, Streck T (2012) Fate of pesticides in combined paddy rice–fish pond farming systems in Northern Vietnam. Journal of Environmental Quality 41:515-525

Barik SK, Mishra S, Ayyappan S (2000) Decomposition patterns of unprocessed and processed lignocellulosics in a freshwater fish pond. Aquatic Ecology 34:185-204

Becker K, Makkar HPS (1999) Effects of dietary tannic acid and quebracho tannin on growth performance and metabolic rates of common carp (*Cyprinus carpio* L.). Aquaculture 175:327–335

Black KD (1998) The environmental interactions associated with fish culture. In: Black KD, Pickering AD (eds.) Biology of farmed fish. Sheffield Academic Press, Sheffield, pp. 284-325

Burford MA, Preston NP, Glibert PM, Dennison WC (2002) Tracing the fate of ^{15}N-enriched feed in an intensive shrimp system. Aquaculture 206:199-216

Burke JS, Bayne DR, Rea H (1986) Impact of silver and bighead carp on plankton communities of channel catfish ponds. Aquaculture 55:59-68

Cai Z, Curtis LR (1990) Effects of diet and temperature on food consumption, growth rate and tissue fatty-acid composition of triploid grass carp. Aquaculture 88:313-327

Chakrabarty D, Das SK, Das KM, Biswas PK (2009) Application of Vermitechnology in Aquaculture. Dynamic Soil, Dynamic Plant 3:41-44

Chakrabarty D, Das SK, Das MK, Bag MP (2010) Assessment of Vermicompost as Direct Application Manure in Fish Farming Ponds. Turkish Journal of Fisheries and Aquatic Sciences 10:47-52

Changguo X, Pingjiu Z, Genxing P, Duosheng Q, Qiuhua C (2006) Changes in diversity, protein content, and amino acid composition of earthworms from a paddy soil under different long-term fertilizations in the Tai Lake Region, China. Acta Ecologica Sinica 26:1667-1674

Chumchal MM, Drenner RW (2004) Interrelationships between phosphorus loading and common carp in the regulation of phytoplankton biomass. Archiv für Hydrobiologie 161:147-158

Condie SA, Webster IT (2002) Stratification and circulation in a shallow turbid waterbody. Environmental Fluid Mechanics 2:177-196

Cooke SE, Hill WR, Meyer KP (2009) Feeding at different plankton densities alters invasive bighead carp (*Hypophthalmichthys nobilis*) growth and zooplankton species composition. Hydrobiologia 625:185-193

Correll DL (1998) The role of phosphorus in the eutrophication of receiving waters: A review. Journal of Environmental Quality 27:261-266

Dabrowska H, Sikora H (1986) Acute toxicity of ammonia to common carp (*Cyprinus carpio* L.). Polish Archives of Hydrobiology 33:121-128

Dabrowska H, Wlasow T (1986) Sublethal effect of ammonia on certain biochemical and haematological indicators in common carp (*Cyprinus carpio* L.). Comparative Biochemistry and Physiology - C Pharmacology Toxicology and Endocrinology 83:179-184

De Silva SS (1993) Supplemental feeding in semi-intensive aquaculture systems. In: New MB, Tacon AGJ, Csavas I (eds.) Farm-made aquafeeds. Proceedings of the FAO/AADCP Regional Expert Consultation on Farm-Made Aquafeeds, 14-18 December 1992. FAO-RAPA/AADCP, Bangkok, Thailand, 434p., pp. 24-60

De Silva SS, Weerakoon DEM (1981) Growth, food intake and evacuation rates of grass carp, *Ctenopharyngodon idella* fry. Aquaculture 25:67-76

Demir N, Kirkagac MU (2005) Plankton composition and water quality in a pond of spring origin in Turkey. Limnology 6:189-194

Dey MM, Bimbao GB, Yong L, Regaspi P, Kohinoor AHM, Ponthana N, Paraguas FJ (2000) Current status of production and consumption of tilapia in selected Asian countries. Aquaculture Economics and Management 4:13-31

Dong X-H, Guo Y-X, Ye J-D, Song W-D, Huang X-H, Wang H (2010) Apparent digestibility of selected feed ingredients in diets for juvenile hybrid tilapia, *Oreochromis niloticus* x *Oreochromis aureus*. Aquaculture Research 41:1356-1364

Dongmeza E (2009) Studies on the nutritional quality of plant materials used as fish feed in Northern Vietnam (PhD thesis). Institute for Animal Productions in the Tropic and Subtropics, Hohenheim Stuttgart, Germany, University of Hohenheim, 55p.

Dongmeza E, Steinbronn S, Francis G, Focken U, Becker K (2009) Investigations on the nutrient and antinutrient content of typical plants used as fish feed in small scale aquaculture in the moutainous regions of Northern Vietnam. Animal Feed Science and Technology 149:162-178

Dongmeza E, Francis G, Steinbronn S, Focken U, Becker K (2010) Investigation on the digestibility and metabolizability of the major nutrients and energy of maize leaves and barnyard grass in grass carp (*Ctenopharyngodon idella*). Aquaculture Nutrition 16:313-326

Dung PTM, Minh LN (2010) Improved household financial literacy as a way to sustainability – initial impacts from book-keeping model in My Duc and potential expansion. In: International Symposium "Sustainable Land Use and Rural Development in Mountainous Regions of Southeast Asia", Hanoi, Vietnam, 21-23 July, Uplands Program, pp. 71

Edwards P, Pacharaprakiti C, Yomjinda M (1994) An Assessment of the role of buffalo manure for pond culture of tilapia. I. On-station experiment. Aquaculture 126:83-95

Edwards P, Demaine H, Innes-Taylor N, Turongruang D (1996) Sustainable aquaculture for small-scale farmers: need for a balanced model. Outlook on Agriculture 25:19-26

Edwards P (2000) Aquaculture, poverty impacts and livelihoods. ODI Natural Resource Perspectives 56:1-4

Elser JJ, Marzolf ER, Goldman CR (1990) Phosphorus and nitrogen limitation of phytoplankton growth in the freshwaters of North America: a review and critique of experimental enrichments. Canadian Journal of Fisheries and Aquatic Sciences 47:1468-1477

Epstein DM, Wurtsbaugh WA, Baker MA (2012) Nitrogen partitioning and transport through a subalpine lake measured with an isotope tracer. Limnology and Oceanography 57:1503-1516

Francis G, Makkar HPS, Becker K (2001) Antinutritional factors present in plant-derived alternate fish feed ingredients and their effects in fish. Aquaculture 199:197-227

Friederichsen JR (2008) Opening up knowledge production through participatory research? Agricultural research for Vietnam's Northern uplands. Development Economics & Policy 63. Peter Lang Internationaler VErlang der Wissenschaft, Frankfurt a.M., 280p.

Fröhlich HL, Ingwersen J, Schmitter P, Lamers M, Hilger T, Schad I (2013) Water and Matter Flows in Mountainous Watersheds of Southeast Asia: Processes and Implications for Management. In: Fröhlich HL, Schreinemachers P, Clemens G, Stahr K (eds.) Sustainable Land Use and Rural Development in Southeast Asia: Innovations and Policies for Mountainous Areas. Springer Verlag, Heidelberg, New York, Dordrecht, London, pp. 109-148

Ghosh C (2004) Integrated vermi-pisciculture - An alternative option for recycling of solid municipal waste in rural India. Bioresource Technology 93:71-75

Glaholt Jr SP, Vanni MJ (2005) Ecological responses to simulated benthic-derived nutrient subsidies mediated by omnivorous fish. Freshwater Biology 50:1864-1881

Hargreaves JA (1997) A simulation model of ammonia dynamics in commercial catfish ponds in the southeastern United States. Aquaculture Engineering 16:27-43

Hoai PM, Sebesvari Z, Minh TB, Viet PH, Renaud FG (2011) Pesticide pollution in agricultural areas of Northern Vietnam: Case study in Hoang Liet and Minh Dai communes. Environmental Pollution 159:3344-3350

Hoffmann V, Gerster-Bentaya M, Christinck A, Lemma M (2009) Rural extension Volume 1: Basics issues and concepts. Margraf Publishers, Weikersheim, 254p.

Hussenot J, Martin J-LM (1995) Assessment of the quality of pond sediment in aquaculture using simple, rapid techniques. Aquaculture International 3:123-133

Jana BB, Sahu SN (1993) Relative performance of three bottom grazing fishes (*Cyprius carpio, Cirrhinus mrigala, Heteropneustes fossilis*) in increasing the fertilizer value of phosphate rock. Aquaculture 115:19-29

Kauschke E, Mohrig W, Cooper EL (2007) Coelomic fluid proteins as basic components of innate immunity in earthworms. European Journal of Soil Biology 43:110-115

Klanjšček J, Geček S, Legović T (2012) Influence of fish feed composition on oxygen utilization during decomposition of aquaculture effluents. Aquaculture Research 43:1845-1860

Kobayashi H, Ohtomi M, Sekizawa Y, Ohta N (2001) Toxicity of coelomic fluid of the earthworm Eisenia foetida to vertebrates but not invertebrates: Probable role of sphingomyelin. Comparative Biochemistry and Physiology - C Toxicology and Pharmacology 128:401-411

Kobayashi H, Ohta N, Umeda M (2004) Biology of lysenin, a protein in the coelomic fluid of the earthworm *Eisenia foetida*. International Review of Cytology 236:45-99

Kolar CS, Rahel FJ (1993) Interaction of a biotic factor (predator presence) and an abiotic factor (low oxygen) as an influence on benthic invertebrate communities. Oecologia 95:210-219

Kumar MS, Burgess SN, Luu LT (2004a) Review of nutrient management in freshwater polyculture. Journal of Applied Aquaculture 16:17-44

Kumar MS, Luu LT, Ha MV, Dieu NQ (2004b) The nutrient profile in organic fertilizers: Biological response to nitrogen and phosphorus management in tanks. Journal of Applied Aquaculture 16:45-60

Kumar MS, Binh TT, Luu LT, Clarke SM (2005) Evaluation of fish production using organic and inorganic fertilizer: Application to grass carp polyculture. Journal of Applied Aquaculture 17:19-34

Lamers M, Anyusheva M, La N, Nguyen VV, Streck T (2011) Pesticide pollution in surface- and groundwater by paddy rice cultivation: A case study from Northern Vietnam. Clean – Soil, Air, Water 39:356-361

Lem A, Tietze U, Ruckes E, van Anrooy R (2004) Fish marketing and credit in Viet Nam. FAO Fisheries Technical Paper 468, FAO, Rome, 92p.

Liao X, Lin D, Mao Y, Cai F (1980) Experiments on enzymatic fiber pellets used as fish feed and protein requirements of grass carp (*Ctenopharyngodon idella* C. et V.). Journal of Fish China 4:217

Lorenzen K, Struve J, Cowan VJ (1997) Impact of farming intensity and water management on nitrogen dynamics in intensive pond culture: a mathematical model applied to Thai commercial shrimp farms. Aquaculture Research 28:493–507

Luu LT (2001) The VAC system in Northern Vietnam. In: Integrated agriculture-aquaculture: A primer. FAO/IIRR/WorldFish Center, Fisheries Technical Paper No 407, Rome, pp. 29-32

Mao Y, Cai F, Lin D (1985) Studies on the daily requirements of protein, carbohydrates, fat, minerals and fiber of juvenile grass carp (*Ctenopharyngodon idella* C. et V.). Transactions of the Chinese Ichthyol Society 4:81

Medina AL, Cova JA, Vielma RA, Pujic P, Carlos MP, Torres JV (2003) Immunological and chemical analysis of proteins from *Eisenia foetida* earthworm. Food and Agricultural Immunology 15:255-263

Merriam JL, McDowell WH, Tank JL, Wollheim WM, Crenshaw CL, Johnson SL (2002) Characterizing nitrogen dynamics, retention and transport in a tropical rainforest stream using an in situ ^{15}N addition. Freshwater Biology 47:143-160

Milstein A, Alkon A, Avnimelech Y, Kochba M, Hulata G, Schroeder GL (1991) Effects of manuring rate on ecology and fish performance in polyculture ponds. Aquaculture 96:119-138

Milstein A (1992) Ecological aspects of fish species interactions in polyculture ponds. Hydrobiologia 231:177-186

Milstein A, Wahab MA, Rahman MM (2002) Environmental effects of common carp *Cyprinus carpio* (L.) and mrigal *Cirrhinus mrigala* (Hamilton) as bottom feeders in major Indian carp polycultures. Aquaculture Research 33:1103-1117

Minh T (2010) Agriculture innovation system in Vietnam Northern mountainous region - Six decade shift from a supply driven to a diversification oriented system. Kommunikation und Beratung 95, Margraf Publishers, Weikersheim, 271p.

Mishra V, Ray R (2009) Dietary diversity, food security and undernourishment: The Vietnamese evidence. Asian Economic Journal 23:225-247

Muendo PN, Milstein A, Dam AA van, Gamal E-N, Stoorvogel JJ, Verdegem MCJ (2006) Exploring the trophic structure in organically fertilized and feed-driven tilapia culture environments using multivariate analyses. Aquaculture Research 37:151-163

Müller JL, Pucher J, Tran TNT, Focken U, Kreuzer M (2012) The potential of vermiculture to produce on-farm feed resources for aquaculture in mountainous areas of North Vietnam In: Stahr K, Fröhlich H, Clemens G, Schreinemachers P (eds.) International Scientific Conference "Sustainable Land Use and Rural Development in Mountain Areas", University of Hohenheim, Stuttgart, Germany, 16-18 April 2012, pp. 113-114

Ng WK, Wee KL (1989) The nutritive value of cassava leaf meal in pelleted feed for Nile tilapia. Aquaculture 83:45-58

Nhan DK, Verdegem MCJ, Binh NT, Duong LT, Milstein A, Verreth JAJ (2008a) Economic and nutrient discharge tradeoffs of excreta-fed aquaculture in the Mekong Delta, Vietnam. Agriculture, Ecosystems and Environment 124:259-269

Nhan DK, Verdegem MCJ, Milstein A, Verreth JAJ (2008b) Water and nutrient budgets of ponds in integrated agriculture–aquaculture systems in the Mekong Delta, Vietnam. Aquaculture Research 39:1216 -1228

Nhi NHY, Preston TR, Ogle B, Lundh T (2010) Effect of earthworms as replacement for trash fish and rice field prawns on growth and survival rate of marble goby (*Oxyeleotris marmoratus*) and Tra catfish (*Pangasius hypophthalmus*). Livestock Research for Rural Development 22:1-12

Normile D (2013) Vietnam Turns Back A `Tsunami of Pesticides`. Science 341:737-738

NRC (National Research Council) (2011) Nutrient Requirements of Fish and Shrimp. National Research Council of the National Academics, The National Academies Press, Washington D.C.

Ohta N, Aizu M, Kaneko T, Sato T, Kobayashi H (2003) Damage to the Gills, Skin and Other Tissues by Lysenin and the Coelomic Fluid of the Earthworm *Eisenia foetida* in Two Teleosts, *Tanichthys albonubes* and *Oreochromis mossambicus*. Journal of Experimental Zoology Part A: Comparative Experimental Biology 295:117-126

Opuszynski K (1972) Use of phytophagous fish to control aquatic plants. Aquaculture 1:61-74

Osborne JA, Riddle RD (1999) Feeding and growth rates for triploid grass carp as influenced by size and water temperature. Journal of Freshwater Ecology 14:41-45

Pucher J, Focken U (2013) Intensification of common carp culture in rural areas of northern Vietnam by plant-based feeds with protein sources of different qualities. In: Poleksic V (ed.) 6th International Conference "Water & Fish". 12-14 June, University of Belgrade, Faculty of Agriculture, Belgrade-Zemun, Serbia, pp. 44-49

Rahman MM, Jo Q, Gong YG, Miller SA, Hossain MY (2008a) A comparative study of common carp (*Cyprinus carpio* L.) and calbasu (*Labeo calbasu* Hamilton) on bottom soil resuspension, water quality, nutrient accumulations, food intake and growth of fish in simulated rohu (*Labeo rohita* Hamilton) ponds. Aquaculture 285:78-83

Rahman MM, Nagelkerke LAJ, Verdegem MCJ, Wahab MA, Verreth JAJ (2008b) Relationships among water quality, food resources, fish diet and fish growth in polyculture ponds: A multivariate approach. Aquaculture 275:108-115

Rai S, Shrestha P (2006) Guidelines to participatory innovation development. PROLINNOVA Nepal Programme, Kathmandu

Reardon T (1995) Sustainability issue for agricultural research strategies in semi arid tropics: Focus on the Sahel. Agricultural Systems 48:345-359

Reinhardt N, Gut T, Lamers M, Streck T (2012) Water Regime in paddy rice systems in Vietnam: Importance of infiltration and bund flow. In: Stahr K, Fröhlich H, Clemens G, Schreinemachers P (eds.) International Scientific Conference "Sustainable Land Use and Rural Development in Moutain Areas", 16-18 April, University of Hohenheim, Stuttgart, Germany, pp. 221-222

Riise JC, Roos N (1997) Benthic metabolism and the effects of bioturbation in a fertilised polyculture fish pond in Northeast Thailand. Aquaculture 150:45-62

Ross LG (2000) Environmental physiology and energetics. In: Beveridge MCM, McAndrew BJ (eds.) Tilapias: biology and exploitation. Kluwer Academic Publishers, Great Britain, pp. 89-128

Saint-Macary C, Keil A, Nielsen T, Birkenberg A, Van LTA, Van DTT, Ufer S, Dung PTM, Heidhues F, Zeller M (2013) Linkages Between Agriculture, Poverty and Natural Resource Use in Mountainous Regions of Southeast Asia. In: Fröhlich HL, Schreinemachers P, Clemens G, Stahr K (eds.) Sustainable Land Use and Rural Development in Southeast Asia: Innovations and Policies for Mountainous Areas. Springer Verlag, Heidelberg, New York, Dordrecht, London, pp. 175-212

Schad I, Roessler R, Neef A, Zarate AV, Hoffmann V (2011) Group-based learning in an authoritarian setting? Novel extension approaches in Vietnam's Northern uplands. Journal of Agricultural Education and Extension 17:85-98

Schad I, Schmitter P, Saint-Macary C, Neef A, Lamers M, Nguyen L, Hilger T, Hoffmann V (2012) Why do people not learn from flood disasters? Evidence from Vietnam's Northwestern mountains. Natural Hazards 62:221-241

Schmitter P, Dercon G, Hilger T, Hertel M, Treffner J, Lam N, Vien TD, Cadisch G (2010a) Linking spatio-temporal variation of crop response with sediment deposition along paddy rice terraces. Agriculture, Ecosystems and Environment 140:34-45

Schmitter P, Dercon G, Hilger T, Ha TTL, Thanh NH, Lam N, Vien TD, Cadisch G (2010b) Sediment induced soil spatial variation in paddy fields of Northwest Vietnam. Geoderma 155:298-307

Schmitter P, Fröhlich HL, Dercon G, Hilger T, Thanh NH, Lam NT, Vien TD, Cadisch G (2012) Redistribution of carbon and nitrogen through irrigation in intensively cultivated tropical mountainous watersheds. Biogeochemistry 109:133-150

Schroeder GL, Wohlfarth G, Alkon A, Halevy A, Krueger H (1990) The dominance of algal-based food webs in fish ponds receiving chemical fertilizers plus organic manures. Aquaculture 86:219-229

Schroeder GL, Alkon A, Laher M (1991) Nutrient flow in pond aquaculture systems. In: Brune DE, Tomasso JR (eds.) Aquaculture and Water Quality. World Aquaculture Society, Baton Rouge, LA, pp. 489–505

Sevilleja R, Torres J, Sollows J, Little D (2001) Using animal wastes in fishponds. In: Integrated agriculture-aquaculture, a primer. FAO/ICLARM/IIRR, FOA Fisheries Technical Paper No 407, Rome, pp. 121–124

Shrestha MK, Yadav CNR (1998) Feeding of napier (*Pennisetum purpureum*) to grass carp in polyculture: A sustainable fish culture practice for small farmers. Asian Fisheries Science 11:287-294

Sinha RK, Herat S, Agarwal S, Asadi R, Carretero E (2002) Vermiculture and waste management: study of action of earthworms *Elsinia foetida*, *Eudrilus euginae* and *Perionyx excavatus* on biodegradation of some community wastes in India and Australia. The Environmentalist 22:261-268

Sogbesan AO, Ugwumba AAA, Madu CT (2007) Productivity potentials and nutritional values of semi-arid zone earthworm (*Hyperiodrilus euryaulos*; Clausen, 1967) cultured in organic wastes as fish meal supplement. Pakistan Journal of Biological Sciences 10:2992-2997

Spataru P (1977) Gut content of silver carp - *Hypophthalmichthys molitrix* (Val.) - and some trophic relations to other fish species in a polyculture system. Aquaculture 11:137-146

Stafford EA, Tacon AGJ (1984) Nutritive value of the earthworm, *Dendrodrilus subrubicundus*, grown on domestic sewage, in trout diets. Agricultural Wastes 9:249-266

Steffens W (1989) Principles of Fish Nutrition. Ellis Harwood Ltd, Chichester, UK

Steinbronn S (2009) A case study: Fish production in the integrated farming system of the Black Thai in Yen Chau district (Son La province) in mountainous North-Western Vietnam - Current state and potential (PhD thesis). Institute for Animal Productions in the Tropic and Subtropics, Hohenheim Stuttgart, Germany, University of Hohenheim, 207p.

Sun Z, Liu X, Sun L, Song C (1997) Earthworm as a potential protein resource. Ecology of Food Nutrition 36:221-236

Tacon AGJ, Stafford EA, Edwards CA (1983) A preliminary investigation of the nutritive value of three terrestrial lumbricid worms for rainbow trout. Aquaculture 35:187-199

Tacon AGJ, De Silva SS (1997) Feed preparation and feed management strategies within semi-intensive fish farming systems in the tropics. Aquaculture 151:379-404

Tan YT (1970) Composition and nutritive value of some grasses, plants and aquatic weeds tested as diets. Journal of Fish Biology 2:253-257

Tuan NN (2010) Development of supplemental diets for carp in Vietnamese upland ponds based on locally available resources (PhD thesis). Institute for Animal Productions in the Tropic and Subtropics, Hohenheim Stuttgart, Germany, University of Hohenheim, 159p.

Udo HMJ, Aklilu HA, Phong LT, Bosma RH, Budisatria IGS, Patil BR, Samdup T, Bebe BO (2011) Impact of intensification of different types of livestock production in smallholder crop-livestock systems. Livestock Science 139:22-29

Van PT, Khoa LV, Lua DT, Van KV, Ha NT (2002) The impacts of red spot disease on small-scale aquaculture in Northern Vietnam. In: Arthur JR, Phillips MJ, Subasinghe RP, Reantaso MB, MacRae IH (eds.) Primary aquatic animal health care in rural, small-scale aquaculture development. FAO Fisheries Technical Paper 406, FAO, Rome, pp. 165-176

Venkatesh B, Shetty HPC (1978) Studies on the growth rate of grass carp *Ctenopharyngodon idella* (Valenciennes) fed on two aquactic weeds and a terrestrial grass. Aquaculture 13:45-53

Vien TD (2003) Culture, environment, and farming systems in Vietnam’s Northern mountain region. Southeast Asian Studies 41:180-205

Viola S (1989) Production of commercial feeds for warm water fish. In: Shilo M, Sarig S (eds.) Fish Culture in Warm Water Systems: Problems and Trends. CRC Press Inc., Boca Raton, Florida, pp. 143-162

Wohlfarth GW, Schroeder GL (1979) Use of manure in fish farming - a review. Agricultureal Wastes 1:279-299

Zoccarato I, Benatti G, Calvi SL, Bianchini ML (1995) Use of pig manure as fertilizer with and without supplement feed in pond carp production in Northern Italy. Aquaculture 129:387-390

9 Summary

In South-East Asia, pond aquaculture plays an important role in the integrated agriculture aquaculture systems of small-scale farmers and contributes to their food security and income. In mountainous regions, pond aquaculture differs in the way it is managed from the aquaculture that is practiced in the lowland regions due to differences in climate and availability of feeds, fertilizers and water. In Northern Vietnam, the traditional pond aquaculture is a polyculture of 5-7 fish species. The macro-herbivorous grass carp (*Ctenopharyngodon idella*) is stocked as the main species and is fed with external feeds that include leaves from maize, banana, cassava and bamboo, and weeds from the fields. Common carp (*Cyprinus carpio*), silver carp (*Hypophthalmichthys molitrix*), bighead carp (*Aristichthys nobilis*), mrigal (*Cirrhinus mrigala*), mud carp (*Cirrhinus molitorella*), silver barb (*Barbus gonionotus*) and Nile Tilapia (*Oreochromis niloticus*) are stocked as secondary species and are often insufficiently nourished by farm by-products that include rice bran, peels of cassava and fermentation residues. Manures from mainly ruminants are used by farmers as feed for fish and as fertilizers for natural food resources. Ponds are managed as a constant water flow-through system. The inflowing water introduces soil particles eroded from the sloping fields of intensively cultured maize and cassava into the ponds, and cause high turbidity that limits both the primary and secondary production. The fish production of this system is low at about 1.5 ± 0.3 t ha^{-1} a^{-1} and is mainly limited by the poor quality of added feeds and fertilisers, low availability of natural food resources, low oxygen production in the ponds and the occurrence of a species-specific disease that causes high mortality in grass carp. To improve the local fish production and thereby increase food security and income of small-scale farmers, changes in the traditional pond management were designed in cooperation with local farmers and were tested in farmers' ponds in the uplands of Northern Vietnam. These changes included the reduction of water flow through the ponds to reduce the introduction of eroded particles and reduce the turbidity. Chemical fertilizers were added to increase the productivity of natural food resources and encourage higher primary production. The disease-prone grass carp was replaced as the main species by common carp that command a similarly high price on the local markets. To feed the omnivorous common carp, supplemental pellet feeds based mainly on locally available resources were applied to the ponds. In a pond trial that lasted seven months, the traditional and modified pond managements were compared for water quality parameters, availability of natural food resources, fish yields, nutrient utilisation efficiencies and monetary net benefit. In a ^{15}N tracer experiment, the nitrogen dynamics in the natural food web in local ponds were compared under the two types of pond management. In cooperation with social scientists, the acceptability of these management modifications by local farmers was evaluated by semi-structured interviews and qualitative methods. In a net cage trial, the suitability of earthworm meal as a replacement for fishmeal in supplemental pellet feeds for common carp was tested. In another net cage trial, the effect of pesticide contaminated grass feeds on the feed intake and health condition of grass carp were tested.

When compared with traditional pond management, the modified pond management system was found to result in reduced water turbidity, deeper phototrophic zones, improved availability of natural food resources, higher primary production and higher fish yield. In addition, the small plankton (1-60 µm size) benefited from the management changes and allowed significantly

higher growth rates of silver carp and bighead carp that are filter feeders. Common carp and grass carp had higher yields due to the changes in the feeding and stocking schemes. Under both types of pond management, nitrogen compounds were assimilated rapidly into the natural food web and there were high rates of sedimentation and re-mobilization of settled nitrogen from the pond bottom. Generally, the modifications to pond management were associated with increased nutrient utilisation efficiencies and resulted in higher net benefits and more stable pond culture conditions. It was shown that plant material from pesticide-treated fields should only be used cautiously as feeds for grass carp because pesticide residues reduce feed intake and adversely affect fish health.

Low cost modifications of pond management were well accepted by the farmers. But modifications involving application of supplemental feeds and chemical fertilisers and that required a continual monetary investment in order to increase fish yield and monetary net benefit, were less well received. The better-educated and more prosperous farmers are more likely to further invest in aquaculture and might act as local adopters. To reduce the costs of supplemental feeds for common carp, earthworm has been shown to be suitable as a replacement for fishmeal in supplemental feeds because earthworm meal in plant-based feeds resulted in higher growth of common carp than fishmeal. Vermiculture might therefore be a suitable additional farming activity in combination with the implementation of pond management modifications. Formation of fish farmer cooperatives might further increase the acceptability of innovations to farmers. The improvements to pond aquaculture that have been developed here may have a beneficial impact on fish production, food security and income of small-scale farmers in the uplands of Northern Vietnam and other regions in South-East Asia if the information is suitably transferred through education programmes that train farmers in technologies that have been specially adapted to conditions in the uplands.

10 Zusammenfassung

Teich-Aquakultur ist ein wichtiger Teil der integrierten Landwirtschaft von Kleinbauern in Süd-Ost-Asien und trägt zur Nahrungs- und Einkommenssicherung bei. Unterschiedliche klimatische Bedingung und Verfügbarkeiten von Futtermitteln, Düngemitteln und Wasser bedingen Unterschiede im Teich-Management in Berg- und Flachlandregionen. Unter dem traditionellen Teichmanagement in den Bergen von Nord-Vietnam werden Teiche mit einer Polykultur von 5-7 Fischarten besetzt. Der Graskarpfen (*Ctenopharyngodon idella*) bildet die Hauptfischart, da dieser makroherbivore Fisch Blattmaterial verdauen kann. Schuppenkarpfen (*Cyprinus carpio*), Silberkarpfen (*Hypophthalmichthys molitrix*), Marmorkarpfen (*Aristichthys nobilis*), Mrigal (*Cirrhinus mrigala*), Schlammkarpfen (*Cirrhinus molitorella*), Silberbarben (*Barbus gonionotus*) und Niltilapien (*Oreochromis niloticus*) sind die Nebenfischarten und nur unzureichend mit z.B. Reiskleie gefüttert. Exkremente von Wiederkäuern und Schweinen werden als Futter und Düngemittel für die Produktion von Naturnahrung (Phyto-, Zooplankton und Makrozoobenthos) eingesetzt. Ständiger Wasserdurchfluss transportiert jedoch erodierte Bodenpartikel von den steilen Mais- und Maniokfeldern in die Teiche und führt zu hoher Wassertrübung, welche die Produktion von Naturnahrung und Sauerstoff beeinträchtigt. Traditionelle Teiche haben daher mit 1.5 ± 0.3 Tonnen ha^{-1} a^{-1} nur einen geringen Fischertrag, welcher begründet ist durch die Verwendung von minderwertigen Futter- und Düngemitteln, durch die geringe Produktion von teichinterner Naturnahrung, durch die geringe Sauerstoffversorgung des Wassers und durch eine arten-spezifische Krankheit, die oft zu Massensterben von Graskarpfen führt. Die Beschreibung diese Krankheit ist Thema einer anderen These. Um die lokale Fischproduktion zu verbessern, wurden Veränderungen des traditionellen Teichmanagements entwickelt und in Teichen von Bauern auf ihre Effekte und Anwendbarkeit getestet. Die Veränderungen bestanden in der Reduktion des Wasserdurchflusses, um so den Eintrag von erodierten Boden-Partikeln in den Teich und damit die Trübung zu verringern. Kunstdünger wurden eingesetzt, um die Produktion Naturnahrung und Sauerstoff zu verbessern. Anstatt mit krankheitsanfälligen Graskarpfen, wurden die Teiche mit dem omnivoren Schuppenkarpfen als Hauptfischart besetzt, der einen ebenso hohen Marktwert hat. Die omnivoren Schuppenkarpfen wurden mit pelletiertem Ergänzungsfutter aus hauptsächlich lokal vorhandenen Rohstoffen gefüttert.

Das traditionelle und das modifizierte Teichmanagement wurden in Teichen von Kleinbauern in Nord-Vietnam über sieben Sommer-Monate verglichen. Hierbei wurden die Wasserqualität, Verfügbarkeit von Naturnahrung, Fischertrag, Nährstoff-Effizienz und der finanzielle Ertrag getestet. In einem Stickstoff-Isotopen-Tracer-Versuch wurden die zwei Aquakultur-Systeme auf die Dynamik von Stickstoff innerhalb des Naturnahrungsnetzes verglichen. Die Akzeptanz dieser Management-Modifikationen wurde bei den Kleinbauern mittels Interviews ermittelt. Die Eignung von Regenwürmern als Fischmehlersatz in pflanzenprotein-basiertem Ergänzungsfutter für Schuppenkarpfen wurde in Netzkäfigen getestet. In einem weiteren Netzkäfigversuch wurden die Effekte von pestizid-kontaminiertem Blattmaterial auf die Futteraufnahme und den Gesundheitszustand von Graskarpfen untersucht.

Im Vergleich zum traditionellen Teichmanagement führten die Modifikationen zu verringerter Wassertrübung, zu stärkeren phototrophen Schichten, zu erhöhten Verfügbarkeiten von Naturnahrung und zu erhöhten Primärproduktionen sowie höheren Fischerträgen. Die verstärkte Produktion kleinerer Plankter unter dem modifizierten Management resultierte in signifikant höherem Wachstum der plankton-filtrierenden Fischarten. Auch Schuppenkarpfen und Graskarpfen zeigten höhere Erträge und Wachstumsraten durch die Verbesserung des Fütterungsregimes. Gelöster Stickstoff zeigte unter beiden Teichmanagements vergleichbar hohe Aufnahmeraten ins Naturnahrungsnetz, aber auch hohe Sedimentierungsraten und hohe Reaktivierungsraten von Stickstoff aus den Sedimenten. Generell führten die Modifikationen zu höherer Nährstoff-Effizienz sowie zu höheren und stabileren Teichkulturbedingungen und finanziellem Ertrag. Es hat sich gezeigt, dass Pflanzenmaterial von pestizid-behandelten Feldern nur mit Bedacht als Futtermittel für Graskarpfen genutzt werden sollte, da die Pestizid-Rückstände sich negativ auf die Futteraufnahme und den Gesundheitszustand auswirken.

Nur diejenigen Modifikationen, die mit geringem finanziellen und Arbeitsaufwand verbunden waren, zeigten hohe Akzeptanz bei Bauern. Der Einsatz von Pelletfutter und Kunstdünger wurde hingegen aufgrund der ständig nötigen Investition nur begrenzt von Kleinbauern angenommen. Es wird erwartet, dass besser gebildete und wohlhabendere Bauern in die neue Teichaquakultur investieren und so als lokale Innovationsverbreiter agieren werden. Regenwurm eignet sich gut als vor Ort erzeugbarer Fischmehlersatz in Ergänzungsfutter für Schuppenkarpfen und könnte so deren Kosten zu reduzieren. Die lokale Regenwurmkultur wird somit als geeignete Zusatztechnologie gesehen, um die Anwendung der verbesserten Teichaquakultur zu erhöhen. Die Etablierung von Kooperativen von Fisch-produzierenden Bauern könnte die Akzeptanz dieser Technologien vergrößern. Es ist zu erwarten, dass die Anwendung der verbesserten Teichaquakultur eine Verbesserung der Fischproduktion, der Nahrungssicherung und der Einkommenssituation der Kleinbauern in den Bergregionen im Norden Vietnams und anderen Regionen in Asien bewirken wird. Hierzu sind Trainingsprogramme gefragt, in denen den Kleinbauern die lokal angepassten Verbesserungen der Aquakultur vermittelt werden.

11 Curriculum Vitae

Personal Data

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Occupation

Since 06/2012	**Researcher at the University of Hohenheim / Life Science Center (Germany)** Development, application and management of large-scale projects in agrarian R&D and technology transfer to SMEs
10/2008 - 06/2012	**Researcher at the University of Hohenheim / Depart. of Aquaculture Systems and Animal Production in the Tropics and Subtropics** Working in the interdisciplinary and trilateral research program “Uplands Program” (SFB 564, funded by DFG) collaborating with Chiang Mai University (Thailand) and Hanoi University of Agriculture (Vietnam)
08/2008 - 10/2008	**Technician at the University of Hohenheim / Depart. of Aquaculture Systems and Animal Production in the Tropics and Subtropics**

Education

01/2009 – 10/2014	**PhD in Agriculture at the University of Hohenheim** PhD Theses: “Nutrient flow in improved upland aquaculture systems in Yen Chau, province Son La (Vietnam)”
10/2004 - 09/2007	**Diplom in Biology at the University of Constance (Germany)** Majors in Aquatic Eco-Toxicology, Fish Ecology, Limnology, Environmental Physics, Microbial Ecology Theses: “Environmental effects on shellfish spat of *Perna canaliculus*”
10/2002 - 09/2004	**Pre-Diplom in Biology at the J.-W.-Goethe-University in Frankfurt a.M. (Germany)**
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References in peer-reviewed journals and books

Pucher J, Steinbronn S, Mayrhofer R, Schad I, El-Mansour M, Focken U (2013) Improved Sustainable Aquaculture Systems for Small-Scale Farmers in Northern Vietnam (Chapter 8). In: Sustainable Land Use and Rural Development in Southeast Asia: Innovations and Policies for Mountainous Areas, (Fröhlich, HL, *et al.* eds.), pp. 281-317, Springer Verlag

Pucher J, Gut T, Mayrhofer R, El-Matbouli M, Viet PH, Ngoc NA, Lamers M, Streck T, Focken U (2014) Pesticide contaminated feeds in integrated grass carp aquaculture: Toxicology and bio-accumulation. Diseases of Aquatic Organisms 108:137-147

Pucher J, Mayrhofer R, El-Matbouli M, Focken U (2014) Effects of modified pond management on limnological parameters in small scale aquaculture ponds in mountainous Northern Vietnam. Aquaculture Research (in press):1-15, doi: 10.1111/are.12465

Pucher J, Mayrhofer R, El-Matbouli M, Focken U (2014) 15N Tracer Application to evaluate Nitrogen Dynamics of Food Webs in two subtropical small-scale Aquaculture Ponds under different Managements. Isotopes in Environmental and Health Studies (in press):1-14, doi: 10.1080/10256016.2014.922963

Pucher J, Mayrhofer R, El-Matbouli M, Focken U (2014) Pond management strategies for small-scale aquaculture in Northern Vietnam: Fish production and economic performance. Aquaculture International (in press), 1-18 doi: 10.1007/s10499-014-9816-0

Pucher J, Ngoc TN, Yen TTH, Mayrhofer R, El-Matboulic M, Focken U (2014): Earthworm meal as fishmeal replacement in plant based feeds for common carp in semi-intensive aquaculture in rural Northern Vietnam. Turkish Journal of Fisheries and Aquatic Sciences 14:557-565

Conference contributions

Wantzen KM, Korn M, Pucher J, Rothhaupt K-O (2005): Einfluss des Flutpulses auf aquatischen Invertebraten im eulithoralen Interstitial des Bodensees. „Treffpunkt Biologische Vielfalt Vl“, Bundesamt für Naturschutz, Skript 164, p. 205-215

Mayrhofer R, Soliman H, Pucher J, Focken U, Trang T, Van KV, El-Matbouli M (2010): Preliminary results in the cause of grass carp disease outbrake in Yen Chau, Son La province, Vietnam. International Symposium "Sustainable Land Use Rural Development in Mountainous Regions of Southeast Asia", Hanoi, Vietnam, 21st - 23rd July

Mayrhofer R, Pucher J, Focken U, Soliman H, El-Matbouli M (2010) Untersuchungen zum saisonalen Graskarpfensterben in Yen Chau, Provinz Son La, Vietnam. XIII. Gemeinschaftstagung der Deutschen, Österreichischen und Schweizer Sektionen der European Association of Fish Pathologists, „Fische und Fischkrankheiten – ein unerschöpfliches Thema“, Krems, Österreich

Pucher J, Mayrhofer R, El-Matbouli M, Focken U (2010): Improvements in pond management and application of low-cost fish feed increase fish production and raise benefit of small scale aquaculture systems in Yen Chau. International Symposium "Sustainable Land Use Rural Development in Mountainous Regions of Southeast Asia", Hanoi, Vietnam, 21st-23rd July

Pucher J, Mayrhofer R, Hung TQ, El-Matbouli M, Focken U (2010): Growth functions of fish species cultured in small scale upland aquaculture systems under traditional and modified pond management schemes in Yen Chau, Northern Viet Nam. Tropentag 2010, “World Food System - A Contribution from Europe”, Zurich, Switzerland, 14th-16th September, p. 463-464

Pucher J, Mayrhofer R, El-Matbouli M, Focken U (2011): Effect of supplemental feeding practice in the culture of common carp in dependency on natural food availability. 9th Asian Fisheries and Aquaculture Forum, “Better Science, Better Fish, Better Life”, Shanghai, China, 21st-25th April, p. 25

Mayrhofer R, Soliman H, Pucher J, Focken U, El-Matbouli M (2011): Grass carp mortality in northern Vietnam – Investigations from 2008-2011. 15th International Conference on Diseases of Fish and Shellfish, Split, Croatia, 12th-16th September

Acharya L, Pucher J, Schad I, Focken U, Hoffmann V (2011): Intensifying Fish Pond Business: An Interdisciplinary Innovation Study on Information Needs of Black Thai Farmers in Chieng Khoi Commune, Vietnam. Tropentag 2011, “Development on the margin”, Bonn, Germany, 5th-7th October

Pucher J, Mayrhofer R, El-Matbouli M, Focken U (2011): Interaction of Natural Food and Supplemental Feeding for Common Carp in Semi-intensively Managed Ponds in the Marginal Uplands of Son La Province, Northern Vietnam. Tropentag 2011, “Development on the margin”, Bonn, Germany, 5th-7th October

Focken U, Dongmeza E, El-Matbouli M, Mayrhofer R, Pucher J, Steinbronn S, Tuan NN (2012): Upland Aquaculture in Yen Chau District (Son La Province, Northern Vietnam): Status, limitations and possible innovations for sustainable development. International Scientific Conference "Sustainable Land Use and Rural Development in Mountain Areas", Hohenheim University, Stuttgart, Germany, 16th-18th April

Mayrhofer R, Soliman H, Pucher J, Focken U, El-Matbouli M (2012): Grass-carp mortality in northern Vietnam. International Scientific Conference "Sustainable Land Use and Rural Development in Mountain Areas", Hohenheim University, Stuttgart, Germany, 16th-18th April

Müller JL, Pucher J, Tran TNT Focken U, Kreuzer M (2012): The potential of vermiculture to produce on-farm feed resources for aquaculture in mountainous areas of North Vietnam. International Scientific Conference "Sustainable Land Use and Rural Development in Mountain Areas", Hohenheim University, Stuttgart, Germany, 16th-18th April

Pucher J, Gut T, Mayrhofer R, Lamers M, Streck T, El-Matbouli M, Focken U (2012): Hydrophobic pesticides on feed material for grass carp: Toxicology and accumulation in fish. International Scientific Conference "Sustainable Land Use and Rural Development in Mountain Areas", Hohenheim University, Stuttgart, Germany, 16th-18th April

Pucher J, Mayrhofer R, El-Matbouli M, Focken U (2012): Potential of aquaculture intensification for small scale Black Thai farmers in Northern Vietnam. International Scientific Conference "Sustainable Land Use and Rural Development in Mountain Areas", Hohenheim University, Stuttgart, Germany, 16th-18th April

Pucher J, Tuan NN, Yen TTH, Mayrhofer R, El-Matbouli M, Focken U (2012): Earthworm meal as alternative animal protein source for full and supplemental feeds for Common Carp

(*Cyprinus carpio* L.). International Scientific Conference "Sustainable Land Use and Rural Development in Mountain Areas", Hohenheim University, Stuttgart, Germany, 16^{th} -18^{th} April

Pucher J, Tuan NN, Yen TTH, Mayrhofer R, El-Matbouli M. Focken U (2012): Feeding Fish Without Fishmeal: Earthworm Meal as Alternative Animal Protein Source in Rural Areas. Tropentag "Resilience of agricultural systems against crises", Göttingen-Kassel/Witzenhausen, Germany, 19^{th} – 21^{st} September

Pucher J, Focken U (2013): Intensification of common carp culture in rural areas of northern Vietnam by plant-based feeds with protein sources of different qualities. Proceedings of the 6th International Conference "Water & Fish". Poleksic, V. ed., pp. 44-49. University of Belgrad, Faculty of Agriculture, Belgrad-Zemun, Serbia, 12^{th} – 14^{th} June

Pucher J, Focken U (2013): ^{15}N tracer studies to evaluate nitrogen dynamics of food webs in two subtropical small scale aquaculture ponds under different management. ASI Jahrestagung. German Association for Stabile Isotope Research (GASIR), Braunschweig, Germany, 30^{th} September - 2^{nd} October

Pucher J, Steinbronn S, Tuan NN, Dongmeza E, Mayrhofer R, El-Matbouli M, Focken U (2013): Improved Sustainable Aquaculture Systems for Small-Scale Farmers in Northern Vietnam. Aquaculture "To the Next 40 Years of Sustainable Global Aquaculture". Elsevier, Las Palmas, Gran Canaria, 3^{rd} - 7^{th} November

Pucher J, Nikolaevskaya E, Focken U (2014) Apparent Digestibility of Earthworm Meal in Plant-based Feeds for Nile Tilapia, *Oreochromis niloticus* (L.). In: Tropentag "Bridging the gap between increasing knowledge and decreasing resources", Prag, Czech Republic, 17^{th}-19^{th} September

Pucher J, Focken U (2014) Sustainable alternative protein resources for aquaculture at different scales of production. GAOL 2014, Ho Chi Minh City, Vietnam, 7^{th}-11^{th} October

Pucher J, Focken U (2014) ^{15}N tracer application to evaluate the nitrogen uptake from natural food webs into fish in two subtropical small-scale aquaculture ponds under different managements. In: Aquaculture Europe, European Aquaculture Society, San Sebastian, Spain, 14^{th}-17^{th} October

Pucher J, Ragg NLC, Dietrich D, Kaspar HF (2014) Effect of periodical changes of water quality parameters on behaviour and health of spat from the Greenshell™ mussel (*Perna canaliculus*). In: Aquaculture Europe, European Aquaculture Society, San Sebastian, Spain, 14^{th}-17^{th} October

Mayrhofer R, Pucher J, El-Matbouli M, Focken U (2014) Surprising effect of experimental bacterial infection of grass carp on δ13C in fish in spite of only minimal intense clinical symptoms. ASI Jahrestagung. German Association for Stabile Isotope Research (GASIR), München, Germany, 15^{th}-17^{th} October