UNCOVERING THE PATHOGENIC LANDSCAPE OF HELMINTH (*OPISTHORCHIS VIVERRINI*) INFECTIONS: THE CONTRIBUTION OF PHYSICAL ENVIRONMENT, SOCIAL ENVIRONMENT, AND HEALTHCARE INTERVENTIONS

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DECLARATION

I hereby declare that this thesis is my original work
and it has been written by me in its entirety.
I have duly acknowledged all the sources of information
which have been used in the thesis.

This thesis has also not been submitted for
any degree in any university previously.

Ong Xueyuan
15 August 2016

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Summary

Extant disease-specific approaches tend to overlook the complex relationships, including the broader environmental and social conditions shaping the pathogenic landscape of diseases. This study uses liver fluke, *O. viverrini*, to examine how a holistic approach can facilitate the understanding of geographical focality of diseases, allowing for issues of re-infection and recalcitrance to control efforts to be addressed.

Waterscapes around a reservoir dam construction in Thailand were examined. First, the impact of the dam and rivers on the intermediate fish host and humans' fish species preferences and fish procurement locations, were determined. The dam construction and subsequent reservoir creation altered fish species assemblages, leading to a considerable increase in fish infection. The presence of a large reservoir also increased the accessibility of fish procurement, thus increasing human infection, especially for villages located close to the reservoir.

Next, the facilitation of *O. viverrini* transmission to fish and humans by rice cultivation and associated water use were investigated. Dam construction facilitated the transmission of *O. viverrini* during rice cultivation as the availability of irrigation water kept the paddies inundated for longer durations, thus increasing the length of the period during which rice paddies could function as habitats for the aquatic snail hosts. Rice cultivation practices facilitated *O. viverrini* transmission by increasing cultivation duration and/or water connectivity through various cultivation practices. Such cultivation practices

were not equally applied across rice paddies, leading to uneven transmission risks in rice paddies positioned at different relative locations to the reservoir.

The influence of dam construction, socioeconomic factors, and disease control efforts was then examined for the causality of human infection. While landscape factors enhance the likelihood of infection, human behavior and social factors determine the ultimate outcome of infection through differences in exposure to (i.e. consumption of) raw fish and in the prevention and treatment priorities of local health centers. Chemotherapy is only palliative, with re-infections quickly occurring if the underlying factors that expose humans to infection are not dealt with. Such factors can be easily masked by existing chemotherapy efforts, but can support the continued persistence of the parasite in the system and re-infection once chemotherapy efforts come to an end.

Opisthorchiasis exhibit high geographic focality due to the close association with the environmental, social, and healthcare factors. A holistic approach to target disease treatment from different angles is necessary for sustainable disease control efforts, and inform the geographies of human pathogenic diseases. While this study focuses the control of opisthorchiasis, recognizing the features of parasitism that are shaping the pathogenic landscape and geographical focality of diseases is an important contribution to the framework of EcoHealth/One Health approach and to the control of other diseases sharing similar contingent causal factors, including other food/water borne diseases and soil-transmitted helminthiases. Integrated control programs should take into account of other communicable and non-communicable diseases, food security, and nutritional statuses etc., as the ultimate health and well-being of the population is determined by a series of interacting factors rather than a single pathogen.

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List of Acronyms and Symbols

A	Chance-corrected within-group agreement
AIDS	Acquired immune deficiency syndrome
ANOSIM	Analysis of similarity
CCA	Cholangiocarcinoma
CI	Confidence interval
HIV	Human immunodeficiency virus
MCA	Multiple correspondence analysis
MRPP	Multi-response permutation procedures
NMDS	Nonmetric multidimensional scaling
NTD	Neglected tropical disease
OR	Odds ratio
PERMANOVA	Permutational multivariate analysis of variance
Q1-Q4	First quartile-fourth quartile
RR	Risk ratio
sd	standard deviation
SIMPER	Similarity percentage

Chapter 1 Introduction

1.1 Holism versus reductionism in healthcare and diseases

According to the World Health Organization (1946), health is defined as "a state of complete physical, mental, and social well-being, and not merely the absence of disease or infirmity". A more practical definition by J. Ralph Audy's (1971) states that health is "a continuing property that can be measured by the individual's ability to rally from a wide range and considerable amplitude of insults, the insults being chemical, physical, infectious, psychological, and social (p. 142)". Both definitions highlight the fact that health is a holistic pursuit of improving resilience to attacks and enhancement of personal overall well-being. Yet, the predominant paradigm of modern medical sciences and health sciences remained largely reductionist, where complex issues are solved by reducing them into simple, basic units (Fang and Casadevall, 2011). The reductionist approach here refers to methodological reductionism (Fang and Casadevall, 2011), which shapes the way diseases are identified and treated. The attribution of a single cause to a disease or dealing with multiple diseases of a person separately are common (Ahn et al., 2006). For infectious diseases, identification of the specific pathogen is often sufficient at explaining the disease, while disease treatment is often heavily reliant on the application of vaccines, antibiotics, or chemotherapy drugs. While such disease-specific approach has contributed immensely to reducing morbidity and mortality, and in the 1980s, even led to the eradication of smallpox (Fenner, 1993), a major limitation of this approach is the tendency to overlook the complex relationships and composite characteristics among risk factors, including the broader environmental and social conditions the pathogen is part of, or what Lambin et al., (2010) term the pathogenic landscape for disease. Consideration of the pathogenic landscape in disease control effort is pertinent in times of global environment change. Unprecedented increases in incidences or expansions in geographical range of infectious diseases have been brought about by the change in patterns of human movement, agriculture and water projects, climate change, and population expansion (Asakura et al., 2015).

Since the work of Maximilien Sorre (1933) and Jacques May (1950) on medical geography in the early twentieth century, other related concepts have stemmed from different disciplines including the landscape epidemiology/natural nidality concept (Pavlovsky, 1966; Reisen, 2010; Meentemeyer et al., 2012), ecosystem approach to health/EcoHealth/disease ecology (Forget and Lebel, 2001; Waltner-Toews, 2001; International Agency for Research on Cancer, 2016), and One Health (Zinsstag et al., 2011; One Health Initiative, 2016). These concepts share many similarities in their recognition of the broader environmental and social factors underlying diseases and health, but can vary in their focus and purpose. Medical geography, ecosystem approach to health, and One Health are aimed at explaining and managing human health issues. Medical geography stresses the importance of place in relation to health (Meade and Earickson, 2000), while an ecosystem approach to health focuses on ecosystem and socioeconomic issues, highlighting the close relationship between ecosystem services and health (Nguyen-Viet et al., 2015). The One Health approach is traditionally health-science driven, and emphasizes links between human and animal health, particularly regarding the transmission of zoonoses (Roger et al., 2016). Landscape epidemiology is not necessarily anthropocentric and its concepts are often applied to epidemiological studies of other plants and animals. Nonetheless, there is much overlap in concepts and applications among these holistic approaches. In the current study, the holistic approach adopted is aimed at broadening of the concept of disease causality to include environmental, social, and healthcare factors. Using concepts from the other holistic approaches, especially the ecosystem approach to health/EcoHealth approach, the pathogenic landscape of particular diseases can be identified and described, and disease outcomes possibly mitigated.

1.2 The necessity of holistic approach in infectious disease control

Renewed interests in the holistic approach to disease control was stimulated by increased incidences and/or the expansion in geographical distribution of emerging infectious diseases from the late twentieth century. Newly emerging infectious diseases often arise from other zoonotic infections as seen in severe acute respiratory syndrome, human immunodeficiency virus (HIV) infection, and influenza (Quinn, 1994; Morens et al., 2004; Keesing et al., 2010), thus underscoring the importance of studying the whole pathogenic landscape of diseases to include human-animal interactions. The geographical ranges of pre-existing infectious diseases have also varied, or their virulence has been altered, in response to environmental and other changes, including the development of drug-resistance (Dheda et al., 2010; Murray et al., 2011; Ashley et al., 2014). Indeed, infectious diseases appear to be locked into a constant evolutionary arms race with their hosts. Infectious diseases can become adapted to the host population, but changes in environmental conditions such as anthropogenic land modification or patterns of human movement can tip the balance and bring about new epidemics or outbreaks (Patz et al., 2000; Morens et al., 2008), stressing the importance of the broader environmental and social conditions on disease emergence and distribution.

Moreover, relationships between humans and pathogens are not always straightforward and pathogens can influence health in indirect ways. While pathogens are typically regarded as deleterious, there is growing interests in the potential of helminth infections to moderate autoimmune diseases (Weinstock 2012; Zaccone and Cooke, 2013). Reduced inflammatory responses in multiple sclerosis patients have been observed in patients who were also infected with intestinal parasites (Correale and Farez, 2007). Even non-pathogenic human microbiota can influence disease burdens by facilitating infection of humans by other pathogens (Kane et al., 2011; Bosch et al., 2013), regulating immune defence (Ichinohe et al., 2011; Silva et al., 2015), and affecting the well-being of the human hosts through their effect on nutrition absorption (Relman, 2011). Consequently, rather than the effect of a single agent, pathogenic infections can be viewed as contingent upon a complex of interacting factors and are thus unlikely to be eliminated purely through chemotherapy, or the standard biomedical response (Fauci and Morens, 2012).

While holistic approaches are gaining popularity among health professionals as responses to the health risks posed by emerging and re-emerging infectious diseases, they are relatively rarely applied to diseases associated with helminths (or the helminthiases), which make up a mere 3% of emerging infectious diseases (Robertson et al., 2014). However, helminthiases are associated with high levels of morbidity in some parts of the world, especially in the tropics, and particularly with certain livelihoods and cultural practices that facilitate contact of people with the parasites. Many helminth diseases in the tropics are now classified as neglected tropical diseases (NTDs): collectively they affect more than a billion people worldwide, but have attracted much less attention into their control and eradication than, for example, diseases such as malaria and HIV-AIDS. NTDs often disproportionally affect the poor or marginalized population in developing countries or rural populations (Songserm et al., 2012), trapping the afflicted in a vicious cycle of poor health outcomes and poverty, and costing billions of dollars in treatment and lost opportunities each year (World Health Organization, 2016). Due to the close association of helminth infections with environmental and social determinants, they often exhibit high geographical focality (Aagaard-Hansen and Chaignat, 2010), with high incidences associated with particular livelihoods or cultural practices that themselves have a strong geography. Without accounting for the underlying conditions determining the distribution of helminthiases, they often remain recalcitrant to disease control efforts.

1.3 The issue of helminthiases persistence exemplifying the need for a holistic approach

Helminthiases, which include foodborne trematodiases, lymphatic filariasis, schistosomiasis, and soil-transmitted helminthiases, are among the most common NTDs (Hotez et al., 2015). The increasing recognition of the burden caused by helminth infections has brought about large-scale control programs by the World Health Organization that have driven nationwide control programs in countries in Asia (Utzinger et al., 2010; Zhou et al., 2010), Latin America (Hotez et al., 2008), and sub-Saharan Africa (Tchuem Tchuenté, 2011), where helminthiases are prevalent. These programs have primarily relied upon chemotherapy for helminthiases control (Prichard et al., 2012). Many chemotherapy programs have relatively limited objectives, resulting in reduced infection levels in the short-term (Lustigman et al., 2012). Re-emergence of the disease, and possibly even development of resistant strains of parasites, is common once a program has been terminated, however (Prichard et al., 2012). Evidence already exists of the reduced efficacy of drugs used to combat lymphatic filariasis (Eberhard et al., 1988) and schistosomiasis (Doenhoff et al., 2009), with frequent treatment involving anthelmintic drugs appearing to hasten the development of drug resistance in some animals (Dorny et al., 1994). While chemotherapy has reduced levels of infection in the short-term, ensuring that positive health benefits extend beyond the cessation of chemotherapy programs has been challenging without improvements in the other factors that predispose populations to helminthiases (Gazzinelli et al., 2012; Hotez and Herricks, 2015). A major increase in schistosomiasis following the construction of dams and irrigation infrastructure has been well-documented (N'Goran et al., 1997), as has the eradication of schistosomiasis in Japan through modernization of agricultural practices (Sasa, 1970) and reduced hookworm infections as a result of improvements in sanitation and housing (Hotez, 2008).

A more holistic approach permits the consideration of factors that underpin the geographical focality of helminthiases, allowing for issues of re-infection and recalcitrance to control efforts to be identified and addressed. A more holistic approach can also help in the elimination of other diseases that might have a high incidence locally and that share some of the same contingent causal factors, such as poor sanitation and surface water management. Identifying these contingent causal factors can also facilitate a better targeting of intervention efforts that also accommodate projected future variations (Thompson and Polley, 2014). Moreover, it allows for the recognition of disease contributing factors that, while being masked by other factors, contribute to the extent and severity of a disease outbreak. For example, relationships between diseases and environmental conditions can be masked by disease control efforts (Tompkins et al., 2016), including those that have mitigated schistosomiasis burdens associated with recent hydro-infrastructure developments (Zhu et al., 2011; Barakat, 2013). Such efforts are palliative and temporary if the underlying factors causing infections, including infection of animal hosts and environmental conditions that promote and maintain pathogenesis, remain (McManus et al., 2010).

The issue of a high geographical focality of both disease and resistance to chemotherapy efforts is exemplified in opisthorchiasis, a NTD caused by the foodborne trematode *Opisthorchis viverrini*. Despite decades of control efforts, infection prevalence has remained high in Thailand overall (Sripa et al., 2011; Sithithaworn et al., 2014), with large variations in the level of infection and burden of disease evident even in relatively small geographical areas (Andrews et al., 2008; Grundy-Warr et al., 2012). Burdens of opisthorchiasis are particularly high in parts of northeast Thailand.

1.4 *O. viverrini* infection as a public health issue in the Mekong Basin

Opisthorchiasis is a major NTD in Southeast Asia, and in the Lower Mekong River Basin in particular. The parasite involved, *O. viverrini*, is one of only three metazoan pathogens classified as a group 1 carcinogen, with sufficient evidence to establish a link between *O. viverrini* and cancer in humans (International Agency for Research on Cancer, 2016). Carcinogenicity of opisthorchiasis stems not only from prolonged infection and re-infection, but also from the repeated treatment involving praziquantel anthelminthic, which can induce DNA damage leading to the development of hepatobiliary abnormalities, including cholangiocarcinoma (CCA) (Pinlaor et al., 2008; Yongvanit et al., 2012). Carcinogenicity from re-infection or repeated chemotherapeutic treatment necessitates the adoption of supporting disease control measures to avoid prolonged reliance on chemotherapy.

In *O. viverrini* endemic regions, CCA is strongly associated with opisthorchiasis (Sripa et al., 2007, Sithithaworn et al., 2014), while in non-endemic regions, CCA is associated with other risk factors that also cause prolonged inflammation of the biliary epithelium, including primary sclerosing cholangitis, choledonchal cysts and hepatolithiasis (Cardinale et al., 2010, Sithithaworn et al., 2014). However, incidence of CCA in endemic regions, including northeast Thailand, is at least 10 times higher than other non-endemic countries, ranging from 67-115 per 100 000 males in northeast Thailand, despite decades of control efforts (Sithithaworn et al., 2014). In fact, *O. viverrini* induced liver cancer is among the leading cause of cancer-associated mortality in the Lower Mekong Basin (Sripa et al., 2012a).

Due to the persistent high infection in northeast Thailand, the Isarn Agenda, a program aimed at CCA prevention and control in northeast Thailand, was introduced in 2012. The program involves fecal examination and ultra-sound scans for CCA for people above 40 years of age and exhibiting high-risk behavior, notably the consumption of raw fish. People found with opisthorchiasis are treated and education programs aimed at discouraging raw fish consumption are also created for primary school children. The Isarn Agenda is not equally applied throughout northeast Thailand, however, as each province has the autonomy to decide on health priorities locally (Laithavewat, 2014 pers. comm., 24 January), which may potentially contribute to the uneven geographical distribution of opisthorchiasis in the region.

1.5 Relationship between *O. viverrini*, the environmental factors, social factors, and disease control efforts

The multi-hosts life cycle of *O. viverrini* and its close relationship with the environmental and social conditions (Wang, 2012) highlights the need for a holistic approach to understand the factors underpinning *O. viverrini* infection and resistance to control efforts. *O. viverrini* is a foodborne parasite with a three-host life cycle. Freshwater *Bithynia* spp. snails and cyprinid fish are the first and second intermediate host respectively. Humans are the definitive host while other fish-eating carnivores, including cats and dogs can serve as reservoir hosts (Centers for Disease Control and Prevention, 2012) (Figure 1.1). Human infection occurs through raw or undercooked cyprinid fish consumption. Hereinafter, for brevity, *Bithynia* spp. snails will be referred to as *Bithynia* snails.

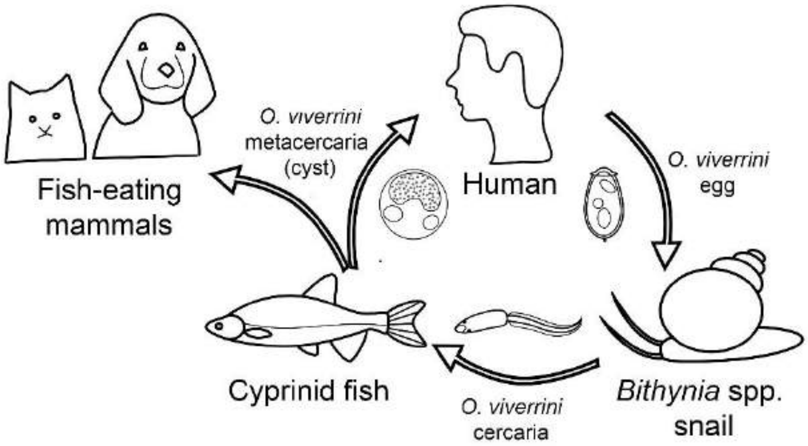


Figure 1.1: Upon ingestion by *Bithynia* snails, *O. viverrini* eggs develop into cercariae, which burrow into cyprinid fish and develop into metacercariae. Humans and reservoir hosts, including cats and dogs, are infected through raw or undercooked cyprinid fish consumption and transmit *O. viverrini* eggs to *Bithynia* snails via defecation.

Livelihoods in the Lower Mekong Basin are closely associated with wetland ecosystems, shaping behavior that can put locals at risk of opisthorchiasis. Extensive rice cultivation in the Lower Mekong River Basin is reliant on the seasonal flood pulses and irrigation systems, and small-scale fishing activities provide a major source of protein and additional income for local communities (Grundy-Warr et al., 2012). Due to highly variable water levels, as a result of strong seasonality in rainfall, natural water bodies have been substantially altered for flood control, irrigation and fishery enhancement through the creation of reservoirs, irrigation canals and ponds (Sripa et al., 2015). Moreover, lowland forests have been converted into rice paddies (Wang et al., 2011). As fish and snails are displaced from their natural habitats and introduced into this human-modified environment, rice paddies, reservoirs and irrigation canals serve as habitats for these intermediate host species, while irrigation canals and drainage systems of rice paddies also enhance the connectivity of rice paddies and adjacent rivers and reservoirs. Species composition and infection prevalence were altered, favoring high *Bithynia* snail abundance in the rice paddies (Wang et al., 2015) and higher *O. viverrini* infection of *Bithynia* snails near the villages, where greater fecal contaminations occur (Kaewkes et al., 2012). While rapid reproduction of *Bithynia* snails occurs during the summer monsoon season, when water levels are high, there exists high variability in peak *Bithynia* density in rice paddies even from within the same province (Lohachit, 2004-2005). This suggests that *Bithynia* reproduction is not directly related to seasons, hinting at the possibility of influence by human water management behavior, including irrigation (Petney et al., 2012). Conversely, little is known about the impact of reservoir and dam construction on infection of the cyprinid fish hosts, although increases in cyprinid fish have been found to be related to reservoir construction in northeast Thailand (Sornmani et al., 1981). Prevalence of opisthorchiasis remains high in the Lower Mekong Basin where host species, suitable habitats (e.g., rivers, reservoirs, and rice paddies), and raw fish consumption cultures co-exist.

Figure 1.2 summarizes the pathogenic landscape for *O. viverrini* where collective influence of factors contributes to shaping the final infection outcome. Existing disease control efforts are based largely on chemotherapy and campaigns aimed at discouraging raw fish consumption, while other factors predisposing a population to infection are seldom dealt with. Modification of surface water through dam construction and rice cultivation may affect the proliferation, transmission, and existence of the *O. viverrini* parasite and its snail and fish hosts. Health center priorities can have a major influence on the success of disease control efforts, while humans determine their likelihood of infection and re-infection by choices they make, how they work in the rice paddies, where they obtained their fish from, and the extent to which they consume raw fish. The transmission of infections from fish to humans, and factors predisposing fish and humans to infection are the main foci of this study, as fish is the most direct route by which humans get infected (Figure 1.1). Given the existing efforts devoted to examining the ecology and infection statues of *Bithynia* snails (e.g. Ngern-klun et al., 2006; Kiatsopit et al., 2012; Prasopdee et al., 2015; Wang et al., 2015), they are not examined in this study.

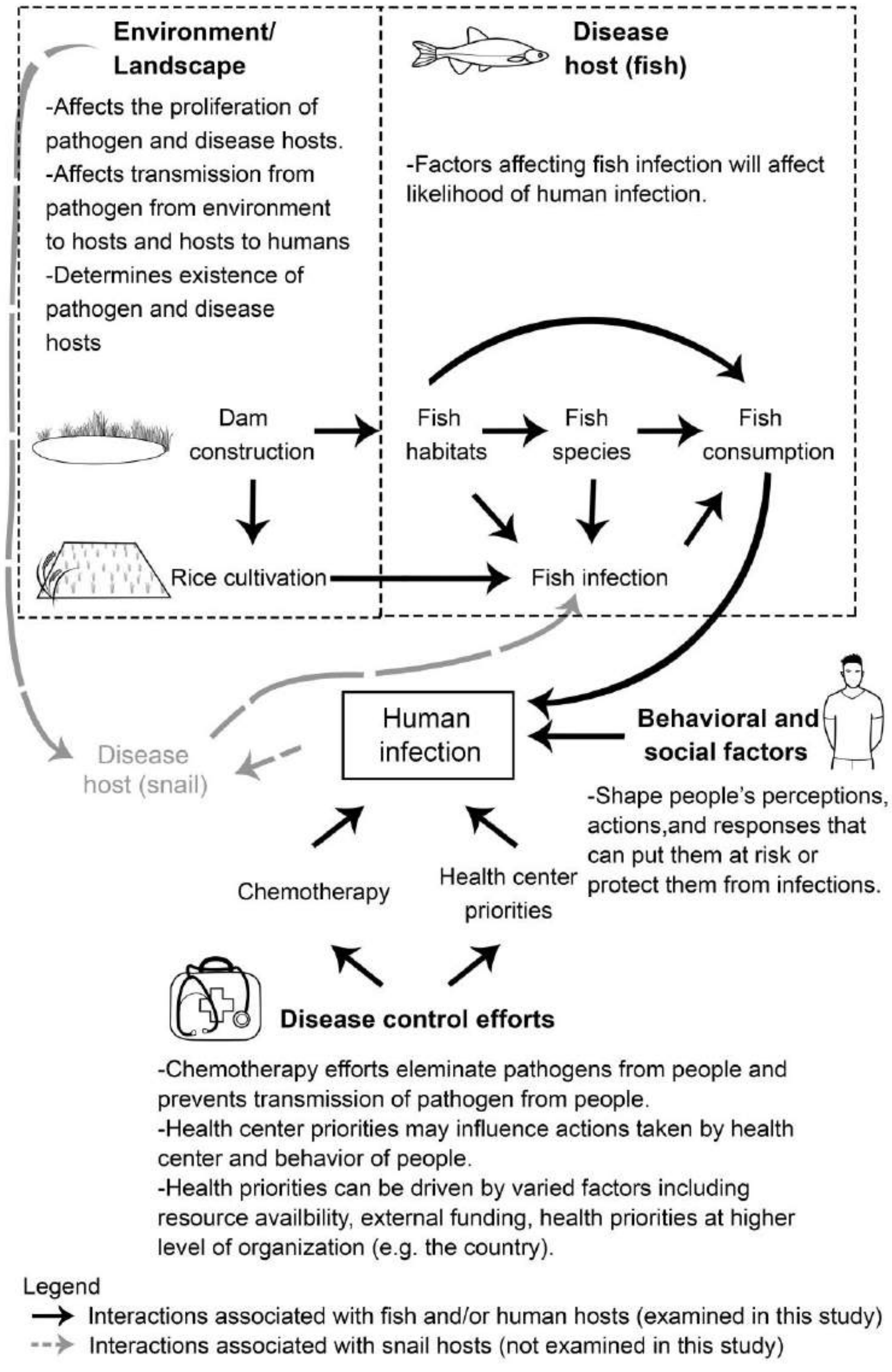


Figure 1.2: Relationships between the underlying conditions in the pathogenic landscape of *O. viverrini*. Land use changes—in the form of surface water modification—affects intermediate hosts of *O. viverrini* while behavioral and social factors affect human hosts. Existing control efforts are largely reliant upon chemotherapy and education campaigns discouraging raw fish consumption, overlooked the other important factors involved in human infection. Given the existing efforts devoted to examining the ecology and infection statues of *Bithynia* snails, they are not examined in this study.

1.6 Research gaps in the understanding of human *O. viverrini* infection

Despite prior research efforts on *O. viverrini* infection, little is known about the interactions of factors underlying the pathogenic landscape of *O. viverrini*. In order to explain the geographical unevenness of disease distribution and why extant disease control efforts have often proved largely unsuccessful, five research gaps have been identified.

First, scant attention has been given to the role of dams and their effect on *O. viverrini* infection, and other water-associated diseases in Southeast Asia, although numerous incidences of disease associated with dams and irrigation in other parts of the world suggest a strong possibility of similar occurrences in Southeast Asia. These include Rift Valley fever (Hassan et al., 2011) and schistosomiasis (N'Goran et al., 1997) in Africa, onchocerciasis in Latin America (Rodríguez-Pérez et al., 2011), and malaria in many parts of the world (Keiser et al., 2005). Given the tremendous land use transformations relating to dams in the Lower Mekong River Basin (Grumbine and Xu, 2011), there is a potential for dams to be "disease triggers" of opisthorchiasis (Ziegler et al., 2013).

Second, opisthorchiasis remained high in northeast Thailand and other parts of the Lower Mekong River Basin despite decades of control efforts (Sithithaworn et al., 2012a; Sripa et al., 2015). As with many helminth disease control efforts in the world, control efforts for opisthorchiasis relied largely upon chemotherapy. Efforts at education campaigns are predominantly focused on the discouragemet of raw fish consumption, but have had limited success (Sripa et al., 2011; Sithithaworn et al., 2014). Chemotherapy effort is unable to prevent re-infection (Suwannahitatorn et al., 2013) and can even facilitate development of CCA from repeated chemotherapeutic treatments (Pinlaor et al., 2008; Yongvanit et al., 2012) or can potentially lead to development of drug resistance (Prichard et al., 2012). It is important to determine the underlying factors predisposing the population to opisthorchiasis so as to better inform policies for suitable measures to reduce infection and/or re-infection.

Third, little is known about the influence of health center priorities on the disease and the disease perceptions of the local population, whom the health centers are providing services for. This is important as health centers in Thailand are the primary healthcare units, and therefore on the frontline of health care provision. Primary healthcare units play pivotal roles in health improvement and health equity (Starfield, 2012) and are especially important for healthcare provision and health awareness in rural areas (Songserm et al., 2012), where helminthiases, including opisthorchiasis, are prevalent. Understanding the influence of health center priorities on the prevalence and impact of particular diseases can help improve understanding of the distribution of diseases, thereby contributing to better informed disease control efforts and health service provision.

Fourth, in addition to dam construction, paddy rice cultivation is responsible for major modification of surface water in the Lower Mekong River Basin. Surface water is the key medium facilitating *O. viverrini* transmission across human, snail, and fish habitats (Ziegler et al., 2013). Although surface water is the key medium facilitating *O. viverrini* connectivity across human, snail, and fish habitats (Ziegler et al., 2013), one of the key elements that has been largely overlooked is the variation in surface water availability and connectivity in different rice paddy patches both spatially and temporally. Existing studies have the tendency to classify rice paddies as the same land use type while overlooking the inherently different cultivation practices applied on rice paddies (e.g. Ngern-klun et al., 2006; Suwannatrai et al., 2011; Kiatsopit et al., 2012). Identifying the unequal contribution of rice paddies in disease transmission is important for informing study designs in rice paddies-associated diseases, to avoid merging paddies of different disease transmission potential during analyses. It also enables recognition of more finely-resolved variations in disease distributions, which is necessary for the study diseases with high geographical focality, such as opisthorchiasis.

Fifth, little is known about the collective influence of risk factors in shaping infection outcomes, as risk factors are often examined in isolation. While risk factors of infection, particular human sociodemographic factors and behavior have been extensively documented (e.g. Kaewpitoon et al., 2012a; Kaewpitoon et al., 2012b; Thaewnongiew et al., 2014, Chudthaisong et al., 2015), little has been done to integrate such information with that of other risk factors or protective factors, including land use modification, hosts infection statuses, or chemotherapy efforts, to derive a more complete understanding of underlying conditions shaping the final infection outcome.

1.7 Research questions, specific research objectives, and framework

In view of the knowledge gaps in opisthorchiasis control and its potential significance to other helminth intervention efforts, the overall aim of this study is to uncover the main factors that contribute to the pathogenic landscape of *O. viverrini* infections. The intention is to improve understanding of the disease while also help to explain why extant disease control efforts have often proved largely unsuccessful, at least in the medium to long term. The findings can be used to inform policies, provide data upon which to base future projections, enable more sustainable disease control efforts, and exemplify the importance of holistic research on helminthiasis.

Six research questions pertaining to the effect of: dam on fish infection; dam on human infection; human behavior on infection, health center influence on infection; rice cultivation on *O. viverrini* transmission; and the collective influence of these environmental, social, and healthcare factors to the pathogenic landscape of *O. viverrini* are raised. To answer these research questions, 11 research objectives are formulated. The corresponding thesis chapters where each research questions are addressed are also highlighted. (Figure 1.3). Figure 1.4 shows where each specific research objective falls into the research framework to form a holistic understanding of the relationships between the environmental, social, and healthcare factors involve in disease transmission.

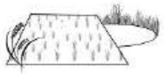
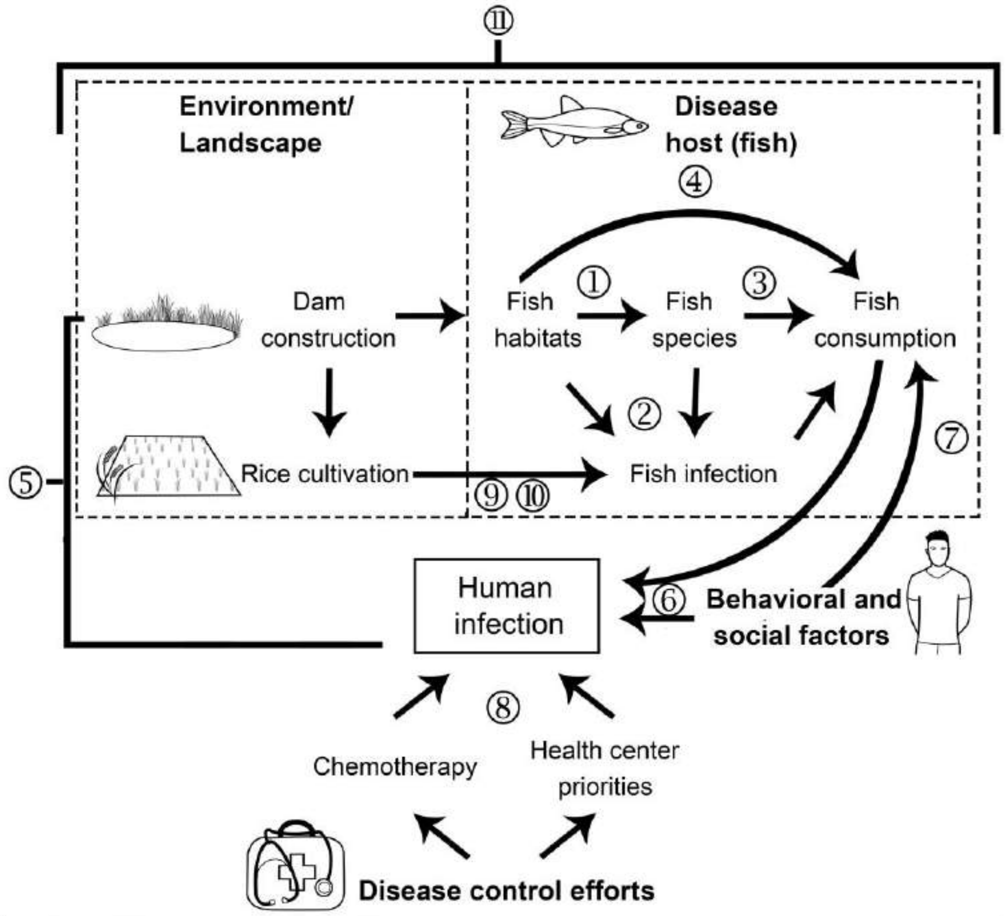
	Research questions	Specific research objectives	Thesis Chapters
	• How does dam construction affect *O. viverrini* infection of fish hosts?	1. Compare fish species biomass assemblages between the reservoir and the rivers.	Chap 4
		2. Compare fish *O. viverrini* infections variation between the reservoir and the rivers.	Chap 4
	• What is the ensuing impact of dam construction on *O. viverrini* infection of the human hosts?	3. Collect villagers' fish species preferences for raw fish dishes.	Chap 4
		4. Measure the proportion of villagers who obtained fish from the reservoir and the factors affecting their procurement location preferences.	Chap 4
		5. Analyze the association between rivers and the reservoir and human *O. viverrini* infection prevalence and intensity.	Chap 5
	• What roles do human behavior play in opisthorchiasis and control?	6. Analyze human behavioral and socioeconomic risk factors associated with human infection prevalence and intensity.	Chap 5
		7. Identify the motivations for consuming or avoiding raw fish consumption and the risk factors associated with such behaviors.	Chap 5
	• How do health centers influence opisthorchiasis in the communities they are providing service for?	8. Analyze the variation of human infection prevalence and intensity according to chemotherapy efforts and identify health center priorities.	Chap 5
	• How do rice cultivation shape the spatial and temporal variation of rice paddies in *O. viverrini* transmission?	9. Identify the roles of rice cultivation process and cropping calendar in shaping the temporal variation in *O. viverrini* transmission risks.	Chap 6
		10. Determine the contribution of cultivation characteristics and associated human behavior on the spatial variation in *O. viverrini* transmission risks.	Chap 6
	• How do the above factors (environmental, healthcare, and behavioural) explain the resistance to existing disease control efforts and influence uneven distribution of disease?	11. Synthesize results from the previous chapters to determine the interaction between factors and their contributions to the pathogenic landscape of *O. viverrini*.	Chap 7

Figure 1.3: Six research questions covering the influence of dam, social factors, healthcare factors, and rice cultivation, their corresponding research objectives, and the thesis chapters where each research objective is being addressed is shown.



Specific research objectives

① Compare fish species biomass assemblages between the reservoir and the rivers.

② Compare fish *O. viverrini* infections variation between the reservoir and the rivers.

③ Collect villagers' fish species preferences for raw fish dishes.

④ Measure the proportion of villagers who obtained fish from the reservoir and the factors affecting their procurement location preferences.

⑤ Analyze the association between rivers and the reservoir and human *O. viverrini* infection prevalence and intensity.

⑥ Analyze human behavioral and socioeconomic risk factors associated with human infection prevalence and intensity.

⑦ Identify the motivations for consuming or avoiding raw fish consumption and the risk factors associated with such behaviors.

⑧ Analyze the variation of human infection prevalence and intensity according to chemotherapy efforts.and identify health center priorities.

⑨ Identify the roles of rice cultivation process and cropping calendar in shaping the temporal variation in *O. viverrini* transmission risks.

⑩ Determine the contribution of cultivation characteristics and associated human behavior on the spatial variation in *O. viverrini* transmission risks.

⑪ Synthesize results from the previous chapters to determine the interaction between factors and their contributions to the pathogenic landscape of *O. viverrini*.

Figure 1.4: Research framework showing the relationships between the environmental, social, and healthcare factors of opisthorchiasis.

1.8 Arrangement and structure of the dissertation

The structure of this thesis and the main content of each chapter are briefly described below. To ensure the logical flow of content, separate introductions, methods, results, and discussion are embedded within each results-informed chapter. This thesis is organized to enable papers to be easily separated from the thesis to be submitted for publication. Chapter 4 has been published in a journal, while Chapter 5 has been accepted by a journal.

Chapter 2 describes and explains the choice of the study area in northeast Thailand which consists of agro-fishing villages around the Ubolratana reservoir impoundment dam.

Chapter 3 describes the sampling sites with respect to the research objectives.

Chapter 4 examines Ubolratana reservoir for the impact of dam construction on the cyprinid fish, and on villagers' fish consumption choices. The impounded section of the river, which forms the reservoir, is compared with the non-impounded region, which forms the river inlets. Cyprinid fish species variation (specific research objective 1) and fish *O. viverrini* infection (specific research objective 2) variation between the reservoir and river inlets are compared. Villagers' preferences for fish species used in raw fish dish preparations are then examined (specific research objective 3), as well as their choices of water body types for obtaining fish (specific research objective 4). This chapter provides insights into the little known impact of dams on diseases in Southeast Asia and exemplifies the value of holistic approach in uncovering interactions between human choices and the environment, which cannot be easily identified using a traditional risk factor analysis approach.

Chapter 5 adopts a holistic approach to uncover the causality of human infection by examining the influences of dam construction on human infection (specific research objective 5), behavioral and socioeconomic factors (specific research objective 6), and disease control efforts (specific research objective 8) on opisthorchiasis. It also examines the association of these risk factors with raw fish consumption behavior (specific research objective 7). Measure of success of control programs is often based on infection levels alone, but does not examine the influence of other underlying factors predisposing people of a certain location to infection. This chapter offers explanation for the uneven distribution of *O. viverrini* and informs policies for better distribution of healthcare resources and sustainable disease control efforts.

Chapter 6 explores the rice cultivation practice in this agro-fishery landscape. Surface water modification by rice paddies is an important route by which fish hosts can be infected with *O. viverrini*. This chapter describes and analyzes the spatial and temporal variation in surface water availability and connectivity in different rice paddy patches, which can give the different rice paddy patches different risks of *O. viverrini* transmission (specific research objective 9 and 10). Given the constraints in existing technology to measuring *Bithynia* snail-shed *O. viverrini* cercariae, the medium of cercariae transport, surface water, is examined instead. Understanding variations in disease transmission risk of different rice paddies patches is consequential to study designs of rice paddies-associated diseases and necessary for the application of a holistic/system approach, which requires detailed information on relationships between risk factors.

Chapter 7 synthesizes results in the previous chapters to examine the collective influence of environmental, social, and healthcare factors on infection outcome (specific research objective 11). It discusses the contribution of such holistic approach to the understanding of helminth diseases and examines the existing limitations and potential to address them in future work.

Chapter 2 Study Area

2.1 Dam construction in the Lower Mekong River Basin, Southeast Asia

The Mekong River Basin supports the livelihoods of more than 70 million people who are largely reliant on natural resource-based livelihoods including fishing and agricultural activities (Mekong River Commission 2015). The Mekong River Basin has the largest inland fishery in the world, with 2.6 million tons of fish and 500 000 of aquatic organisms caught annually (Mekong River Commission 2015). The Upper Mekong River Basin originates in the Tibet-Qinghai plateau in China while the Lower Mekong River Basin is shared by five countries in Southeast Asia including Cambodia, Lao People's Democratic Republic, Myanmar, Thailand, and Vietnam. Water resource development, especially the development of irrigation systems to increase agricultural yield, is an integral part of rural development in these countries (Hoanh et al., 2009). The Mekong River Basin is characterized by alternating wet and dry seasons, with the wettest period from June to November accounting for 80% of its annual discharge and driest period occurring in March and April (Lu et al., 2008).

In the Lower Mekong River tributaries, more than 30 dams have been constructed and the construction of more than 85 dams have been proposed (Grumbine and Xu, 2011; Sarnsamak, 2013) (Figure 2.1). Such waterscapes have diverse and multi-scalar social, economic, political, and environmental geographic issues associated with it. For instance, research on dams in the Mekong region reveals that they provide more benefits to urban centers and industrial users than to the predominantly rural societies where the dams are located, which often have a high dependence on wild-capture fisheries for food and livelihood security (Molle et al., 2009; Ziv et al., 2012). Research has hence examined issues of social and environmental justice concerning the geographic implications of dams (Barrington et al., 2012) and of the “water-energy-food nexus” (Middleton et al., 2015). The wealth-creating ability of dams has encouraged a growth in dam construction in the Mekong region, advocated by national energy and water ministries, international development agencies, and an expansive hydropower industry comprising state, quasi-state, and transnational cooperations (Molle et al., 2009; Glassman, 2010). The local population, while reaping economic benefits from dam construction to a certain extent, is affected by dam construction in the form of population displacement (Heming and Rees, 2000; Fujikura et al., 2009), loss of ecosystem services (Dugan et al., 2010), and potentially increase in disease risks (Patz et al., 2000).

Current investigations into the impact of dams in Southeast Asia have focused mainly on fishery and livelihoods of the nearby communities (Orr et al., 2012; Vaidyanathan, 2011). Despite the numerous incidences of disease associated with dams in other parts of the world, such as Rift valley fever (Hassan et al., 2011), schistosomiasis (N’Goran et al., 1997), onchocerciasis (Rodríguez-Pérez et al., 2011), and malaria (Keiser et al., 2005), the influences of dam construction and reservoir creation on the occurrences of infectious diseases in this region have been largely overlooked (Sithithaworn et al., 2012a; Mekong River Commission, 2016).

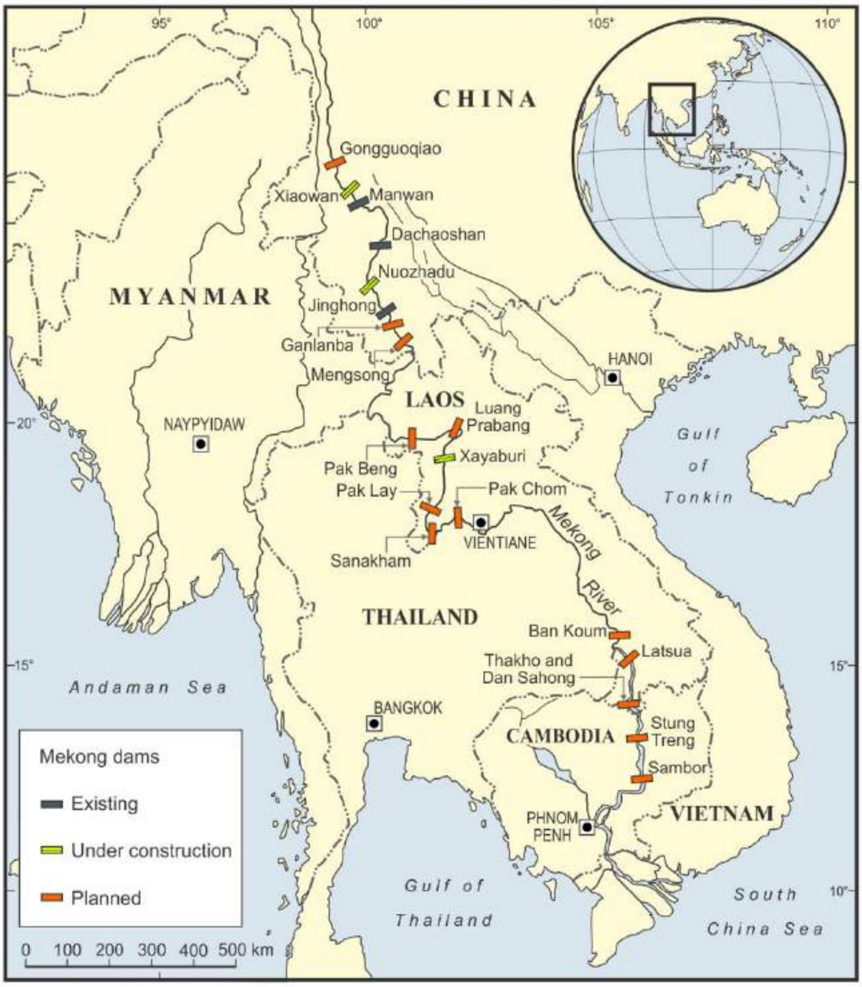


Figure 2.1: Proposed and existing dams of the Mekong river. Only dams to be constructed on the main river stem is shown. Dams that will be constructed on the tributaries are not shown (Ziegler et al., 2013) .

2.2 General background of study area in northeast Thailand

Thailand became the first country in Southeast Asia, in the 1950s, to develop large hydropower dams. Such construction was driven by loans offered by international development agencies to combat communist activities in Southeast Asia (Greacen and Palettu, 2007). Most of the large reservoirs in Thailand are created for the purpose of hydroelectricity generation, while fishery enhancement serves as a secondary benefit for the local fishermen (Jutagate et al., 2012). To date, Thailand's hydropower generation is derived from dam developments within the country and other countries along the Mekong river including China, Myanmar, and Laos (Kuenzer et al., 2013). Thailand also funds hydropower development in countries along the Mekong river (Matthews 2012; Kuenzer et al., 2013). As such, the influence of Thailand's hydroelectric demand can impact a larger geographical region that the immediate area surrounding the dam, further highlighting the close association of such waterscapes with the social and political dynamics in the region.

Northeast Thailand, the poorest region of Thailand, has been targeted for economic development through extensive dam construction and irrigation projects since the late 1950s (Sneddon, 2002). Water development projects in northeast Thailand are relevant to understanding the impact on the Mekong river as northeast Thailand makes up 85% of the river basin in Thailand that drains to the Mekong River (Molle et al., 2009). The first large hydroelectric dam constructed in the northeast Thailand was the Nam Pung Dam which was completed in 1965, followed by the Ubolratana Dam, then the Sirindhorn Dam, and Chulabhorn Dam. Other large reservoir dam systems in northeast Thailand included Lam Pao Dam, Lam Takhong Dam, and Lam Pra Plerng Dam (Molle et al., 2009). In 1975, funds were made available at the subdistrict level for the construction of several thousands of ponds and weirs in villages (Bruns, 1991), greatly altering the surface water connectivity, availability and seasonal variation in water levels in the region. Such developments create major land use modification and alter existing epidemiological relationships (Srivardhana, 1987), exacerbating the proliferation of diseases endemic in this region, including opisthorchiasis (Ziegler et al., 2013). While opisthorchiasis control programs have been implemented since 1984 (Jongsuksuntigul & Imsomboon, 1997; Sithithaworn and Haswell-Elkins, 2003), there is great geographical unevenness in terms of the application, sustainability, and effectiveness of such control programs (Sripa et al., 2015) (more in Chapter 5).

This study was conducted at the Ubolratana reservoir (16º30'-55'N, 102º20'-40'E) situated in northeast Thailand. The Ubolratana reservoir is a eutrophic reservoir with a maximum surface area of approximately 410km^2, at 182m above mean sea level (Kakkaeo, 2004), with a mean depth of 16m (Jutagate et al., 2012). It was created by the construction of a hydroelectric dam along the Pong river, a tributary of the Mekong river, in 1965 (Petr, 1985). To the north of the reservoir lies the province of Nong Bua Lamphu, and to the south, the province of Khon Kaen (Figure 2.2). Samplings sites for this study, including fish sampling, human behavior surveys and interviews, human infection sampling, and environmental data collection, were conducted in the villages around the reservoir. The sampling sites will be described in details in Chapter 3.

The Ubolratana reservoir, and in general, the whole of northeast Thailand, has a tropical monsoonal climate and experiences major changes in water availability throughout the year. Due to the orographic effect, northeast Thailand falls under a rain shadow, where the air is drier and precipitation is usually lower than rest of Thailand (Wilk et al., 2001), except during the rainy season brought about by the southwest monsoon. The cool-dry season occurs during the months of November to February, and the hot-dry season, during the months of March to May (Wang et al., 2016b). Hereinafter, the cool-dry and hot-dry season will be collectively referred to as the dry season. The rainy season occurs from June to October where floods are common, especially during the late rainy season, in the months of August and September (Wilk et al., 2001). While the seasonal rainfall is high at 1104mm during the rainy season, rainfall during the cool-dry and hot-dry seasons are respectively 14 times and 5 times lower than that of the rainy season (Thai Meteorological Department, 2012). Given the irregular water supply in the region across the year, in addition to hydroelectricity generation, Ubolratana reservoir also serves to control floods, enhance water availability and food security (Petr, 1985).

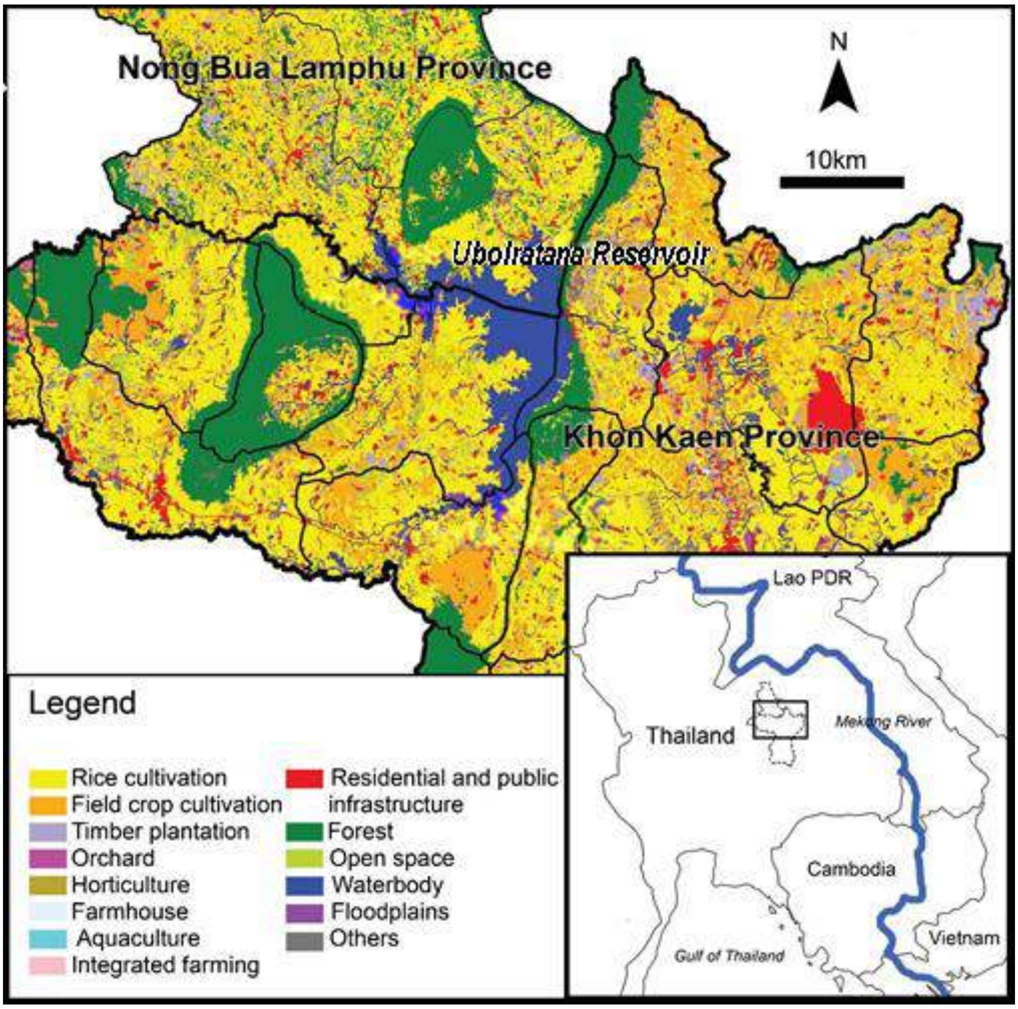


Figure 2.2: Ubolratana is part of the lower Mekong River Basin, which spans Cambodia, Lao People's Democratic Republic, Thailand, and Vietnam. Rice cultivation is the major land use type around the reservoir, and in fact, the majority of northeast Thailand. Adapted from Land Development Department of Thailand (2010) and Land Development Department of Thailand (2011).

After the construction of the Ubolratana reservoir, increased human migration into the region brought about major land use changes. Forest cover in the Nam Pong River Basin, which is the river basin of the Pong river and Ubolratana reservoir, declined by more than 65% since 1965, as forests are being converted into agricultural lands (Wilki et al., 2001) (Figure 2.2). Paddy rice cultivation is the dominant land use type around the Ubolratana reservoir, except to the right of the reservoir, which is occupied by the Nam Phong National Park (Figure 2.2). The conversion of forests to agricultural lands and the conversion of rivers to reservoir lead to changes in species composition of the first and second intermediate hosts of *O. viverrini* (Baran and Myschowoda, 2009) (more in Chapter 6). *Bithynia* snails, the first intermediate hosts of *O. viverrini*, are found in highest species abundance in the rice paddies as compared to rivers, ponds (Wang et al., 2015), and the Ubolratana reservoir (Kittivorachate and Yangyuen, 2004). Fish species change following impoundment of the reservoir as the riverine species are replaced by lacustrine species. Many species of cyprinid fish, the second intermediate hosts of *O. viverrini*, are able to adapt to such open water environment, leading to their increase in the reservoirs (Sornmani et al., 1981; Welcomme et al. 2006).

In addition to the physical geographic uneveness, there appears to be unevenss in the distribution of *O. viverrini* among different sociodemographic groups. For instance, males were found to be more likely to die from *O. viverrini* infection (Haswell-Elkin et al., 1991), exerting economic toll on the family as they are often the main income earners (Sripa et al., 2012b). Fishermen were found with higher *O. viverrini* infection (Forrer et al., 2012; Kaewpitoon et al., 2012a) due to the practice of making raw fish dishes to celebrate the day’s catch (Grundy-Warr et al., 2012). Highland villages are potentially at lower risk of infection due to limited accessibility to freshwater fish and cultural variation from different ethinic groups with less preference for raw fish dishes (Forrer et al., 2012; Wang et al., 2013).

2.3 Justification for choice of study area

The Ubolratana reservoir is chosen for the study of *O. viverrini* infection because the reservoir spans two provinces, the Khon Kaen province and Nong Bua Lamphu province, with different health jurisdictions, allowing for comparisons between the variation in application of *O. viverrini* control programs, and healthcare focus by the different health jurisdictions.

The extensive farming and fishing activities, coupled with deeply-rooted raw eating culture, and the resistance to change in eating habits in this region (Kaewpitoon et al., 2007; Grundy-Warr et al., 2012), allow for the examination of environmental and social influence on the persistence of opisthorchiasis. As Ubolratana reservoir and its surrounding area examined in this study are also part of the wetland ecosystem in the Lower Mekong River Basin, where intensive hydroelectric and irrigation projects have extensively modified the natural waterscapes (Srivardhana, 1987; Sneddon 2002), a focus on the implications of constructed wetlands on human health in the Ubolratana reservoir has potentially much broader applications in the region and beyond.

Moreover, in addition to *O. viverrini*, a wide range of water-related diseases is present in Southeast Asia. These include Cryptosporidium, Giardia lamblia, and Entamoeba species, which are able to cause diarrheal-related diseases; *Plasmoidum* species that cause malaria via mosquito vectors; and *Schistosoma* blood flukes that infect humans through contact with water (Petney and Taraschewski, 2011). There are also at least 12 species of zoonotic helminths, including *O. viverrini*, where aquatic snails and fish serve as the first and second intermediate hosts respectively (Hortle, 2008). Given the considerable impact of dam construction on surface water availability and fish ecology, dam construction and irrigation projects can potentially exacerbate these diseases or at least have complex implications for the study of their life cycles, socioecological connections, and public health. Ubolratana reservoir thus serves as an ideal site to study the environmental/land use, social, and healthcare influence on *O. viverrini* and other water-related disease transmission, allowing issues of epidemiological impact of dams and disease recalcitrance to control efforts to be addressed.

Chapter 3 Sampling Locations

To address the various research objectives, samples were collected from sites around the Ubolratana reservoir. The sampling sites were described below. To ensure logical flow of content, other methods of data collection and statistical analyses were described in the materials and methods section of each results-informed chapter (Chapters 4-6).

3.1 Cyprinid fish sampling locations

To address research objectives 1-4, data for fish species assemblage and fish infection were collected. This allows for the examination of the effects of impoundments on fish infection and human fish consumption behavior. Cyprinid fish were collected four times over the year, from June to July 2012, September to October 2012, January 2013, and June 2013. These sampling periods coincided with the early rainy season in June, late rainy season in September, and the dry season in January to account for the major changes in water level.

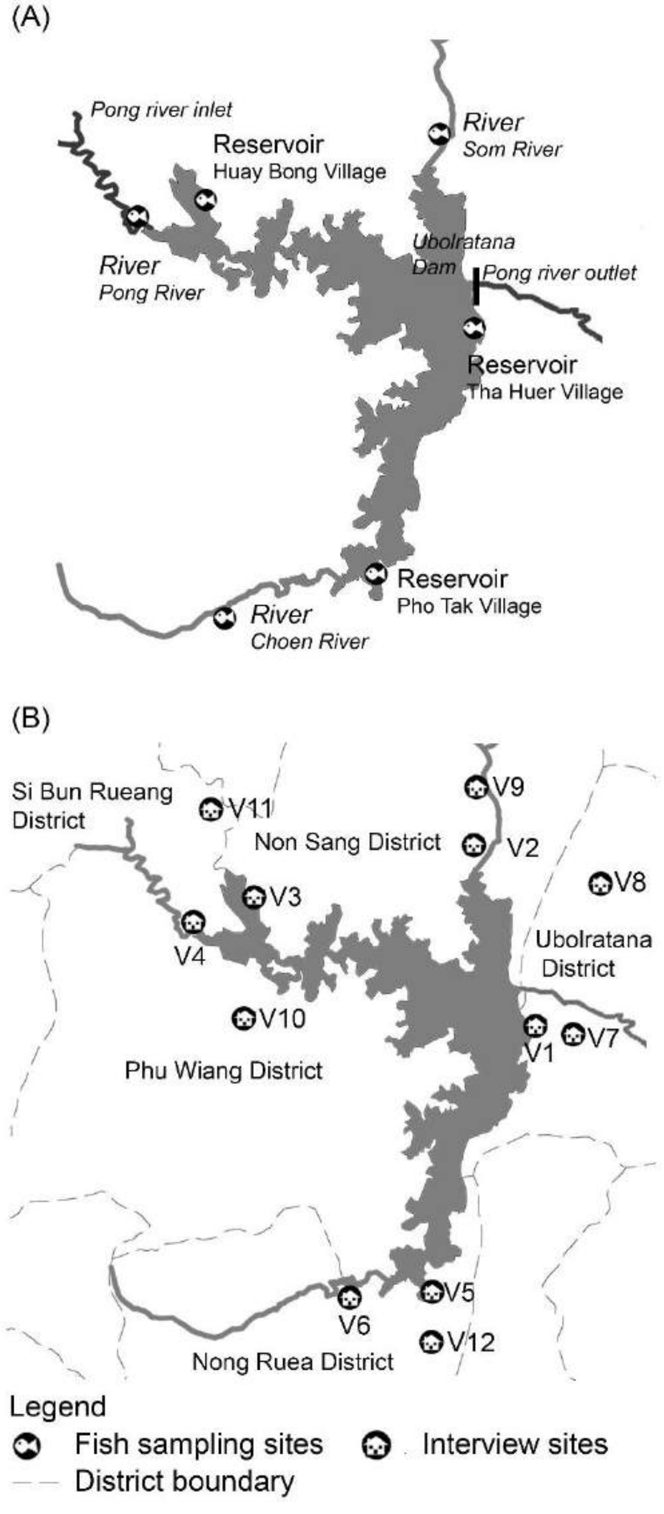


Figure 3.1: (A) Fish sampling sites at the three main river inlets and three locations of the Ubolratana reservoir. (B) Locations of 12 villages sampled for villagers' fish species preference for raw fish dishes and fish procurement locations.

3.2 Human infection sampling locations

To address research objectives 5-8, data for human infection prevalence and intensity, and human socio-economic and behavioral factors were collected. Field collection was conducted from August 2014 to December 2014. Fecal samples and questionnaire-based surveys on socio-economic and behavioral factors of participants were collected in August 2014, and any infected individuals identified from the results were treated during the months of November and December 2014.

Fecal samples and questionnaires were collected from four villages (Figure 3.2). Two of the villages, Sai Mun and Huay Bong, are located in the province of Nong Bua Lamphu, to the north of the reservoir. The other two, Fa Luem and Pho Tak, are in the province of Khon Kaen, to the south of the reservoir (Figure 3.2). In order to examine the influence of the physical environment on human *O. viverrini* infection, villages of varied levels of exposure to fish infected with *O. viverrini* were sampled. Two of the villages sampled, one in the north and one in the south of the reservoir, are located along the river inlets in the study area, and two, one in the north and one in the south of the reservoir, are located along the shore of the main body of the reservoir. Hereinafter, the villages are referred to as north (N)-river, N-reservoir and south (S)-river, and S-reservoir. *O. viverrini* infection prevalence and intensity were compared between villages located along the river inlets and reservoir to highlight and examine possible environmental influences. Samples in the north and south of the reservoir were compared to determine the association of infection with inter-provincial health jurisdiction.

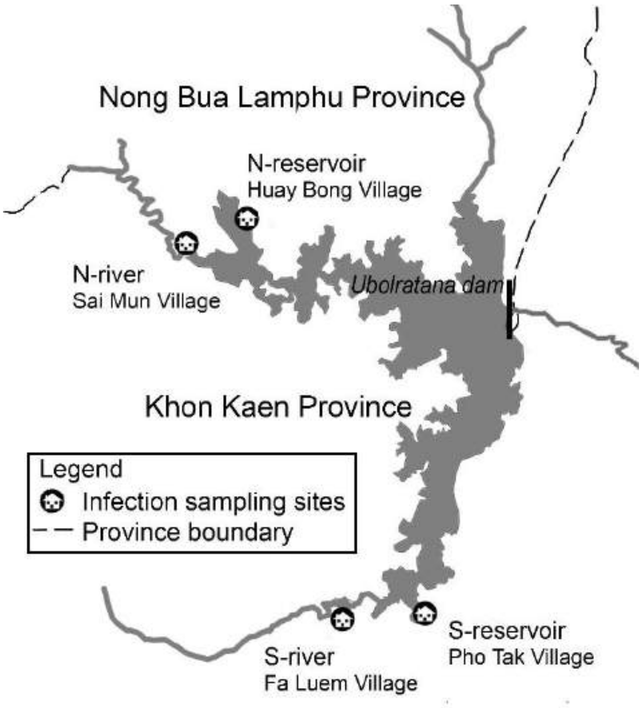


Figure 3.2: Sampling sites for human infection study, two of which are located to the north of the reservoir, and two to the south.

3.3 Rice cultivation sampling locations

To address research objectives 9 and 10, rice cultivation practices and associated human behavior were collected via anonymous door-to-door semi-structured interviews. Samples were collected from farmers belonging to the same villages where fish sampling and human infection sampling were conducted and additional sites around the reservoir. This allow for understanding water use practices related to *O. viverrini* transmission and human behavior related to rice cultivation. Interviews were conducted from February 2015 to April 2015 with 58 rice farmers (Figure 3.3).

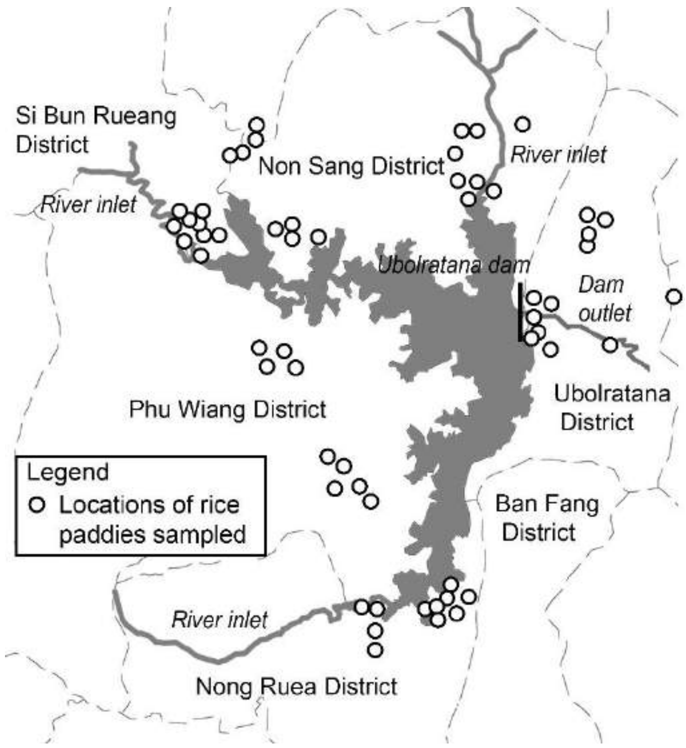


Figure 3.3: Locations of rice paddies belonging to farmers interviewed.

Chapter 4 Dam influences on *O. viverrini* transmission: Fish infection and human fish consumption behavior

Preface

Chapter 4 has been reworked for submission to the Annals of the American Association of Geographers. The paper has been published, along with four co-authors (Ong et al., 2016) (Appendix 1).

4.1 Introduction

Research on interactions between people and environments is central to geography (Harden, 2012), and an evolving literature in relation to links between ecosystems, human landscapes, and public health (Myers et al., 2013; Richter et al., 2015). Of special concern is interactions between humans and waterscapes pertaining to human constructed dams (Fonstad, 2013). Dams are often examined regarding costs and benefits in terms of ecomonic, social, and environmental impacts on different localities and communities (White 1988; Rosenberg et al., 1995). Economically, they do generate significant benefits through revenue derived from hydroelectric power generation and energy exports, and may contribute to drinking, industrial, and agricultural water usage and flood control.

However, dam constructions are responsible for the modification of hydrologic regimes and alteration of biological community interactions and structures for both impoundment dams (Magilligan and Nislow, 2005; Han et al., 2008; Guo et al., 2012), and run-of-the river dams (Csiki and Rhoads, 2010; Anderson et al., 2015). Besides, impoundment dams and their associated irrigation schemes can provide new breeding habitats for vectors and hosts of emerging or re-emerging infectious diseases, triggering public health issues (Patz et al., 2000).

In Southeast Asia, a neglected and "underestimated" public health problem is opisthorchiasis, an infection caused by the liver fluke *O. viverrini* (Sripa et al., 2011). Human infection occurs through consumption of raw or undercooked infected freshwater fish. The connections between water-bodies, livelihoods, and food security are of direct relevance for the 65 million people or so who live in the Lower Mekong River Basin where there is still a high dependence on farming-fishing for incomes and on aquatic natural resources (Baran et al., 2007; Ziv et al., 2012). In a water scarce area, the introduction of a constant supply of water such as reservoirs may encourage the survival and proliferation of *O. viverrini* and its hosts, by expanding its ecological niche previously limited by water availability (Wang et al., 2011). Prevalence of *O. viverrini* infection remains high in the Lower Mekong River Basin where host species, suitable habitats (e.g. rivers, reservoirs, and rice paddies), and raw fish consumption cultures exist together.

O. viverrini infection is associated with a number of hepatobiliary diseases, and is a fundamental risk factor for CCA, a fatal bile duct cancer (Sripa et al., 2011). At least 10 million people in Thailand and the Lao People's Democratic Republic are estimated to be infected with *O. viverrini*, and northeast Thailand has the highest reported incidence of CCA in the world (Sithithaworn et al., 2012a). To date, most attention has been placed on researching *O. viverrini* from epidemiological and clinical perspectives (Sithithaworn and Haswell-Elkins, 2003; Sayasone et al., 2011). Efforts have also been made to understand the ecology of the first intermediate host, *Bithynia* snails (Lohachit, 2004-2005; Wang et al., 2015), and the influences of environmental factors on *Bithynia* snail distribution (Suwannatrai et al., 2011) and human *O. viverrini* prevalence (Forrer et al., 2012; Wang et al., 2013). While mono-disciplinary specialist studies are very necessary, there are areas of interactions between humans and the environment that can only be understood with holistic studies.

A growing body of literature on EcoHealth and One Health calls attention to a holistic and trans-disciplinary approach to explore complex human health issues (Zinsstag et al., 2011; Myers et al., 2013; Richter et al., 2015). Although such approach is still relatively novel in relation to *O. viverrini,* attention has been given to a range of ecological, cultural, and social connections concerning the public health in the Lawa Lake area in northeast Thailand (Sripa et al., 2015). Given the tremendous land use transformations relating to dams, more research is needed on the effects of dams on fisheries, food, and human health, and whether or not dams can be "disease triggers" (Zeigler et al., 2013). Specifically, studies on the effects of impoundment on fish infection, human fish consumption behavior, and their interactions have been scarce, although infected fish are the direct route by which *O. viverrini* is transmitted to humans upon consumption.

Understanding disease transmission and risk necessitates scrutiny of intricate human-waterscape interactions. This is the approach adopted in this chapter to examine the role of impoundment on fish to human *O. viverrini* transmission around the Ubolratana reservoir area in northeast Thailand. Two types of water bodies are compared: the impounded section of the river, which forms the reservoir, versus the non-impounded region, which forms the river inlets of the reservoir. Cyprinid fish of the two water body types are examined for their differences in species biomass assemblage and *O. viverrini* infection to determine if different waterscapes harbor fish of different characteristics for human consumption. Villagers' preferences for fish species for raw fish dish preparations are examined, as well as their choices of water body types for obtaining fish. Specifically, this chapter addresses four questions.

1. Do fish species biomass assemblages vary between the reservoir and the rivers?
2. Do fish infections vary between the reservoir and the rivers?
3. Do villagers have preferences for using certain fish species for raw fish dish preparations?
4. What is the proportion of villagers who obtained fish from the reservoir and what are the factors affecting their procurement location preferences?

   Collectively, these questions contribute to a better understanding of the interactions between the parasite, intermediate host, human behavior, and the environment for *O. viverrini* transmission.

4.2 Materials and methods

4.2.1 Sampling of cyprinid fish

At the fish sampling locations (Figure 3.1 in Section 3.1), lift nets, locally known as *Sa-dung*, were used for most of the fish collection (Figures 4.1A and

4.1B). Alternatively, the brushwood fishing method was employed (Figures 4.1C and 4.1D) in the Som River during the dry season because of its inconsistent water flow, and in other sampling sites during the late rainy seasons due to fishing restrictions and strong water currents. Fishing gears were set near the sides and the middle of the rivers and at the edge of the reservoir where fishermen commonly work. To enable an equivalent comparison across the sites, fishermen were asked to use two-inch nets for fish collection and store the fish on ice. Then, fish were purchased from the fishermen and transported back to the laboratory for analysis.

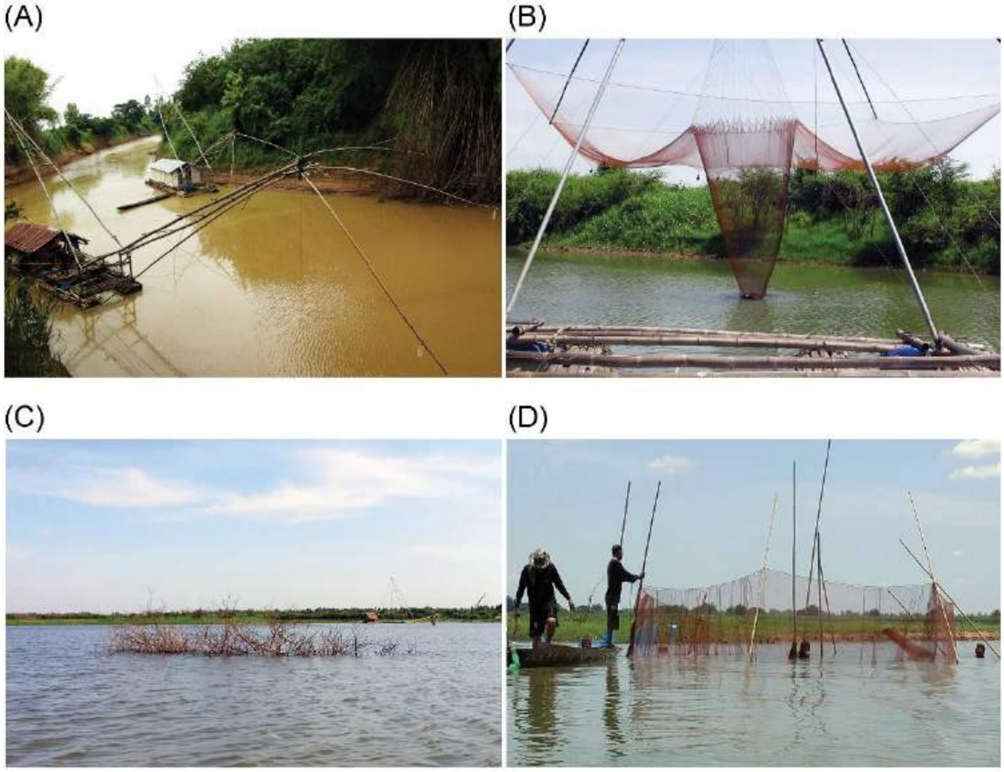


Figure 4.1: Fishing methods used. Lift net fishing, where (A) the poles connected to a square net were lowered into the water; (B) the poles of the lift net were lifted and the net drawn into a funnel shape to gather fish in the middle of the net. Brushwood fishing, where (C) tree branches were placed in the water for fish to gather; (D) nets were placed around the tree branches and the branches were slowly removed to trap the fish in the middle of the circle.

4.2.2 Determinig fish species biomass assemblage

Fish samples from all seasons were grouped to obtain species biomass, relative species abundance, and the frequencies of species encounters at the reservoir and the rivers. To determine if fish assemblage varied between the two water body types, species biomass was used instead of species abundance because the amount of fish consumed by humans may be more dependent on the size than the number of fish. Fish collected across the four sampling seasons were grouped as villagers consume fish throughout the year; the data collected across the sampling seasons thus served as a reflection of the fish species and potential *O. viverrini* parasites consumed. In addition, it is through long-term consumption of *O. viverrini* infected fish, rather than a one-time exposure, that CCA may develop (Parkin et al., 1993; Pinlaor et al., 2004). However, to comprehend if species assemblage of different sampling seasons exhibited similar patterns as the overall species biomass assemblage, samples were also compared by seasons.

Multivariate analyses for community ecology from the vegan package in R (Clarke, 1993; Oksanen, 2013) were employed to determine if fish species biomass assemblage varied between the reservoir and the rivers. Bray-Curtis dissimilarity matrix was used. The analysis of similarity (ANOSIM), permutational multivariate analysis of variance (PERMANOVA) with *adonis*, and multi-response permutation procedures (MRPP) were employed to test for significance of the dissimilarity. Similarity percentage (SIMPER) was tabulated to determine the key fish species contributing to the difference in community structure. Non-metric multidimensional scaling (NMDS) was employed to visualize the level of similarity among the samples of the two water body types in a two-dimensional space. All the statistical analyses were carried out in R unless otherwise specified.

An underlying assumption of this study is that common fish species were likely to be consumed more frequently as they form an important component in the local diets of villagers. Comparing the common species in the reservoir and the rivers thus became essential. Species that were encountered only once in one sampling site were considered as rare species in this study, and were excluded from the above multivariate analyses. Using common species also enabled consistent interpretation of community similarity between different sampling sites (Marchant, 2002).

4.2.3 Examining cyprinid fish for *O. viverrini* infection

To determine fish infection, metacercariae, which is the infective stage of *O. viverrini* parasite, were extracted from the whole fish using acid pepsin digestion (Sithithaworn et al., 1997). For brevity, each fish species within individual sampling site was termed as a "set" of fish. Fish within a set were digested together to obtain the number of *O. viverrini* metacercariae, also referred to as cysts. As fish were digested together, the cyst retrieved represented the average number of cyst found in a mixed pool of both infected and non-infected fish, reflecting the situation when raw fish dishes were prepared and consumed. The level of infection was then quantified using density, computed as the average number of cysts per kilogram of fish, as well as intensity, defined as the average number of cysts per fish in this study.

Density provided a useful measure for comparison with the human consumption behavior because the amount of consumption was likely to be more affected by fish weight than fish number. To examine if fish infection densities varied between the reservoir and the rivers, *t*-test with unequal variances was used on $\log(x+1)$ transformed data, as data were not normally distributed and some data values were less than one. All species of fish were used in this analysis. Samples were also analyzed by seasons to understand if each sampling season exhibited different fish infection densities from the overall infections between the reservoir and the rivers.

4.2.4 Determining villagers' fish species preference for making raw fish dish

Permissions were obtained in order to undertake the research in the villages following ethnical approval recommendations from the Institutional Review Board of the National University of Singapore (Reference code: 12-333E). Participant information sheets were translated into Thai to explain to the participants about the purpose of the study. As a measure of household consumption, one adult from each of the 251 households was approached. A total of 100 males and 151 females were interviewed, their age ranging from 21-90 years old with the mean age of 53.99, se $\pm$ 0.90.

The three most commonly consumed raw fish dishes in northeast Thailand are *koi pla*, *pla som*, and *pla ra*. The viability of the metacercariae in these fish dishes is dependent on the sodium level and duration of fermentation. As koi pla is consumed fresh, most of the metacercariae are viable. Pla som is lightly fermented for two days to one week, and metacercariae are found to be viable for up to 69 hours of fermentation (Prasongwatana et al., 2013). The likelihood of obtaining a live metacercaria from pla ra is very low, due to its long period of fermentation for at least a few months (Prasongwatana et al., 2013). The villagers who consumed koi pla and/or pla som were therefore further asked about their fish species choices for raw fish preparations, with the aid of fish photographs labelled with common names in Thai.

4.2.5 Assessing villagers' preferences for fish procurement locations

Locations from which the 251 households obtained their fish were inquired and grouped into four categories: reservoir, river, market, and others. The categories of reservoir and river indicated that villagers caught or purchased their fish from the reservoir and the rivers, respectively. The category of market represented villagers who purchased fish from the market or mobile fish sellers but did not know the source of the fish. The category of others denoted that fish were obtained from other water body types, such as rice paddies or irrigation canals.

The location preference composition in each village was expressed as percentages for hierarchical clustering analysis to identify clusters of villages with similar preferences for fish procurement locations. The analysis was performed using the pvclust package with the *euclidean* distance measure and the group average cluster method, and approximately unbiased (AU) *p*-values were tabulated using multiscale bootstrap resampling (Suzuki and Shimodaira, 2006). AU *p*-values above 95%, generated using 1000 bootstrap replicates, were used to indicate that the clusters observed did not occur by chance.

Additionally, the locations of villages and their proximities to the reservoir and the rivers, from which the villagers reported to have obtained their fish, were analyzed in ArcGIS10.0 (Environmental System Research Institute, 2010). Reasons for obtaining fish from certain water body types were also compiled through the surveys.

4.3 Results

4.3.1 Biomass assemblages of cyprinid fish

Eleven cyprinid fish species were obtained from the rivers and the reservoir. Fish species biomass assemblages (Figure 4.2A) and relative species abundances (Table 4.1) varied between the two water body types. *Henicorhynchus siamensis* dominated the rivers by biomass (85%) and abundance (83.3%). In the reservoir, *Labiobarbus leptocheilus* (27.0%) and *Cyclocheilichthys armatus* (24.0%) were the most abundant fish (Table 4.1), while *L. leptocheilus* (22%) and *H. siamensis* (21%) dominated in terms of biomass (Figure 4.2A). Similar to the findings for the whole year, *H. siamensis* consistently dominated the rivers across the seasons, and was found in greater proportion in the rivers than in the reservoir (Figures 4.2B-D). Similar species were obtained in the reservoir across the seasons although the most dominant species varied (Figures 4.2B-D).

Other species of fish obtained from the rivers were in relatively low abundance, although *C. armatus*, *Osteochilus hasselti*, and *Puntioplites falcifer* were encountered in four out of seven samples (57.1%) from the rivers (Table 4.1). *C. armatus* was encountered in all eleven samples taken from the reservoir, and *H. siamensis*, *L. leptocheilus*, and *P. falcifer* were also commonly found in the reservoir, evident in more than 90% of the samples obtained (Table 4.1). Conversely, *Barnonymus gonionotus*, *Chirrhinus mrigala*, and *Puntius brevis* were obtained only from the reservoir, and *Hampala macrolecipoda* only from the rivers (Figure 4.2 and Table 4.1). As *C. mrigala* and *H. macrolecipoda* were found only once from one sampling site, they were considered as the rare species in this study.

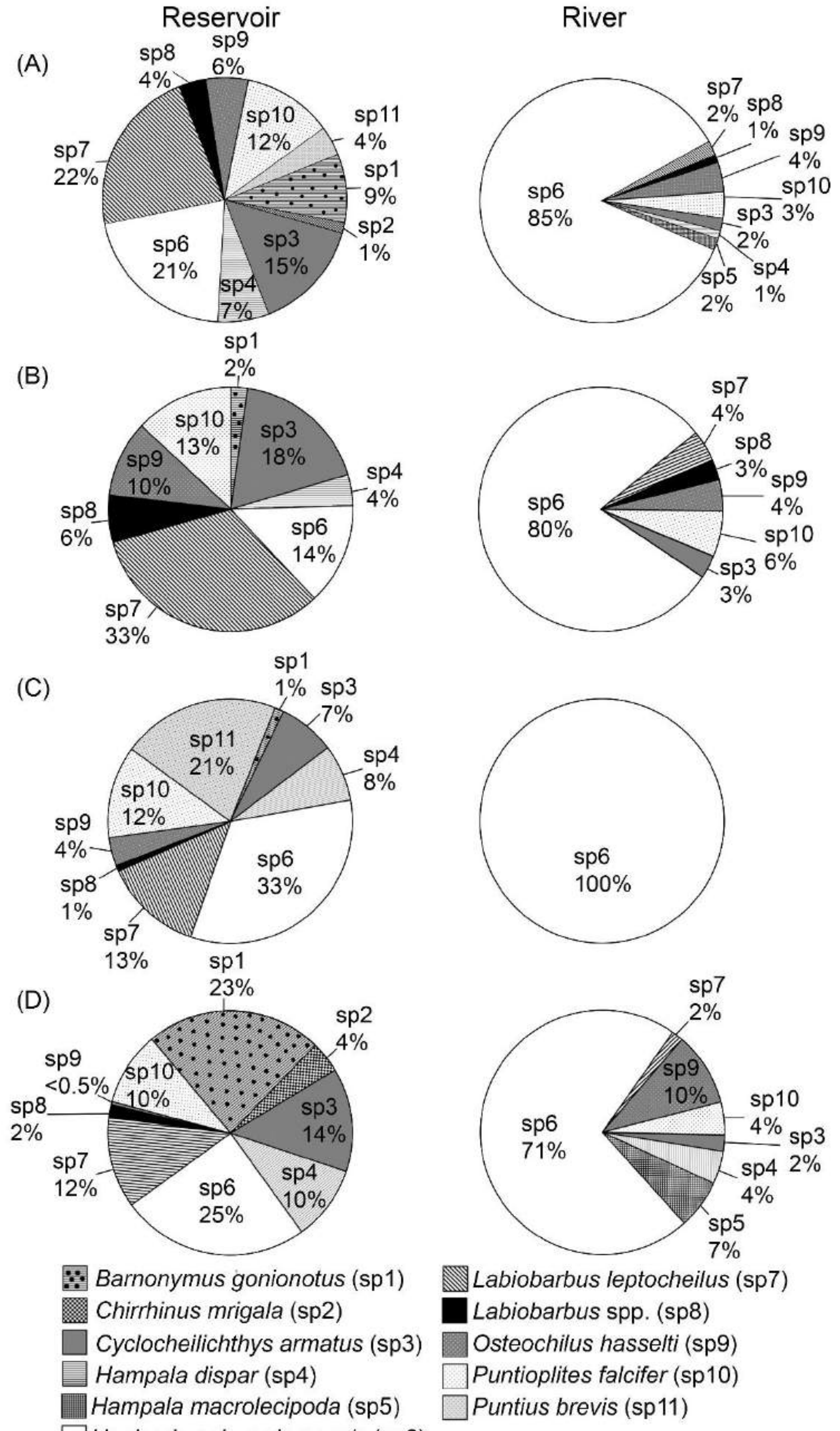


Figure 4.2: Differences in cyprinid fish species biomass assemblages between the reservoir and the rivers in (A) all seasons; (B) early rainy season; (C) late rainy season; and (D) dry season.

Table 4.1: Comparison of biomass, abundance, and frequency of encounters of fish species between the reservoir and the rivers.

Fish species	**Total weight (kg)**		**Relative species abundance (%)**		**Samples with fish encounters (%)**	
	Reservoir	**River**	**Reservoir (*n* = 2915)**	**River (*n* = 432)**	**Reservoir (*n* = 11)**	**River (*n* = 7)**
Barnonymus gonionotus	3.25	0.00	4.9	0.0	45.5	0.0
Chirrhinus mrigala	0.50	0.00	<0.1	0.0	9.1	0.0
Cyclocheilichthys armatus	5.25	0.33	24.0	3.9	100.0	57.1
Hampala dispar	2.40	0.20	2.7	0.9	54.6	14.3
Hampala macrolecipoda	0.00	0.30	0.0	1.2	0.0	14.3
Henicorhynchus siamensis	7.48	16.32	11.3	83.3	90.9	100.0
Labiobarbus leptocheilus	7.87	0.37	27.0	3.2	90.9	42.9
Labiobarbus spp.	1.30	0.20	9.5	2.1	81.8	14.3
Osteochilus hasselti	2.01	0.75	4.7	2.3	54.6	57.1
Puntioplites falcifer	4.20	0.64	9.1	3.0	90.9	57.1
Puntius brevis	1.39	0.00	6.8	0.0	18.2	0.0
Total	35.65	19.11	-	-	-	-

Comparing fish biomass assemblages among seasons of the same water body type rendered no significant difference, suggesting that fish biomass assemblages of the same water body type were similar across the seasons (Table 4.2). When fish biomass assemblages were compared between the two water body types across the whole year, the differences were significant with all three assessments, although species overlap between the two water body types rendered moderately low values in *R*, R^2 and *A* (Table 4.3). When the two water body types were analyzed by seasons, significant difference was only detected in the early rainy season using PERMANOVA and MRPP (Table 4.3). The NMDS plot indicated that river samples were clustered to the right while reservoir samples were clustered to the left, with some mixing in between the two groups (Figure 4.3), confirming that fish species assemblages were more similar within the same water body type than between the rivers and the reservoir.

Table 4.2: Comparing fish biomass assemblages among sampling seasons for the reservoir and the rivers using the analysis of similarity (ANOSIM), permutational multivariate analysis of variance with adonis, and multi-response permutation procedures (MRPP).

Water body	**Season with sample size**	**ANOSIM**		**Adonis**		**MRPP**	
		R	***p*-value**	R^2	***p*-value**	***A***	***p*-value**
Within river	Early rainy-3 Late rainy-2 Dry-2	0.10	0.28	0.40	0.23	0.08	0.25
Within reservoir	Early rainy-5 Late rainy-3 Dry-3	0.19	0.11	0.25	0.23	0.01	0.64

Note: A = chance-corrected within-group agreement. A value of 1 represents complete homogeneity within groups and a negative value represents greater heterogeneity than expected by chance within groups.

Table 4.3: Comparing fish biomass assemblages between the reservoir and the rivers by seasons using the analysis of similarity (ANOSIM), permutational multivariate analysis of variance with adonis, and multi-response permutation procedures (MRPP).

Season	Water body type with sample size	ANOSIM		Adonis		MRPP	
		R	***p*-value**	***R*²**	***p*-value**	***A***	***p*-value**
All seasons	Reservoir-11 River-7	0.26	0.01	0.27	0.002	0.14	0.004
Early rainy season	Reservoir-5 River-3	0.44	0.06	0.45	0.04	0.20	0.03
Late rainy season	Reservoir-3 River-2	0.33	0.21	0.39	0.19	0.24	0.22
Dry season	Reservoir-3 River-2	0.08	0.39	0.33	0.30	0.05	0.31

Note: *A* = chance-corrected within-group agreement. A value of 1 represents complete homogeneity within groups and a negative value represents greater heterogeneity than expected by chance within groups.

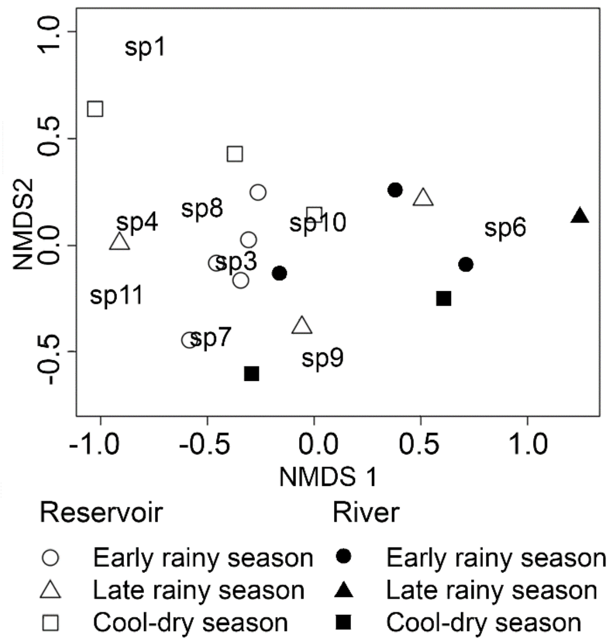


Figure 4.3: Nonmetric multidimensional scaling (NMDS) plot of fish species biomass assemblages (Stress = 0.11). The sampling sites with similar species biomass assemblages are close to each other in the plot. Fish species are labeled to show the influence of particular species on the positioning of each sample. Rare species of this study are excluded from the analysis. The labels (e.g., sp1, sp3) denote the same species in Figure 4.2.

Analysis from SIMPER suggested that the strongest discriminating species for the differences in the two water body types was *H. siamensis*, which contributed to 43.0% of the differences in biomass assemblage. *P. falcifer* contributed to a further 10.6% of the differences, *L. leptocheilus* 9.8%, and *C. armatus* 8.5%. This was in agreement with the NMDS plot illustrating that *H. siamensis* was largely responsible for the placement of the river samples toward the right of the plot (Figure 4.3). While *H. siamensis* was found across all six sampling sites and in seventeen out of the eighteen samples, its strong influence on community differences suggested that the greater biomass of *H. siamensis* obtained from the rivers (Figure 4.2) accounted for the differences in the community structures of the two water body types.

4.3.2 *O. viverrini* infection in cyprinid fish

The average fish infection was higher in the reservoir than in the rivers (Table 4.4). The density and the intensity of infection for the reservoir were respectively 24.65 cyst/kg and 0.35 cyst/fish, compared to respectively 0.06 cyst/kg and 0.003 cyst/fish for the rivers (Table 4.4). Fish overall *O. viverrini* infection density was found to be statistically significantly higher in the reservoir than in the rivers (*p*-value = 0.02), although the difference in infection intensity for the two water body types was only marginally significant (*p*-value = 0.07). Six out of ten fish species were found to be infected in the reservoir, with *L. leptocheilus* having the highest infection density (97.20 cyst/kg), followed by *C. armatus* (17.14 cyst/kg) and *Hampala dispar* (3.75 cyst/kg). Conversely, only one out of eight species, i.e., *H. siamensis* (0.06 cyst/kg), was found to be infected in the rivers. Similar to the patterns observed in overall fish infection density between the two water body types, fish infection was consistently lower in the rivers than the reservoir in all sampling seasons (Table 4.5).

Table 4.4: *O. viverrini* infection density and intensity in fish obtained from the reservoir and the rivers.

Fish species	**Density (cyst/kg)**		**Intensity (cyst/fish)**	
	Reservoir (se)	**River (se)**	**Reservoir (se)**	**River (se)**
Barnonymus gonionotus	0.31 (±0.07)	-	0.01 (±3x10^{-3})	-
Chirrhinus mrigala	0.00	-	0.00	-
Cyclocheilichthys armatus	17.14 (±7.43)	0.00	0.15 (±0.06)	0.00
Hampala dispar	3.75 (±16.28)	0.00	0.15 (±0.44)	0.00
Hampala macrolecipoda	-	0.00	-	0.00
Henicorhynchus siamensis	0.00	0.06 (±0.16)	0.00	4x10^{-3}
Labiobarbus leptocheilus	97.20 (±22.33)	0.00	1.08 (±0.17)	0.00
Labiobarbus spp.	0.00	0.00	0.00	0.00
Osteochilus hasselti	0.50 (±2.86)	0.00	0.01 (±3x10^{-3})	0.00
Puntioplites falcifer	3.10 (±1.45)	0.00	0.06 (±0.03)	0.00
Puntius brevis	0.00	-	0.00	-
Average	24.65 (±3.78)	0.06 (±0.05)	0.35 (±0.04)	3x10^{-3} (±1x10^{-3})

Note: se = standard error; - = no fish available

Table 4.5: Comparison of fish *O. viverrini* infection density between the reservoir and the rivers by seasons.

Fish species	**Early rainy season**		**Late rainy season**		**Dry season**	
	Reservoir (se)	**River (se)**	**Reservoir (se)**	**River (se)**	**Reservoir (se)**	**River (se)**
Barnonymus gonionotus	0.00	-	0.00	-	0.36	-
Chirrhinus mrigala	-	-	-	-	0.00	-
Cyclocheilichthys armatus	5.81 (±2.33)	0.00	2.00 (±16.67)	-	43.03 (±24.29)	0.00
Hampala dispar	11.43 (±30.55)	-	0.00	-	0.83 (±0.63)	0.00
Hampala macrolecipoda	-	-	-	-	-	0.00
Henicorhynchus siamensis	0.00	0.17 (±0.37)	0.00	0.00	0.00	0.00
Labiobarbus leptocheilus	137.84 (±41.07)	0.00	0.00	-	0.00	0.00
Labiobarbus spp.	0.00	0.00	0.00	-	0.00	0.00
Osteochilus hasselti	0.00	0.00	0.00	-	20.00	0.00
Puntioplites falcifer	5.41 (±2.49)	0.00	0.00	-	0.85 (±0.75)	0.00
Puntius brevis	-	-	0.00	-	-	-
Average	47.28 (±7.08)	0.14 (±0.09)	0.15 (±2.94)	0.00	6.24 (±4.16)	0.00

Note: se = standard error, and not available for values with one set of fish; - = no fish available.

4.3.3 Fish species preferences for use in making raw fish dishes

As different fish species were found to have different infection levels, villagers' choices of fish species for making raw fish dishes had implications on the likelihood of human infection. Out of the 251 respondents, 30.7% ate raw koi pla and/or pla som. The total percentages of fish species choices for making kio pla and pla som added up to above 100% (Figure 4.4) because more than one species was often used.

H. siamensis (66.7%), *H. dispar* (30.1%), and *C. armatus* (26.2%) were commonly used for koi pla, while *O. hasselti* (4.8 %) and *Labiobarbus* spp. (7.1%) were less preferred (Figure 4.4). Villagers who consumed kio pla referred to the "crunchy" texture of a raw fish that made it delicious when it was chopped up with various ingredients (e.g. herbs, spring onions, garlic, chili, lime juice) added for flavor and eaten soon afterwards. Some villagers preferred *C. armatus* for koi pla because the bitterness of its intestines added flavor to the dish. The most commonly used species for pla som was *H. siamensis* (56.7%), and *P. brevis* (40.9%) was another popular choice; both species were found with low *O. viverrini* infection densities. The least commonly used species for pla som was *Labiobarbus* spp. (18.3%). *O.hasselti* was more commonly used for pla som (28.3%) than for koi pla, likely due to different dish preparation processes. As the fish was diced together with the bones during koi pla preparation, *O. hasselti* was the least preferred species because its bones were thicker and harder to be consumed. Villagers were less selective for fish species for pla som because mixed species of fish could be grinded together to produce fish paste, or simply bigger fish were fermented whole, without being grinded.

In addition to taste, many villagers commented that their choice of fish species depended on the species that they managed to catch. Thus, several fish species could be used together for raw fish dishes, and this could include species with relatively high *O. viverrini* infection densities, such as *L. leptocheilus* and *C. armatus* (Figure 4.4).

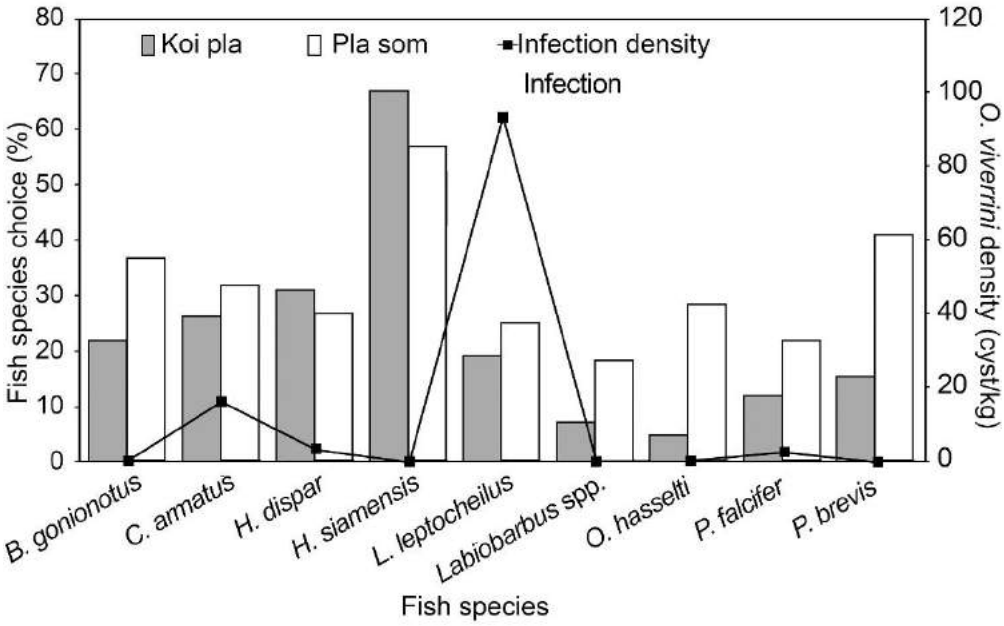


Figure 4.4: Villagers' fish species choices for preparing raw fish dishes of *kio pla* and *pla som* (bar graph) and the overall *O. viverrini* infection density of each fish species sampled from the study area (line graph).

4.3.4 Villagers' fish procurement locations

Location and proximity in relation to water bodies is relevant to procurement. Hierarchical clustering grouped the twelve villages into three distinct clusters according to similarities in fish procurement locations (Figure 4.5). Cluster 1 consisted of villages V1, V3 and V5, all of which were located within 1 km buffer of the reservoir (Figure 4.6A). Cluster 2 consisted of V7, V9, V10 and V11, all of which were located beyond 3 km buffer from the reservoir (Figure 4.6B). Cluster 3 consisted of V4 and V6, both located within 1 km buffer of the main river inlets (Figure 4.6A).

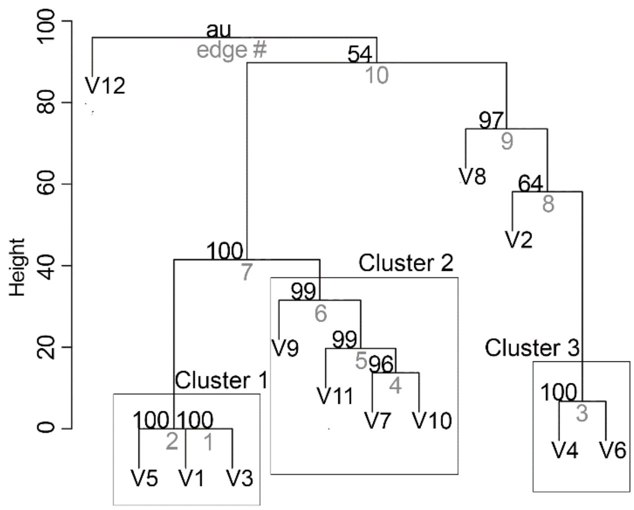


Figure 4.5: Hierarchical clustering of fish procurement location preferences of 12 villages (V1 to V12). Clusters were highlighted by rectangles.

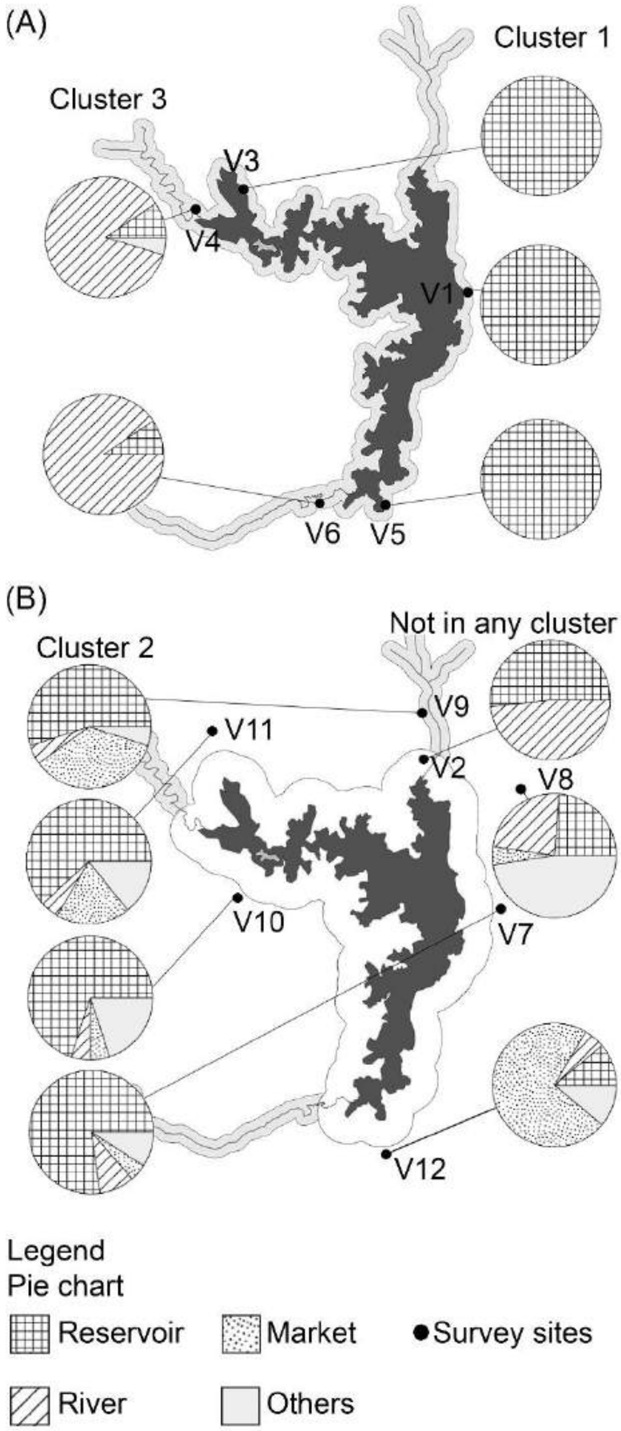


Figure 4.6: Composition of the fish procurement location preferences of villages grouped by hierarchical clustering into (A) Clusters 1 and 3, with 1 km buffer of the reservoir and rivers shown, and (B) Cluster 2 and those not grouped into any of the clusters, with 1 km buffer of the rivers and 3 km buffer of the reservoir illustrated.

All Cluster 1 villagers reported getting their fish from the reservoir (Figure 4.6A), due to the close proximity to and consistent supply of fish from the reservoir. In Cluster 2, at least 55% of the villagers were reliant on fish from the reservoir, resulting in Clusters 1 and 2 being much more similar to each other than to Cluster 3 (Figure 4.5). However, unlike Cluster 1, where all villagers obtained their fish directly from the reservoir, respondents from Cluster 2 also procured fish from a variety of sources under the others category (Figure 9B). Cluster 2 villages were further away from the reservoir, making it a less immediate choice for fishing than it was for villagers in Cluster 1. Some respondents in Cluster 2 would get their fish from the reservoir if there were insufficient fish from other water body types.

Most of the Cluster 3 villagers reported getting their fish from the rivers (Figure 4.6A), with 85.7% of the villagers from V4 and 90.5% from V6. This finding was in contrast to V2, whose spatial location is just slightly beyond 1 km from the river but was not grouped into any of the three clusters, as well as to V9, whose spatial location is within 1 km from the river but was grouped into Cluster 2 (Figure 4.6B). In V2, 51.7% of the people obtained their fish from the reservoir while 48.3% from the river. The difference in fish procurement locations between V2 and Cluster 3 villages of V4 and V6 could be due to the inconsistent water supply from the Som River, where V2 is located. More respondents from V2 than from V4 and V6 stated that the river was unable to provide them with sufficient fish. Indeed, according to the accounts of several villagers in V9, which is located at the upstream of V2, the water flow of the Som River was inconsistent, presenting an issue for them to obtain fish from the river, particularly during the dry season. Consequently, V9 had higher proportion of villagers obtaining fish from the market but much lower proportion of villagers obtaining fish from the river than V2, although both villages are located at approximately 1 km away from the Som River.

It is noteworthy that V8 and V12 exhibited very different patterns of fish procurement location preferences. Similar to the Cluster 2 villages, V8 is located more than 3 km from the reservoir. However, 23.8% of the V8 villagers responded to obtain fish from their nearby small river tributaries, resulting in a higher proportion of people obtaining fish from the river category than the Cluster 2 villages. V8 also had the highest proportion of villagers (47.6%) obtaining fish from the others category. Alternatively, more than 70% of the V12 villagers purchased their fish from the market or mobile sellers (Figure 4.6B). The origin of those fish was unknown although it was possible that some of the fish might have been caught from the Ubolratana reservoir.

4.4 Discussion

4.4.1 Cyprinid fish biomass assemblage and *O. viverrini* infection

Dams and impoundments have been documented to ecologically impact on fish species assemblages (Han et al., 2008; Gao et al., 2010; Li et al., 2013). They alter river flow regimes, leading to the replacement of species adapted to moving water by species adapted to still water (Morley, 2007; Li et al., 2013). Fish species can also be altered due to the inhibition of longitudinal migration, or the loss of floodplains needed for spawning, feeding, or serving as nursery grounds (Dugan et al., 2010). Surveys of fish species composition in the Ubolratana reservoir found a decrease from 76 species prior to the dam construction in 1965 to 37 species in 1982, with fish species of strict riverine characters decreased the most after impoundment (Petr, 1985). In this study, cyprinid fish species assemblages differed between the rivers and the reservoir (Figure 4.2; Table 4.1). *H. siamensis* predominated the rivers, accounting for more than 80% of the biomass assemblage and species abundance, because it is a riverine species whose life cycle depends on lateral migrations to the floodplains during the rainy season (Suvarnaraksha et al., 2011). Alternatively, *H. siamensis* only accounted for 11.3% of the fish species abundance in the reservoir, after *L. leptocheilus* (27.0%) and *C. armatus* (24.0%) (Table 4.1).

That the overall *O. viverrini* infection in fish species (Figure 4.4) was less in *H. siamensis* than in *H. dispar* and *C. armatus* in this study echoes the findings in prior work. A review on *O. viverrini* relevant research (Wang, 2012) showed that *H. siamensis* infection was reported only once among the papers reviewed as compared to ten times for *H. dispar* and five times for *C. armatus*. It was, however, unclear if the lack of infection for *H. siamensis* was due to the absence of *H. siamensis* in those studies or the lack of infection, as some of those studies reported only fish found with positive infection. Recent work in Cambodia (Touch et al., 2013) found 10.9% infection in *H. siamensis*, lower than 31.3% in *H. dispar* and 84.6% in *C. armatus*, although prevalence, as opposed to density, was reported. Conversely, *L. leptocheilus*, a species with high infection in this study, was not commonly found with high infection in prior work.

The average fish infection density of 24.65 cysts/kg in the reservoir was significantly higher than that of 0.06 cysts/kg in the rivers (Table 4.4). This was because, two of the three most dominant species in the reservoir, *L. leptocheilus* and *C. armatus*, were also the species with the highest *O. viverrini* infection densities (97.20 and 17.14 cysts/kg respectively) (Table 4.4). In contrast, *H. siamensis*, which dominated the rivers (Figure 4.2), was found with much lower infection density (0.06 cyst/kg) (Table 4.4). Of all the infected fish species, *B. gonionotus, H. dispar*, and *P. falcifer* were also more frequently encountered in the reservoir than the rivers (Table 4.1). The fish infection density obtained in the reservoir is consequential to human infection risk due to the high worm recovery of 50% in its final host, as seen in experimental animals (Sripa and Kaeweks, 2000). This can give rise to substantial worm burden in people through long-term raw fish consumption.

These findings are significant as they validate the potential epidemiological implications of dam constructions in the endemic regions of *O. viverrini*, which has received scientific postulation relating to the potential implications dams have on parasite life-cycles (Ziegler et al., 2013). They are also important in view of the fact that reservoirs affect fish composition (Fernando and Holcik, 1982; Baran and Myschowoda, 2009), which echoes the more widespread consequences of hydropower and reservoir development in the Lower Mekong River Basin. Numerous fish species in the Lower Mekong River Basin adapted to the annual flood pulse and migratory paths of many common species can be adversely affected by such hydropower development (Baran and Myschowoda, 2009; Anderson et al., 2015). Thus, if the remaining fish species that thrive in the reservoir are more commonly found to be infected with *O. viverrini*, it is likely that human-induced hydropower and reservoir transformations to the landscape are providing suitable conditions for the perpetuation of a life cycle associated with human infection and disease. A focus on land use dynamics and landscape ecology are critical to the multi-disciplinary explorations of food-borne (and water-borne) parasite life cycles and human infection.

4.4.2 Human preferences for fish species and fish procurement locations

Fishing is embedded into the everyday lives of the villagers to supplement incomes and diets (Grundy-Warr et al., 2012). Fish species choice for raw fish dish is a consequence of both fish taste and availability. Similar to prior work that has suggested *H. siamensis* as an important and commonly consumed fish species in the Lower Mekong region (van Zalinge et al., 2004; Suvarnaraksha et al., 2011), this study shows that *H. siamensis* was the preferred fish species by more than 65% and 55% of the villagers respectively for making koi pla and pla som (Figure 4.4). Although *H. siamensis* has low *O. viverrini* infection density in the Ubolratana area, villagers do consume other fish species with relatively high *O. viverrini* infection. More than 25% of the villagers have used *C. armatus* and *H. dispar*, which have been found with relatively high infection densities both in this study (Figure 4.4) and in prior work (Sithithaworn et al., 1997; Touch et al., 2009), and more than 20% of the villagers have used *L. leptocheilus*, the species with the highest infection density in this study (Figure 4.4; Table 4.4), for making raw fish dishes. These species of fish should be avoided as consuming them may increase the likelihood of human *O. viverrini* infection.

The conversion of river habitats into reservoirs through dam impoundments can, however, decrease the relative abundance of the riverine species *H. siamensis*, resulting in the dominance of potentially high *O. viverrini* infected fish species, such as *L. leptocheilus* and *C. armatus*. Since mixed species of fish tend to be used for making raw fish dishes and the use of fish often depends on what villagers can catch, dam impoundments in *O. viverrini* endemic areas may increase the risk of human *O. viverrini* infection through the increasing possibility of consuming fish species highly infected with *O. viverrini*.

Fish procurement locations are determined foremost by proximity to homes, followed by fish availability. Such practice is natural in rural wetlands and rice-fish cultures, as around any one village, there are different water bodies being affected by pronounced rainy and dry seasons. Among the various water body types, the reservoir supplies fish to a larger number and a broader spatial extent of villages beyond the area that is immediately boarding the reservoir. This is because, if villagers who live further away from the reservoir cannot acquire sufficient fish from their surrounding water bodies such as rice paddies, they would still obtain fish from the reservoir, or the market, for which the source of fish is presumably also from the reservoir. Given that the findings of this chapter demonstrate the notable differences in fish *O. viverrini* infections between the reservoir and the rivers, the reservoir has the potential to increase the likelihood of eating *O. viverrini* infected fish by people across different villages. The reservoir is thus an important water body source to target for efforts to bring down *O. viverrini* infection.

4.4.3 Knowledge, attitudes, and practices of raw fish consumption

Consumption of raw, undercooked and fermented fish is deeply embedded into the ways of life and cultural landscape of large parts of the Lower Mekong region, spanning the borders of Thailand, Lao People's Democratic Republic, Cambodia, and Vietnam (Grundy-Warr et al., 2012; Xayaseng et al., 2013). Some forms of consumption seem to be clearly related to livelihoods; for instance, koi pla can be easily prepared at fish landing sites by local fishers as a cheap and tasty dish after a mornings' fishing activity. It is thus a common practice for fishers, mostly male, to consume koi pla in the fields or near fish landing sites. Additionally, it is common for fishermen to defecate directly into the water while they spend long hours fishing on their mobile boathouses. An infected human can carry *O. viverrini* eggs in the feces, thereby facilitating the persistence of *O. viverrini* in the landscape. Alternatively, pla ra is more regularly eaten to accompany other dishes in the house, or additive to particular dishes and spicy salads that are popular throughout northeast Thailand (Kaewpitoon et al., 2007).

Existing epidemiological and public health studies suggest that attitudes are influenced by "generation to generation" practices in different parts of the Lower Mekong region. For instance, Xayaseng et al., (2013) revealed that consuming raw or insufficiently cooked fish dishes "is a long-standing tradition that has been practiced for many generations" and relates to "many social and cultural ceremonies" in rural southern Laos. Thus, dealing with the issue of eating practices is essentially dealing with "practices-as-performance" (Schatzki, 1996), including sociality and individual activity during collective celebrations, such as the *Sonkran* water festival in April, the annual rocket festival at the beginning of the rainy season in May and June, weddings, and even celebrating a day's fishing. In the context of this study, the "raw attitudes" relating to eating behaviors are more difficult to tackle precisely because they relate to long established and situated "practices" within the rural wetlands of the Lower Mekong region (Grundy-Warr et al., 2012), and food cultures are vitally linked to the geographies of everyday life as well as the historically contextual lifestyles and socio-ecological connections of the region.

4.5 Conclusion

This chapter investigated the interplay of waterscapes, fish hosts, and human behavior on *O. viverrini* infection risk in the Ubolratana reservoir area in northeast Thailand. The reservoir and its river inlets harbored different fish species of varied *O. viverrini* infection densities, with the overall fish infection higher in the reservoir than the rivers. The reservoir supplied fish to a larger number and a broader spatial extent of villages beyond the boarding area of the reservoir. Coupling the results of *O. viverrini* infection in various fish species with findings on human raw fish consumption behavior shed lights on the potential risks of human infection. In particular, reservoirs had the implications in increasing human *O. viverrini* infection likelihood through the potential change of fish species assemblages after impoundments, the higher *O. viverrini* infection densities of the fish found within, the accessibility for villagers to procure fish for consumption, and the supply of fish to the surrounding villages.

A holistic approach focusing on dams, fish, food, and in the subsequent chapters, on public health and role of rice cultivation in infection transmission, can productively add useful insights into multi-disciplinary understandings of water- and food-borne disease dynamics. While this study focuses on one dam and reservoir site, established for five decades, there are implications for literally thousands of potential sites, albeit with differing physical and socio-environmental characteristics. Detailed comparative reservoir research needs to be done because there are different environmental conditions relating to distinct reservoirs, which may favor different species of fish over others.

Chapter 5 The pathogenic landscape of human *O. viverrini* infection: Role of physical and social environment, and healthcare influence

Preface

This chapter has been reworked as a research paper for publication in PLOS Neglected Tropical Diseases with contributions from five co-authors (Appendix 1).

5.1 Introduction

Helminthiases are the most common NTDs in Southeast Asia (Hotez et al., 2015). They disproportionally affect the poor or marginalized population in developing countries, trapping the afflicted in a vicious cycle of poor health outcomes and poverty (World Health Organization, 2016).

Many existing disease control programs for such disease have primarily relied upon chemotherapy for helminthiases control (Prichard et al., 2012) but chemotherapy effects are temporary if underlying factors predisposing people to infection remains (McManus et al., 2010) Helminth infections, and indeed many infectious diseases, are strongly influenced by environmental and socio-economic conditions, and by human behavior and the effectiveness of health service provision (Hotez and Herricks, 2015), or what Lambin et al. (2010) term the pathogenic landscape for disease.

The pathogenic landscape predisposes the local population to diseases and accounts for the high geographical focality of many helminth diseases. This is because the complex and highly dynamic process of infection, where a change in one underlying factor can drastically alter the situation for the other conditions will lead to the uneven distribution of diseases even in places with seemingly similar conditions. Such unevenness is observed in opisthorchiasis, an infection caused by the foodborne trematode *O. viverrini*, where large variations in the disease burden may be observed in a relatively small geographic area (Andrews et al., 2008; Grundy-Warr et al., 2012). Despite the close association of helminth parasite life cycle and life strategies with the physical environment and animal hosts, the EcoHealth/One Health approach has rarely been applied to study of helminthiasis (Robertson et al., 2014). Yet, understanding such factors that underpin infections with a high geographical focality can provide important contributions to the framework of One Health approach to a broader range of diseases, enabling intervention efforts that are tailored to local pathogenic landscapes, and in particular finely resolved vulnerabilities to the disease (Thompson and Polley, 2014).

O. viverrini is closely associated with wetland (rice)-based agriculture where drainage canals can facilitate infection of fish hosts by snail-shed cercariae (Wang et al., 2015). Small-scale freshwater fishing activities provide a major source of protein and additional income for local communities (Grundy-Warr et al., 2012), while raw fish consumption has led to the persistence of opisthorchiasis in many parts of the region despite decades of control efforts (Sripa et al., 2011; Sithithaworn et al., 2014). The latter have, to date, largely been restricted to chemotherapy and education campaigns, where the measure of success of control programs is limited to prevalence reduction instead of reinfection rate and long-term sustainability (Sithithaworn et al., 2012a). Despite the close relationship of opisthorchiasis with the physical and social environment, research on the range of factors that underpin the cycle of infection and reinfection has largely been neglected (Forget and Lebel, 2001; Sripa et al., 2015).

The focus of this chapter is the pathogenic landscape for opisthorchiasis, in particular the epidemiological role of dam construction and subsequent reservoir creation, socio-economic conditions and behavior, and variations in the efficacy of the provision of health services. This study illustrates the causes of an uneven distribution of disease burden, identifies contributing factors of infection while controlling for existing chemotherapy control efforts, and has the potential to facilitate improved health intervention efforts that take into account the high geographical focality of opisthorchiasis. The approach and results have wider applicability, to the study of other NTDs, especially those with complex, environmentally sensitive life cycles.

5.2 Materials and methods

5.2.1 Participants and sample collection procedures

Permission for fieldwork at the four villages (Figure 3.2, detailed in Section 3.2) was obtained from the subdistrict health centers. Meetings were held with heads of the health centers and health center workers to explain the purpose, procedures, risks, and benefits of the study. Health center workers were briefed, using Thai language, on the participant information sheet and the need to obtain written consent from the participants, and on how to administer the questionnaire, and to obtain fecal samples. After fecal examination, deworming medication was provided to the health centers for treatment of participants who were tested positive with intestinal parasites. Those infected with *O. viverrini* were treated with praziquantel at an oral dose of 40 mg/kg. All medications were administered by certified nurses from the health centers.

Participating households were selected from information provided by the local health center using a random number generator. All members from the selected households who were 21 years or older at the time of the survey were invited to participate. Using StatCalc in Epi Info 7.1.5 software at confidence interval level of 95% and margin of error at 5%, a sample size of 125 was needed for each village. A total of 756 participants from the four study villages were eventually invited. Ethical approval for this study was obtained from the Institutional Review Board of National University of Singapore (Reference code: A-14-122) and Khon Kaen University, Thailand (Reference code: HE571229).

5.2.2 Determining human infection status

Current infection status involving *O. viverrini*, other foodborne parasites, and soil-transmitted helminths were determined from the analysis of fecal samples. A single fecal sample was provided by each participant. The samples were returned to the health center on the same day and kept on ice. Samples were transported to the laboratory the following morning where they were stored in the freezer at -20^{o}C until analyzed for their parasite content. To increase the number of fecal samples returned, each village was visited on two consecutive mornings for the transportation of samples. Samples were processed using the formalin-ether concentration technique (Elkins et al., 1990) and examined under the microscope by experienced laboratory technologists. *O. viverrini* eggs were counted and recorded, and evidence of other intestinal parasites noted. Infection prevalence was tabulated by dividing the number of infected people with the total number of people sampled, while infection intensity was determined as the number of *O. viverrini* eggs per gram (epg) of fecal sample. Infection statuses of participants were provided to the head of the health centers along with medications for the treatment of *O. viverrini* and other intestinal parasites. Information on past treatment of *O. viverrini* was obtained from both health center records and completed questionnaires (the latter were used to identify participants who received *O. viverrini* treatment from institutions other than health centers, including hospitals).

5.2.3 Collecting socio-economic information

A questionnaire-based survey was conducted to determine the association of socio-economic and behavioral factors with *O. viverrini* infection prevalence and intensity (Appendix 2). Variables used in this study were selected based upon existing studies on *O. viverrini* risk factors (Forrer et al., 2012; Kaewpitoon et al., 2012), while the set of possible responses in the multiple-choice questionnaire were formulated based on preliminary semi-structured interviews conducted with 251 respondents in the catchment of the Ubolratana reservoir.

Socio-economic information, including the demographic factors age and gender, relating to participants was provided by the health centers. Age was tabulated based on the year of birth of the participant and was expressed as a continuous variable. Other data, including level of education and occupation, were obtained through the questionnaires. As each participant may have more than one occupation, the various occupation types were each presented as an explanatory variable. Per capita income was established by dividing the household income by the number of members in the household and expressed as a binary variable above or below the estimated poverty line of 2430.5 baht. This figure was arrived at by averaging the estimated poverty line values for 2014 from the National Economic and Social Development Board of Thailand for the provinces of Nong Bua Lamphu (2347 baht) and Khon Kaen (2514 baht) (National Economic and Social Development Board of Thailand, 2015). Participants were given the option of whether they wished to disclose information on their income.

Levels of awareness of the hazard of *O. viverrini* infection and patterns of consumption of the raw fish dishes Koi pla (freshly prepared raw fish salad), Mum pla, and Pla som (both of which are lightly fermented raw fish dishes), which are commonly eaten in the study area, were determined through the questionnaire survey. Participants were also asked for the reasons behind their consumption/non-consumption of raw fish.

5.2.4 Collecting information on healthcare focus, perceptions, and chemotherapy history

The Isarn Agenda, a program aimed at CCA prevention and control in northeast Thailand, was introduced in 2012. The program involves fecal examination, ultra-sound scan for CCA above 40 years of age, exhibiting risky behavior, notably the consumption of raw fish. People found with opisthorchiasis are treated. Education programs are also created for primary school children. The Isarn agenda is not equally applied throughout northeast Thailand, however, as each province has the autonomy to decide on health priorities locally. In Khon Kaen province, in the southern part of the study area, only two districts, which are not included in this study, adopted the Isarn agenda, while other districts opted to focus on non-communicable diseases, such as cardiovascular diseases and diabetes. All districts in Nong Bua Lamphu, in the northern part of the study area, adopted the Isarn agenda. Thus, of the four villages examined in this study, the N-river and N-reservoir villages adopted the Isarn agenda, and the S-river and S-reservoir village did not.

The prevalence and intensity of infections in the four study villages were compared with past *O. viverrini* infection diagnostic tests conducted by the health centers. Unlike mass drug administration efforts for other helminth parasites, praziquantel anthelmintic were given as a treatment for *O. viverrini* infection only for positive cases of infection. As such, past attempts in *O. viverrini* diagnostic tests can also be used to determine past chemotherapy efforts. Furthermore, information on local health priorities and perceptions of opisthorchiasis was also obtained from the heads of the health centers.

5.2.5 Statistical analysis

Criteria for inclusion in the analyses included providing consent, not having withdrawn from the study, provision of suitable stool sample, and having a completed questionnaire. The prevalence and intensity of *O. viverrini* infections together with the reasons for/for not consuming raw fish were analyzed for their association with environmental factors, notably the type of water body from which fish used in raw fish dishes are sourced, and other socio-economic factors. The possible influence of interventions by health centers, and variations in their level of implementation, was also investigated. Bivariate analyses were first performed on each explanatory variable; variables with *p*-values below 0.2 were next entered into multivariate models. To examine prevalence, data from all participants were used in analyses. To examine intensity, only participants who tested positive for infection were included. To examine reasons for consumption, only participants who consumed raw fish were used for the analyses; conversely, in examining reasons for non-consumption, only participants who do not consume raw fish were used. Logistic regression was employed for analyzing infection prevalence, reasons for consumption, and reasons for non-consumption. The models were simplified with backward elimination and variable deletion determined using a chi-squared test for non-significant difference in deviance. Quasi-Poisson regression was used for analyzing infection intensity in the case of overdispersion. The model was simplified with backward elimination and variable deletion determined using F-test for non-significant difference in deviance. In addition, chi-squared test was used to test for variation in proportion of raw fish consumption by location, gender, and *O. viverrini* awareness.

Variations in level of infections of fish by water body type (reservoir or river inlet) were also analyzed, using data from Chapter 4. A *t*-test with unequal variances was used on log (x+1) transformed data, as data were not normally distributed. Differences in levels of infections in fish according to water body type (Chapter 4) are compared with results from this study. Results from these analyses were used as a basis for examining the role of factors that have contributed to the pathogenic landscape for opisthorchiasis in the study area. All statistical analyses were carried out in R unless otherwise specified.

5.3 Results

5.3.1 Summary of data collected

Of the 756 participants invited, 632 suitable samples were obtained (83.60%). The mean age of participants is 52.6 years. Among the participants, 54.2% were females and 45.8% were males. Comparison of the *O. viverrini* infection prevalence and intensity of the four villages showed that the S-river village had the highest prevalence at 40.21%, while the N-reservoir village had the highest infection intensity at 99.41 epg (Figure 5.1A). When the villages were grouped according to their provincial health jurisdiction, infection prevalence in the north villages in Nong Bua Lamphu province (5.45%) was statistically significantly lower than that of the south villages in Khon Kaen province (26.42%) (Figure 5.1B). When villages were grouped according to their proximity to water body types, infection prevalence did not vary much between villages located close to the reservoir and to the river inlets, but infection intensity was significantly higher for the reservoir villages at 93.72 epg than for the river villages at 38.54 epg.

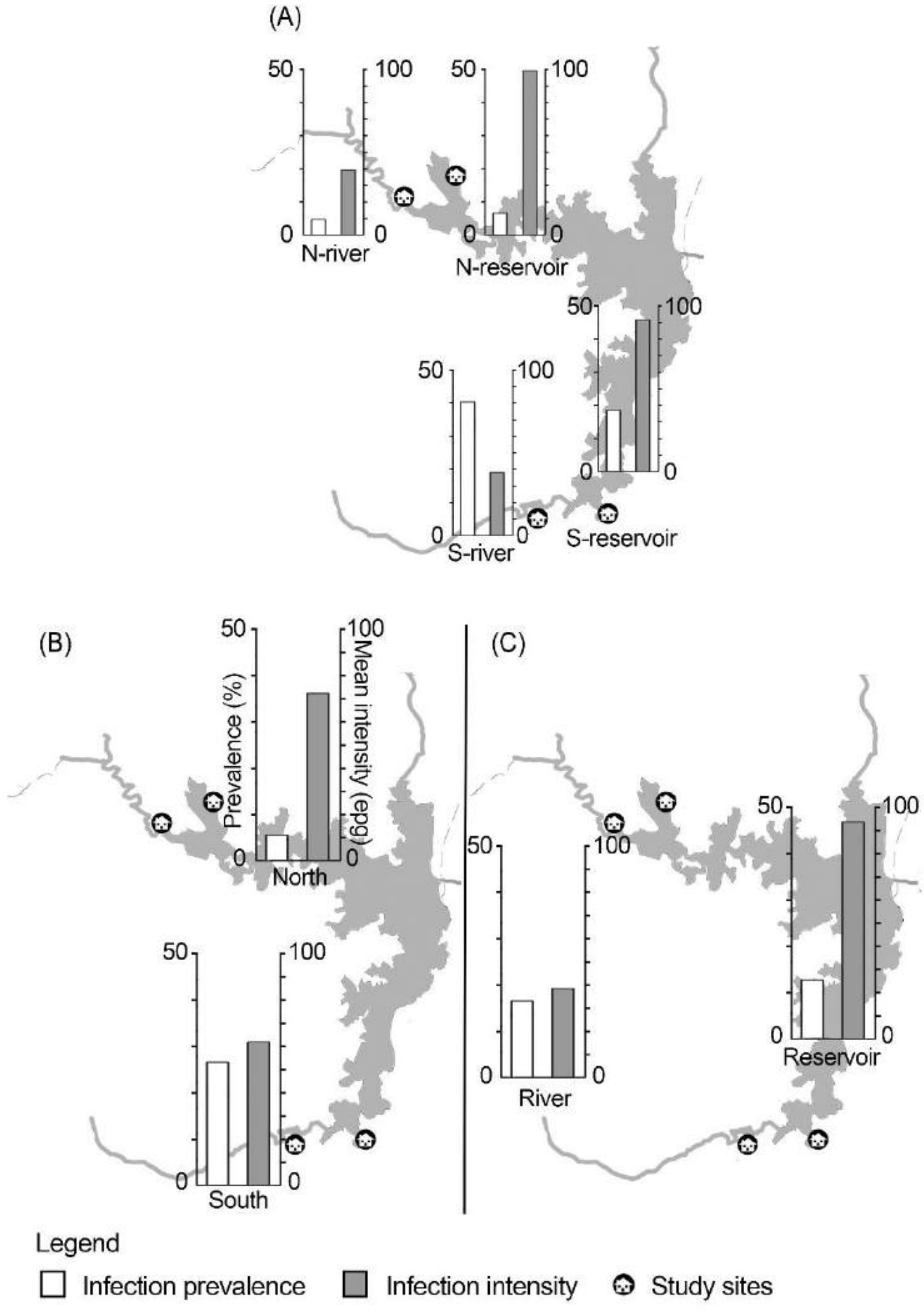


Figure 5.1: Comparisons of the spatial variation in *O. viverrini* infection prevalence and mean intensity by (A) villages, (B) provincial health jurisdictions, and (C) water body types. Bivariate and multivariate analyses showed significant differences in infection prevalence between health jurisdictions and in infection intensity between water body types.

Bivariate analyses of the infection status of other foodborne parasites and soil-transmitted helminths revealed that *O. viverrini* infection prevalence was higher in participants who were also infected with other parasites, particularly those infected with soil-transmitted helminths (Figure 5.2A). Participants who had raw fish consumption behavior were found to be significantly associated with higher *O. viverrini* prevalence (Figure 5.2A), while the intensity of infection was significant higher for participants who had not been dewormed (121.19 epg) than for those who had been (56.7 epg) (Figure 5.2B). However, such associations were not observed in the multivariate models. Past *O. viverrini* deworming history did not greatly influence *O. viverrini* infection prevalence (Figure 5.2A), and no statistically significant association was observed between *O. viverrini* awareness and both the *O. viverrini* infection prevalence and intensity.

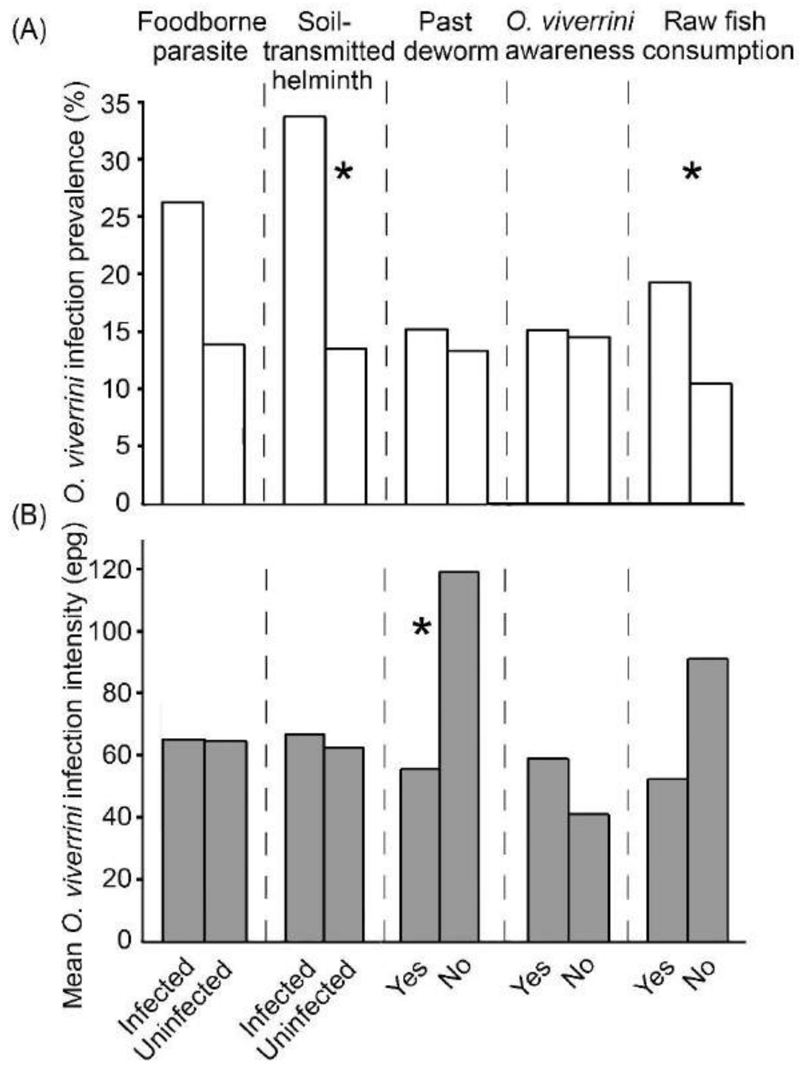


Figure 5.2: Association of (A) *O. viverrini* infection prevalence and (B) mean infection intensity with participants of other infections, deworm history, awareness, and raw fish consumption behavior. Significant differences in infection prevalence or intensity for bivariate analyses are indicated by the asterisks. None of these variables were, however, associated with *O. viverrini* infection in the multivariate models.

The mean age of participants found infected with *O. viverrini* was 56.1 years while the mean age that of the uninfected was 52.0. Age was positively associated with infection prevalence in the multivariate model. Among other socio-economic factors (Figure 5.3), both the bivariate and multivariate analyses suggested that gender was significantly associated with infection prevalence, while farming as an occupation and poverty line were significantly associated with infection intensity.

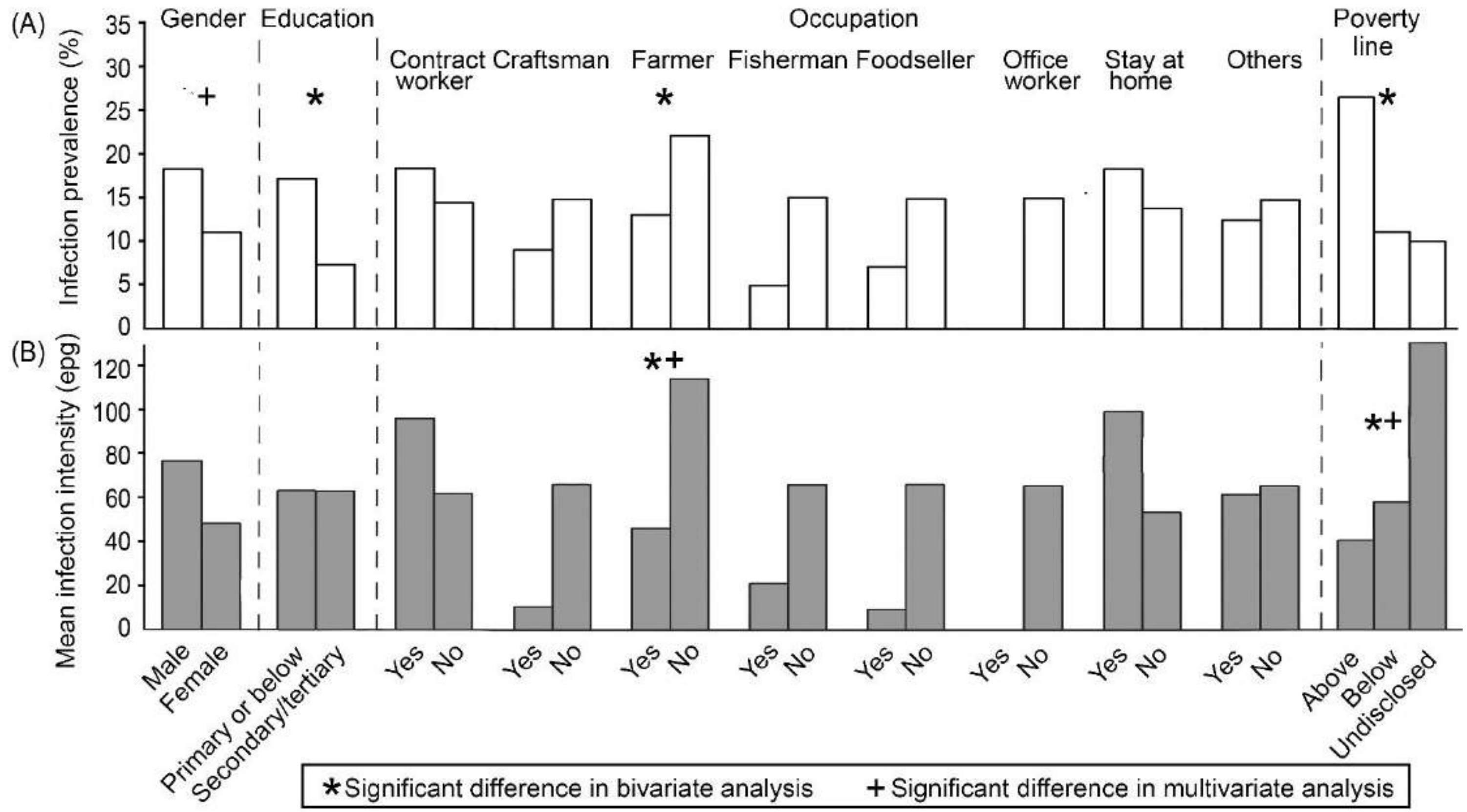


Figure 5.3: (A) *O. viverrini* infection prevalence and (B) mean intensity of infected participants of various socio-economic factors. Infection prevalence is significantly different between gender in both bivariate and multivariate analyses while infection intensity is significantly different between farming as an occupation and also people of different poverty level in both bivariate and multivariate analyses.

5.3.2 Risk factors for infection prevalence and intensity and reasons for/for not consuming raw fish

Bivariate analyses indicated that the variables of soil-transmitted helminths, location, age, gender, education, farming as an occupation, poverty line, and raw fish consumption were significantly associated with *O. viverrini* infection prevalence (p-value < 0.05). Other foodborne parasitic infection was associated with *O. viverrini* infection prevalence at p-value < 0.20. These variables were hence entered into a multivariate regression model.

Results of the multivariate logistic regression model showed that the likelihood of infection was higher among villagers in the south, increased with age, and was the greatest in males and in those who consumed raw fish (Table 5.1). In addition, the consumption of raw fish is higher (63.6%) in the two villages located in the province of Nong Bua Lamphu (N-river and N-reservoir) when compared with the two villages studied in the province of Khon Kaen (S-river and S-reservoir) (52.2%) (χ^2 = 7.31, df = 1, p-value = 0.01). Males were also more likely to consume raw fish (63.5%) than females (54.2%) (χ^2 = 4.83, df = 1, p-value = 0.03). *O. viverrini* awareness was found to be negatively associated with raw fish consumption, with 28.6% of participants who were unaware of *O. viverrini* reported not eating raw fish and 71.4% who reported eating, as compared to 48.7% of participants who were aware of *O. viverrini* reported not eating raw fish and 51.3% who reported eating (χ^2 = 7.86, df = 1, p-value = 0.01).

Table 5.1: Logistic regression analysis of infection prevalence. Odds of infection was higher among villagers in the south, increased with age, and was the greatest in males and in those who consumed raw fish.

Independent variable		Adjusted OR	95% CI	*p*-value
Spatial distribution	Location			
	North	1.00		
	South	5.73	3.32-10.27	< 0.01
Socio-economic factors	Age			
	> 21 years old	1.03	1.01-1.06	< 0.01
	Gender			
	Male	1.78	1.07-3.00	0.03
	Female	1.00		
Attitudes and practices	Raw fish consumption			
	Yes	2.71	1.57-4.83	< 0.01
	No	1.00		

Note: OR = odds ratio; CI = confidence interval. Only variables that are statistically significant in the model are displayed.

The variables of past *O. viverrini* deworming, water body type, farming as an occupation, and income relative to the poverty line were significantly associated with infection intensity ($p < 0.05$). Other foodborne parasitic infection and soil-transmitted helminth infection were associated with *O. viverrini* infection prevalence at *p*-value < 0.20. Consequently, these variables were examined together in a multivariate Quasi-Poisson regression model. Results of the multivariate Quasi-Poisson regression model revealed that increased infection intensity was found in participants from villages located closest to the reservoir, participants who were not farmers, and participants who chose not to disclose their income information (Table 5.2). Comparison of spatial variation in human and fish infection indicated that, similar to human infection intensity, fish infection density was significantly higher in the reservoir water body type than the river water body type (t = 2.66, df = 10.22, *p*-value = 0.02), but not significantly different between the north and south (t = -0.04, df = 9.39, *p*-value = 0.97).

Table 5.2: Multivariate Quasi Poisson regression analysis of infection intensity. Increased risk of infection intensity was found in participants from villages located closest to the reservoir, participants who were not farmers, and participants who chose not to disclose their income information.

Independent variable		**Adjusted RR**	**95% CI**	***p*-value**
Spatial distribution	Water body			
	River	1.00		
	Reservoir	2.09	1.12-4.02	< 0.01
Socio-economic factors	Occupation - Farmer			
	Yes	0.44		
	No	1.00	0.25-0.78	< 0.01
	Poverty line			
	Above	1		
	Below	1.02	0.48-2.19	0.96
	Undisclosed	2.71	1.34-5.63	< 0.01

Note: RR = risk ratio; CI = confidence interval. Only variables that are statistically significant in the model are displayed.

Participants who were unaware of *O. viverrini* were more than twice as likely to state that they ate raw fish because it tasted delicious, while those living in the S-river and S-reservoir villages were more likely to state that they ate it out of habit. The odds of eating raw fish because of friends decreased with every one-year increase in age. Males were also more than twice as likely to eat raw fish because of friends as females (Table 5.3).

Table 5.3. Logistic regression analyses of the reasons for consumption (delicious, habit, or eat with friends) among participants who consume raw fish dishes.

Reasons for consumption	Independent variable	Positive response (%)	OR	95% CI	*p*-value
Delicious	*O. viverrini* awareness				
	Yes	55.62	1		
	No	76.46	2.59	1.15-6.42	0.03
Habit	Location				
	North	27.27	1		
	South	50.88	2.76	1.68-4.59	< 0.01
Eat with friends	Age				
	> 21 years old	26.1	0.97	0.95-0.99	0.01
	Gender				
	Male	35	2.28	1.30-4.8	< 0.01
	Female	18.66	1		

Note: OR = odds ratio; CI = confidence interval. Only variables that are statistically significant in the models are displayed.

The likelihood of not eating raw fish in order to avoid being infected by *O. viverrini* was at least eight times higher among participants who were aware of the risks of infection. When asked about the reason for selecting the option of avoiding *O. viverrini* despite being unaware *O. viverrini*, some of the participants explained that they had been encouraged by health volunteer workers or nurses to avoid eating raw fish because of the parasite, even though they were unsure about what the parasite was. Participants who knew about *O. viverrini* were also about seven times more likely to avoid eating raw fish due to other health reasons. Participants who did not know about *O. viverrini* were more likely to avoid eating raw fish because they dislike it. In addition, participants who said that they disliked raw fish were more likely not to have received treatment in the past, or are younger, or live in either N-river or N-reservoir village (Table 5.4).

Table 5.4: Logistic regression analyses for the reasons for non-consumption (avoid *O. viverrini*, avoid due to other health reasons, dislike) among participants who do not consume raw fish dishes.

Reasons for non-consumption	**Independent variable**	**Positive response (%)**	**OR**	**95% CI**	**p-value**
Avoid O. viverrini	*O. viverrini* awareness				
	Yes	71.63	8.04	4.19-16.80	< 0.01
	No	13.33	1		
Avoid due to other health reasons	*O. viverrini* awareness				
	Yes	26.67	6.96	1.62-27.08	< 0.01
	No	4.96	1		
Dislike	Past *O. viverrini* deworm				
	No	41.82	2.78	1.05-8.41	0.05
	Yes	14.63			
	Location				
	North	41.05	2.63	1.11-6.70	0.03
	South	25.29	1		
	Age				
	> 21 years old	33.89	0.97	0.93-0.99	0.03
	O. viverrini awareness				
	Yes	24.82	1		
	No	53.33	3.19	0.91-11.53	0.07

Note: OR = odds ratio; CI = confidence interval. Only variables that are statistically significant are displayed.

5.3.3 Healthcare focus, perceptions, and chemotherapy history

In the S-river village, no attempt to determine *O. viverrini* infection, including fecal examination, had been performed for at least 10 years. The priorities of the health center of the S-river village focused on the health effects of pesticide use and respiratory tract infections (Table 5.5). By comparison, in the S-reservoir village, fecal tests of *O. viverrini* infection were performed in 2007 and 2008, with infection prevalence estimated at 0% and 2%, respectively (Table 5.5). Because of funding constraints, the direct smear technique was employed and only relatively few people were tested in the village. A recent fecal examination done in 2014 reported infection prevalence to be low at 0.25%. As such, similar to the S-river village, the S-reservoir health center staff did not view opisthorchiasis as a top priority; instead, diabetes and hypertension were the main concerns.

In the N-river village, fecal examination was performed in 2011 and 2012 with infection prevalence estimated at 5.25% and 2.26%, respectively. The local health center prioritized teenage pregnancy and parasitic diseases as the top health concerns, with campaigns aimed at reducing rates of teenage pregnancy organized by health center staff. In the N-reservoir village, fecal examination was performed in 2012 and 2013, and *O. viverrini* prevalence was estimated at 8.24% and 0.27%, respectively. Different from the south villages, the local health centers of both N-reservoir and N-river villages used the single Kato-Katz thick smear technique for *O. viverrini* infection test. Hypertension and diabetes, the top health concerns of the S-reservoir village, were also prioritized by the local health center staff of the N-reservoir village as the major health concerns, among work related injuries and gastrointestinal diseases (Table 5.5).

Table 5.5: Summary of key findings on health centers' chemotherapy efforts and current top health concerns. Infection intensities were not collected by the health centers.

Village	Past chemotherapy	Diagnostic tool	*O. viverrini* prevalence	Top health concerns
S-river	None in the past 10 years	-	-	Toxicity from pesticides and respiratory tract infections
S-reservoir	2007 2008 2014*	Direct smear Direct smear Direct smear	0% 2% 0.25%	Diabetes and hypertension
N-river	2011 2012	Single Kato-Katz thick smear Single Kato-Katz thick smear	5.25% 2.26%	Teenage pregnancy and parasitic diseases
N-reservoir	2012 2013	Single Kato-Katz thick smear Single Kato-Katz thick smear	8.24% 0.27%	Diabetes, hypertension, work related injuries, and gastrointestinal diseases

* The 2014 examination was conducted for other villages in the same sub-district of the S-reservoir village.

5.4 Discussion

5.4.1 Waterbodies as a contributing factor to the pathogenic landscape for *O. viverrini* infection

The lack of significant difference in infection prevalence between villages located close to the river inlets and to the Ubolratana reservoir was not reflected in infection intensity, with the intensity of infection among people from villages located closest to the reservoir over twice that of villagers living alongside river inlets. The pattern of infection intensities among humans thus matches fish infection intensities, with higher overall fish infection associated with the reservoir when compared with river inlets (Chapter 4), and with no significant difference in fish infection in the north and south of the reservoir. Distance to water body had an impact on where villagers tended to source the fish used in raw fish dishes: villagers who lived close to the river tended to procure fish from the river, while those living close to the reservoir tended to procure fish from the reservoir. As fish in the reservoir is more plentiful, people who lived farther away from both river inlets and reservoir also tended to rely upon fish caught from the reservoir (Chapter 4). Differences in fish infection levels depending on water body can affect the level of exposure of humans to the risk of infection, as is evident in the results: the average intensity of infection in the S-river village was low despite the lack of chemotherapy effort, and lower than both N-reservoir and S-reservoir villages, despite the recent chemotherapy efforts in the N-reservoir village in particular.

Opisthorchiasis-induced inflammation can lead to the development of various hepatobiliary abnormalities, of which *O. viverrini*-induced advanced periductal fibrosis (APF) and CCA are driven by common cellular mechanisms, marked by elevated level of plasma interleukin-6 (Sripa et al., 2012b). Participants with the most elevated level of plasma interleukin-6 were found to have an increased risk of 19 and 150 times of developing APF and CCA respectively as compared with other *O. viverrini* infected individuals with no detectable plasma interleukin-6 (Sripa et al., 2012b). The risk of developing APF was found to increase with increased infection intensity (Elkins et al., 1996; Mairiang et al., 2012) and duration of infection (Elkins et al., 1996), although Sripa et al., (2012b) found no such link between infection intensity and the risk of developing APF. Even at low levels of infection, APF may be prevalent (Mairiang et al. 2012). This hints at the possibility of CCA development even in individuals with low infection intensity, owing to prolonged inflammation, making infection intensity a critical measure to consider when determining the risk of CCA development. During the course of five months of this study, a participant was diagnosed with CCA and passed away shortly after diagnosis. The participant had a history of raw fish consumption, no record of past *O. viverrini* treatment, and had tested negative for infection in this study. The apparent absence of *O. viverrini* eggs in the fecal sample could have been due to a low intensity of infection or bile duct obstruction (Sithithaworn et al. 1991).

To date, there is little information on the association between human infection intensity and fish infection variation in different water body types. In fact, infection intensity is much less frequently reported than infection prevalence in *O. viverrini* studies (e.g. Kaewpitoon et al., 2012a; Yong et al., 2012; Yeoh et al., 2015). The same is the case for other helminthiases, including soil-transmitted helminthiases. For example, the majority of studies in Latin America report only prevalence (Saboyá et al. 2013). This is problematic because infection intensity enables a very different understanding of the disease transmission and life strategies as compared with infection prevalence, in addition to being a factor in the most severe forms of infectious disease, including the risk of developing CCA in the case of *O. viverrini* infections. In the case of this study, using only infection prevalence as the measure of success for intervention effort can problematically lead to individuals with high infection intensities in low prevalence areas being overlooked. The findings in this study thus echo the concept of One Health, highlighting the close relationship between the health of humans and that of the health/infection status of the animal hosts and physical environment. It identifies the reservoir as an important source to target for opisthorchiasis intervention efforts and also underscores the importance of considering infection intensity in the understanding of the parasite's transmission pathways. Comparative multilocality studies are necessary to gain useful insights into the similarity or difference in relationships between opisthorchiasis and the environment in such reservoir systems.

5.4.2 Socio-economic and behavioral influencing factors

Higher infection prevalence in males than in females accords with findings from some previous studies (Kaewpitoon et al., 2012b; Thaewnongiew et al., 2014). Little difference in prevalence between genders has also been reported (Kaewpitoon et al., 2012a; Haswell-Elkin et al., 1991), although Haswell-Elkin et al., (1991) notes that the frequency of high infection intensities may be higher among males. Males are also more likely to die from opisthorchiasis. As males are often the main income earners in families in Thailand, opisthorchiasis can exert a disproportionate economic toll on those affected (Sripa et al., 2012b). One reason for a higher infection prevalence and intensity among males is likely to be their socializing behavior: raw fish dishes are often available for consumption at social gatherings of males. Infection levels also tended to increase with age from 21 years in this study. This finding is at odds with existing results, which indicate a plateauing of infection levels in the late teens followed by a decline in later life (Sripa et al., 2011; Sithithaworn et al., 2014).

In some studies, fishermen and/or farmers were found to have higher infection prevalence (Forrer et al., 2012; Kaewpitoon et al., 2012a). This is because local fishermen often make a dish of koi pla from their catch to celebrate that day's fishing (Grundy-Warr et al., 2012). Farmers may also harvest fish from their rice paddies and prepare and consume the catch on the spot. Conversely, in this study, infection in fishermen and farmers was not significantly higher than for other occupations. In this study, higher infection intensities were found only in participants who were not farmers, and who chose not to disclose their income information. Consequently, the socio-economic and demographic factors selected in this study could not identify the specific groups of people at risk of higher infection intensity. Recent chemotherapy efforts in three of the four villages may have weakened links with the range of factors that result in infections.

While there was no significant difference in infection prevalence and intensity with *O. viverrini* awareness, *O. viverrini* awareness appeared to reduce the proportion of people who reported consuming raw fish. Participants who were aware of *O. viverrini* were also more likely to avoid raw fish consumption in order to avoid opisthorchiasis and other health issues, while participants who were unaware of *O. viverrini* were more likely to avoid consumption due to personal dietary preferences. Awareness campaigns may be able to affect personal health decisions to a certain extent, although more holistic effort is needed to tackle this long-standing issue.

5.4.3 Variation in efficacy of health center focus

The pattern of villagers residing in the south of the reservoir being much more likely to be infected than villagers in the north may reflect inter-provincial differences in health priorities and treatment efforts. Use of praziquantel to treat infections can result in a sharp decline in prevalence (Lovis et al., 2012). For example, praziquantel administration brought about an immediate decline of *O. viverrini* prevalence from approximately 60% to 14%, while infections among the control, untreated group increased from 65% to 71% within the same time frame (Saowakontha et al., 1993). Likewise, a similar decline in prevalence (67% to 16%) during three years of praziquantel administration is reported in Sripa et al., (2015). Favorable results following chemotherapy-based treatment efforts do not necessarily imply long-term success of a campaign, however. Resurgence of infection has been observed soon after the cessation of a campaign (Suwannahitatorn et al., 2013).

This study revealed disparate healthcare concerns and opisthorchiasis control efforts. While the particular focus of the health center can be tailored to the needs of the villages within the sub-district (Bureau of Policy and Strategy, 2009), funding allocation for healthcare is decided at provincial level. As the S-river and S-reservoir villages are part of districts in Khon Kaen province, where the Isarn Agenda was not implemented, limited funding was made available for opisthorchiasis control efforts.

The lack of fecal examination for *O. viverrini* infection for the past decade may account for the high infection prevalence recorded in the S-river village. In the S-reservoir village, where a relatively limited treatment program was in place, infection prevalence was second only to the S-river village. Direct smear was used in the S-reservoir village to test for infections as it is the most affordable, despite it being the least sensitive method (Keiser and Utzinger, 2009). The low sensitivity of the test may have led to erroneous results in the form of low prevalence data. Due to an apparent low prevalence of *O. viverrini* and increasing prevalence in chronic diseases, particularly diabetes and hypertension, it is not unexpected that the local health center staff increasingly prioritize such chronic diseases as their top health concerns. Coupled with the affordability and simplicity of testing for diabetes and hypertension, regular blood sugar tests and blood pressure tests are offered by the health centers, which may in turn shift the health focus of the villagers to such chronic diseases. Indeed, during the course of this study, the villagers and health center staff of the south villages have expressed that *O. viverrini* infection is not an issue of concern in the village. Coincidentally, fecal examinations were carried out by the health center staff in 2014, the same year of this present study, to survey *O. viverrini* infections in villages within the sub-district of the S-reservoir village. As the health center knew about our intent of sampling in the S-reservoir village, the health center sampling was conducted in all villages of the sub-district except the S-reservoir village. Their survey reported an overall infection prevalence of 0.25% for those villages, which was close to the prevalence observed for the S-reservoir village in 2007 and 2008 (Table 5.5). Nevertheless, the prevalence was in stark contrast to the much higher levels obtained in this study (i.e. 18.45% for the S-reservoir and 40.21% for the S-river, Figure 5.2A), with the disparity likely due to the difference in sensitivity of fecal examination methods employed. Unfortunately, disparate healthcare focus, coupled with limited funding and a less sensitive opisthorchiasis screening method may have given villagers—and health center staff —a false impression of the importance of opisthorchiasis. Villagers who consume raw fish may be lulled into a false sense of security when any tests for *O. viverrini* infection generate negative results, despite the frequent consumption of raw fish, as mentioned by several villagers interviewed.

Fecal examinations were carried out by the health centers concerned on a greater number of individuals in the N-river and N-reservoir villages. The diagnostic tests for *O. viverrini* infection also relied upon the more sensitive single Kato-Katz thick smear. Infection prevalence in the N-river village, at 5.3% and 2.3% in, respectively, 2011 and 2012, was close to the 4.6% prevalence obtained in this study. Similarly, close results were found for the N-reservoir village (8.2% and 0.3% in, respectively, 2012 and 2013, compared with 6.4% in this study). Despite the increased focus on opisthorchiasis and CCA after the implementation of Isarn Agenda and the lower infection prevalence, no significant differences in awareness of the risks of *O. viverrini* infection was observed, and the proportion of participants who reported eating raw fish remained high in these two north villages.

This study underscores the influence of health center focus on *O. viverrini* infection prevalence. While most of the prior work has emphasized on human behavior and socio-economic risk factors for helminth diseases including opisthorchiasis, healthcare focus and provision can greatly affect the risk of infection and the vulnerability of local populations (Keiser and Utzinger et al., 2009). Healthcare focus and provision can also shed light on the varying stakeholders' values determining the pathogenic landscape of diseases. Stakeholders' values can influence the outcome and direction of healthcare provision as illustrated in the variation in provincial funding and health center focus in this study. In the cases of other disease intervention efforts that substantially rely on external donor funding, there can be potential conflicting interests between local population, funding donors, or even pharmaceutical companies (Shiffman, 2006). The influences of healthcare focus and interests of other stakeholders thus need to be considered when deciphering the factors contributing to disease risks.

The holistic approach in this study has identified important features of helminth parasitism, specifically, opisthorchiasis, which include the connectivity of animal hosts and humans facilitated by waterbodies and human behavior; human behavioral and physical environmental conditions that facilitated reinfection; and the influence of healthcare interventions on infection prevalence. Identification of such features of parasitism is an important contribution to the framework of EcoHealth/One Health approach (Thompson and Polley, 2014), where consideration of helminth diseases has largely been overlooked (Robertson et al., 2014).

5.5 Conclusion

The types of waterbodies from which fish for consumption in raw form are sourced, socio-economic and behavioral factors, and variation in efficacy of the provision of health centers all play critical roles in creating and maintaining the pathogenic landscape in which *O. viverrini* infections occur. Socializing behavior can, on the one hand, promote raw fish consumption, as observed in males who tend to have higher infection and are significantly more likely than females to cite eating with friends as the reason for consumption, but on the other hand, alter risk perception through first-hand knowledge of deaths and spreading of health information. While underlying socio-economic, demographic and behavioral factors have been relatively well-documented in previous work, this is not the case for the physical environment and healthcare factors identified in this study as relevant to *O. viverrini* infection and potentially to other helminth infections.

Humans interact with the environment reciprocally, thereby influencing their risks of disease infection. Human modifications of the environment, particularly in the form of dam construction and reservoir creation, have changed the aquatic habitats for the *O. viverrini* intermediate fish hosts. As *O. viverrini* infection intensities in the fish vary across different water body types, humans affect their risks of consuming *O. viverrini* infected raw fish through fish procurement location preferences.

In opisthorchiasis studies and that of other helminthiases, infection intensity is still much less frequently reported than infection prevalence, despite the role of infection intensity in intensity-dependent mortality. The importance of considering infection intensity in a cross-sectional infection study is exemplified in this study, owing to the critical role of intensity in the most serious forms of many infectious diseases, including opisthorchiasis, and in providing insights into parasite transmission risks.

Healthcare focus can directly affect human infection prevalence through chemotherapy and indirectly guide villagers' risk perceptions through the choices of health campaigns or monitoring programs. Chemotherapy in the case of helminthiases such as opisthorchiasis is only palliative, with re-infections quickly occurring if the underlying factors that expose humans to infection are not dealt with—i.e. if the symptoms rather than the ultimate causes of parasite burdens remain the focus of attention. Such factors are easily overlooked, but can support the continued persistence of the parasite in the system and re-infection once chemotherapy efforts come to an end. There is thus a need for a holistic approach to integrate the factors accounting for the broader pathogenic landscape within which diseases such as opisthorchiasis persist.

Chapter 6 The uneven influence of rice cultivation on surface water management and facilitation of *O. viverrini* transmission

6.1 Introduction

Paddy rice cultivation is of major importance to the livelihoods and food security of more than half of the global population, whose staple diet is rice (Mohanty, 2013). Globally, over 160 million hectares of land is being dedicated to paddy rice cultivation (Food and Agriculture Organization of the United Nations, 2014), where rice paddies and their associated land use types, including irrigation systems, can pose severe disease risks. One of the main causes of disease risks is the extensive surface water modification and alteration of water transport and connectivity. Surface water modification by rice cultivation provides new habitats for disease vectors and hosts, resulting in the increase in severity and range of many diseases including malaria (Mboera et al., 2015), opisthorchiasis (Wang, 2012), schistosomiasis (Liang et al., 2007), and Japanese encephalitis (LeFlohic et al., 2013). Extensive surface water modification by rice cultivation can also serve as a medium for parasite transmission, as observed in schistosomiasis (Liang et al., 2007) and opisthorchiasis (Wang, 2012), and even for waterborne diseases not restricted to rice cultivation such as cholera (Lantagene et al., 2014), salmonellosis, and shigellosis (Trang, 2007; Abaidoo et al., 2010).

Paddy rice cultivation and its associated irrigation systems are responsible for major surface water modification in the Lower Mekong River Basin, where natural waterbodies have been altered for flood control, irrigation, and fishery enhancement through the creation of reservoirs, rice paddies and ponds (Wang et al., 2011; Sripa et al., 2015). Since water resource development was adopted as a key strategy towards stimulating agricultural and economic development by Thailand's development agencies in the late 1950s, many large dams, including the Ubolratana dam, and thousands of small dams, irrigation systems, and weirs have been constructed (Sneddon, 2002). Such construction creates permanent waterbodies in a landscape formerly characterized by major changes in water level between the rainy and dry seasons. Consequently, the massive changes in surface water availability and water body creations can encourage disease proliferation that would be otherwise limited by seasonal changes (Sithithaworn et al., 2012a; Ziegler et al., 2013). One of such diseases associated with surface water modification is opisthorchiasis, an infection by liver fluke, *O. viverrini*. *O. viverrini* is a foodborne parasite, where transmission to humans occurs via the consumption of infected raw cyprinid fish. Cyprinid fish is infected by the cercaria stage of *O. viverrini*, which is shed from infected *Bithynia* snails, and the snails are usually found in high abundance in the rice paddies (Petney et al., 2012; Wang et al., 2015). Opisthorchiasis has proven to be largely recalcitrant to exisiting disease control efforts (Sripa et al., 2011; Sithithaworn et al., 2014), as long as landscape conditions—rice paddies and water bodies—and human behavior continue to support the life cycle of *O. viverrini* (Wang et al., 2011; Grundy-Warr et al., 2012).

Although surface water is the key medium facilitating *O. viverrini* connectivity across human, snail, and fish habitats (Ziegler et al., 2013), one of the key elements that has been largely overlooked by scholars is the variation in surface water availability and connectivity in different rice paddy patches both spatially and temporally. Studies examining the spatial distribution of *Bithynia* snails on rice paddies (e.g. Ngern-klun et al., 2006; Suwannatrai et al., 2011; Kiatsopit et al., 2012) have the tendency to classify rice paddies as the same land use type while overlooking the inherently different cultivation practices applied on rice paddies, which can give rise to different risks of *O. viverrini* transmission spatially. While several studies have looked at the temporal variation in *Bithynia* snail host abundance or infection in rice paddies (Lohachit 2004-2005; Kiatsopit et al., 2012; Wang et al., 2015), little is known about the exact mechanisms of water use and surface water availability in facilitate uneven transmission risks of rice paddies.

The epidemiological significance of water use and surface water availability in rice cultivation is evidenced by the association of paddy rice cultivation with diseases transmission or vectors proliferation observed in other diseases. For example, rice growth stages were found to affect mosquito abundance (Duik-Wasser et al., 2007); irrigation using water from contaminated river sources increases the risk of cholera in rice farmers during the Haiti cholera outbreak (Lantagne et al., 2014); and uncultivated rice paddies that were overgrown with weeds were found to provide breeding ground for mite and tick borne diseases (Kuo et al., 2012). Given the unequal contribution of rice paddies in disease transmission, it is necessary to differentiate such variations in rice cultivation to better understand the roles of rice paddies in disease transmission. Moreover, understanding variations in disease transmission risk in rice paddies of different characteristics is consequential to research designs of rice paddies-associated diseases and necessary for the application of a holistic/system approach, which requires detailed information on relationships among risk factors.

Given the constraint in existing technology to measure snail-shed *O. viverrini* cercariae, Wang et al., (2016b) used surface water, the medium of cercariae transport, as the proxy for understanding *O. viverrini* transmission. Similarly, surface water will be adopted in this study to determine cercariae transmission from rice paddies to fish hosts. As methods of rice cultivation vary according to geographical regions, rice cultivation process and cropping calendar of farmers around the Ubolratana reservoir were obtained to understand the local methods of cultivation. Additionally, rice cultivation characteristics and associated human behavior were collected to identify cultivation characteristics with increased transmission risks. Together, mechanisms facilitating the spatial and temporal variation of rice paddies in *O. viverrini* transmission can be determined. Specifically, this chapter addresses two questions.

1. What is the rice cultivation process and cropping calendar of this study site? How is temporal variation of *O. viverrini* transmission shaped by such cultivation process and surface water changes?
2. What is the underlying patterns of association among the cultivation characteristics? How do cultivation characteristics and associated human behavior shape the spatial variation in *O. viverrini* transmission risks of rice paddies?

6.2 Materials and methods

6.2.1 Investigating rice cultivation process and cropping calendar at the Ubolratana reservoir

The rice cultivation process and the cropping calendar of farmers were obtained via interviews. Rice paddies locations of farmers interviewed were documented in Figure 3.3, Section 3.3. Farmers were asked to describe their cultivation process, which included the main cultivation activities such as land preparation, sowing/transplanting, irrigation and drainage, and harvesting. The cropping calendar was created by obtaining information on the duration of cultivation activities and the time of the months such cultivation activities were performed. Information on the cultivation process and the cropping calendar was used to derive surface water changes across the year and provide understanding of the temporal variation in water level and water use of the rice paddies.

6.2.2 Identifying cultivation characteristics with increased transmission risks

To identify the rice cultivation characteristics with increased transmission risks, variables were identified through literature reviews on rice cultivation in Thailand (e.g. Tanabe, 1981; Little et al., 1996; Khunthasuvon et al., 1998; Hassan, 2000; Titapiwatanakun, 2012) and ascertained through interviews with the farmers. Four cultivation characteristics—cultivation frequency, drainage of water from paddies, presence of fish in paddies, and presence of irrigation ponds—were identified as variables with increased risks of facilitating *O. viverrini* transmission. They have increased risks in facilitating transmission for the following reasons: increased cultivation frequency can increase the number of habitats available for *Bithynia* snails (Wang et al., 2011; Ziegler et al., 2013); drainage of water from rice paddies enhance water connectivity between rice paddies and surrounding water bodies (Wang et al., 2016b); and the presence of fish in paddies or the presence of irrigation ponds serve as a source of raw fish for consumption (Sithithaworn et al., 2012b). The four variables are presented individually, in maps for the visualization of their spatial distribution. Other rice cultivation characteristics identified in this study include: flooding of paddies, inundation duration, surface elevation, and water application strategy (Figure 6.1). All data were obtained via interviews except for surface elevation, which was measured using the Differential Global Positioning System; and paddy inundation duration, expressed in weeks, which was derived from the cropping calendar.

To investigate the contribution of cultivation-associated human behavior to *O. viverrini* transmission, behavioral information was collected from the following variables: 1) drainage of water from paddies—to determine where water was drained to; 2) presence of fish in paddies—to obtain the proportion of farmers consuming raw fish from fish caught in the paddies; 3) presence of irrigation ponds—to determine the presence of fish in ponds, how those fish were introduced, and proportion of farmers consuming raw fish caught in the ponds; and 4) water application strategy—to determine the source of irrigated water if irrigation was used (Figure 6.1). Additionally, locations of defecation by rice farmers were obtained, as *O. viverrini* eggs are transmitted via fecal matter. Permissions were obtained to undertake the research following ethical approval recommendations from the Institutional Review Board of the National

University of Singapore (Reference code: 12-333) and Khon Kaen University, Thailand (Reference number: HE571458).

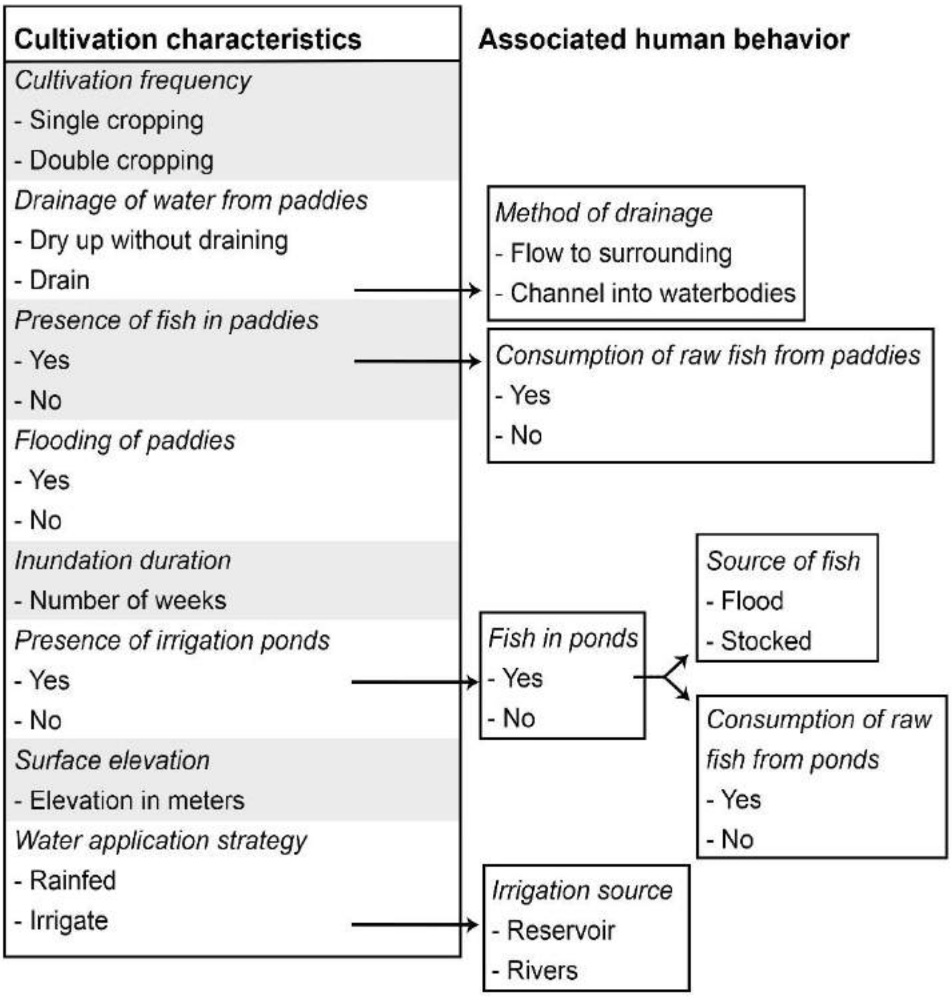


Figure 6.1: Summary of variables collected. Cultivation characteristics are shown in the left column along with the respective factors of each cultivation characteristics. Associated human behavior of each cultivation characteristics are displayed to the right of the cultivation characteristics column. Farmers who irrigated using irrigation ponds were asked for the water source of their ponds. If rain is the sole water source, they are classified as rainfed paddies instead of irrigated paddies in the cultivation characteristic—water application strategy.

6.2.3 Statistical analyses

To visualize the underlying patterns of association among cultivation characteristics, cultivation characteristics were explored using the multiple correspondence analysis (MCA) (Costa et al., 2013). Continuous variables including surface elevation and inundation duration were grouped into categories: first quartile (Q1), second quartile (Q2), third quartile (Q3), and fourth quartile (Q4). Other variables including cultivation frequency, drainage of water from rice paddies, flooding of paddies, presence of fish in paddies, presence of irrigation ponds, and water application strategy, were presented as categories. The four characteristics identified with increased risks of facilitating transmission—cultivation frequency, drainage of water from rice paddies, presence of fish in paddies, and/or presence of irrigation ponds—were each used as a dependent variable. Based on the result from MCA, variables found to be clustered with each of the dependent variables were analyzed for statistical significance using logistic regression. In situations where multicollineariity exist, bivariate tests, either fisher's exact test or *t*-test, were used instead. If the dependent variable was not found to be clustered with any of the other variables, statistical analyses were not performed. Additional bivariate tests based on the results from the earlier statistical analyses, were performed between some variables to better understand the relationships between independent variables. Depending on the results from the analyses, cultivation characteristics associated with each other were grouped. This groups were classified as either "high risk group", or "low risk group", depending on their facilitation of transmission. Collectively, such information allows for the understanding of the mechanisms of rice cultivation contributing to the spatial and temporal variation of *O. viverrini* transmission in rice paddies. All statistical analyses in this chapter are carried out in R.

6.3 Results

6.3.1 Cultivation process and cropping calendars

Rice is cultivated up to twice a year around the Ubolratana reservoir. The main-season cultivation occurs during the rainy season, while the off-season cultivation occurs during the dry season. To prepare rice paddies for cultivation, the rice paddies are first cleared, ploughed and levelled (Figure 6.2A). Rice plants are introduced into the rice paddies via either transplanting (Figure 6.2C), using rice seedling prepared in the nurseries (Figure 6.2B), or direct sowing (Figure 6.2D). During the development of the rice plants, rice paddies are kept inundated.

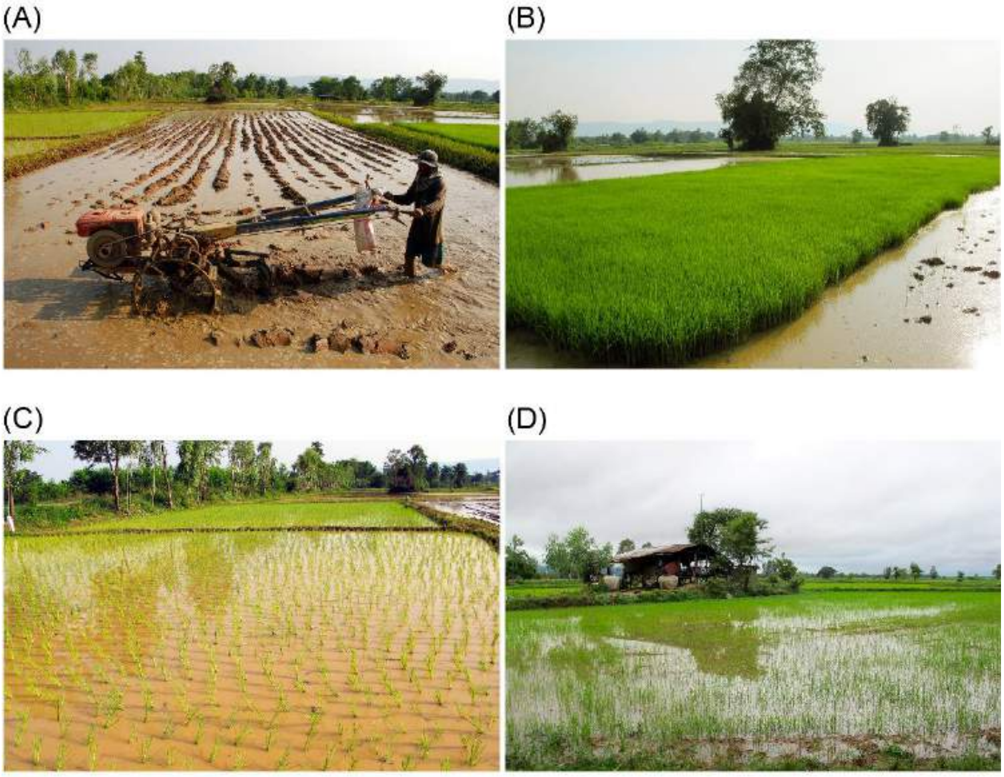


Figure 6.2: Rice cultivation activities. (A) Ploughing in preparation for cultivation. (B) Nursery for transplanting. (C) Transplanted rice paddy. (D) Sowed rice paddy.

The rice paddies are kept inundated by either irrigation or rain. Irrigation methods differ according to the relative location of the rice paddies to the

Ubolratana dam. Farmers whose rice paddies are located downriver of the dam obtain water released from the reservoir via irrigation canals (Figure 6.3A). According to farmers' accounts, they are required to pay 90 baht for every hour of water released into their irrigation canals. Farmers whose rice paddies are upriver from the dam may obtain water from the reservoir through pumps and pipelines constructed by the Thai Royal Irrigation Department for agriculture purposes (Figures 6.3B and C). Alternatively, they may pump water directly from the rivers or reservoir using personal equipment (Figure 6.3D). According to the farmers, they practice rainfed cultivation because their rice paddies are located in places where it is physically challenging or costly to channel irrigation water due to the higher elevation of their rice paddies. In the months of August and September, the late rainy season, some rice paddies (Figure 6.3E) and the surrounding houses (Figure 6.3F) can be flooded, connecting *O. viverrini* eggs from fecal matter with snail habitats and fish habitats.

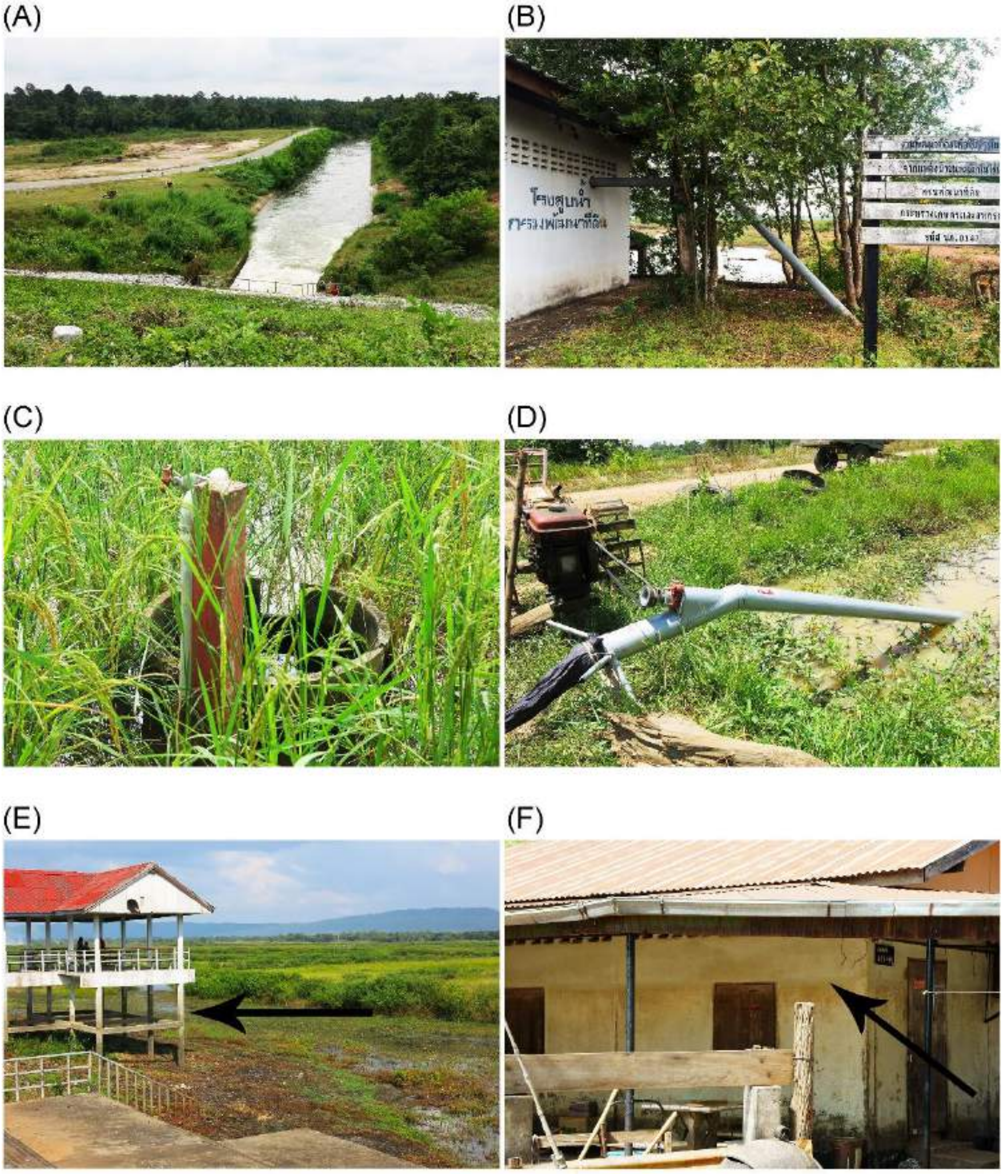


Figure 6.3: (A) Irrigation canal at the Ubolratana dam for paddies downriver of the dam. (B) Pump station for paddies upriver of the dam. (C) Irrigation using water from the pump station. (D) Irrigation using a personal pump, which can be used for pumping water directly from the reservoir, rivers or irrigation ponds. (E) The black arrows show the flood line at rice paddies and (F) flood line of a home.

Rice paddies are drained a few weeks before harvesting (Figure 6.4B) during which, the rice plants are ripening (Figure 6.4A). Fish may be caught by farmers from the rice paddies (Figure 6.4C) after the draining of paddies, or from irrigation ponds (Figure 6.4D), and may be prepared raw with seasonings, to be consumed immediately. Some farmers (31.11%) practice open defecation near the rice paddies, which can facilitate the introduction of *O. viverrini* eggs into the rice paddies (Table 6.1), while others defecated in the toilets, connected to septic tank systems, in the paddies or at home.

Figure 6.4: (A) Ripening stage. (B) Harvesting. (C) Fish trapping after draining of rice paddy. (D) Fish trapping from irrigation pond.

Table 6.1: Percentage of rice farmers defecating at the various locations while working in the rice paddies.

Defecation location while working in the rice paddies	**Percentage**
Around the paddies	31.11
Return home	15.56
Toilet in paddies	53.33

Cropping calendars obtained from interview with farmers show the duration of each of the major cultivation processes. The main-season rice cultivation, locally referred to as *na bi*, is between May and December during the rainy season (Figure 6.5). Frequency of land preparation, which includes ploughing, harrowing and levelling commences in May and peaks in early June. The duration of cultivation is approximately seven months; farmers who begin cultivation in May will harvest in November while those who begin in June will harvest in late November or in December. Transplanting occurs on a later date than sowing as rice need to be first grown in the nurseries. Rice plants are inundated within a month from sowing, kept inundated during the development, and the paddies are drained within several weeks from harvesting. The inundation duration of the rainfed and irrigated rice paddies are similar, which ranges from May to December (Figure 6.5).

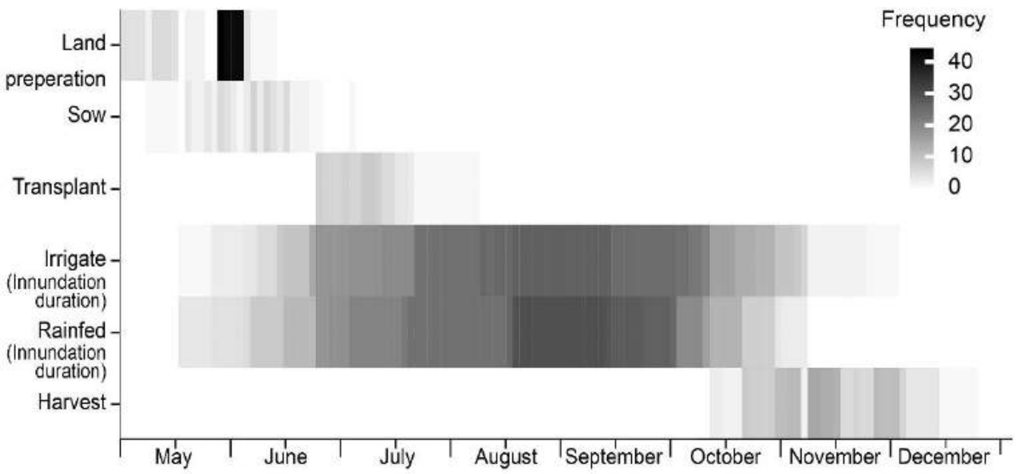


Figure 6.5: Main-season rice cropping calendar. The number of farmers (i.e. frequency) performing a particular farming activity on a particular date was indicated by varying shades of gray, with the darkest shade representing the highest frequency. Land preparation and sowing occur during May or June, while transplanting occurs in July. Rice is harvested during October to December and the paddies are inundated from May to October/November.

Off-season rice cultivation, locally referred to as *na prang*, is practiced by 19 out of the 58 farmers interviewed. Cultivation occurs between December and June during the dry season (Figure 6.6). Sowing is employed by most of the farmers interviewed except for one who uses transplanting. As transplanting is employed by only one farmer, it is not shown in the figure. Irrigation is employed by all farmers although one of the farmers interviewed also uses rainwater to supplement irrigation to reduce the cost needed for irrigation. The farmer's rice paddies are located at the dam outlet where he is required to pay for the use of irrigation water. Cultivation period is approximately five to six months long, one to two months shorter than the main-season cultivation. Farmers who begin cultivation in December will harvest by April or May while those who begin cultivation in January will harvest by May or June (Figure 6.6).

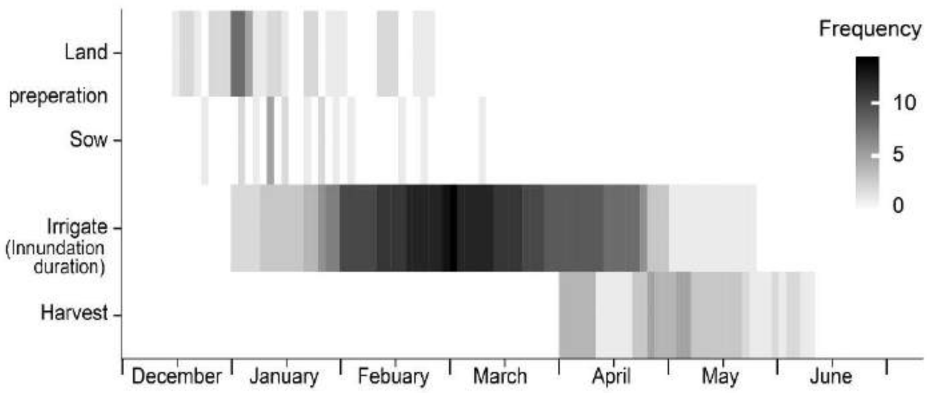


Figure 6.6: Off-season rice cropping calendar. The number of farmers (i.e. frequency) performing a particular farming activity on a particular date is indicated by varying shades of gray, with the darkest shade representing the highest frequency. Land preparation commences between December and February, and sowing between December to March. Rice is harvest during April to June and the paddies are inundated from January to May.

6.3.2 Determining the underlying patterns of associations among the cultivation characteristics and their spatial distribution

Among the farmers interviewed, 67.24% cultivated rice once a year during the rainy season, while the other 32.76% cultivated twice, once during the rainy season and once during the dry season (Figure 6.7). Majority of the farmers drained their rice paddies (79.31%) while the rest of the farmers allowed their rice paddies to dry up naturally. Among the farmers who drained their rice paddies, 43.47% let the water flow into the surrounding areas, which could infiltrate into the soil or travel as surface flow into the reservoir or rivers, while 56.53% channeled water directly to the rivers or reservoir via tubes. Cyprinid fish were found in 72.41% of the rice paddies examined in this study where 24.00% of the farmers consumed the fish raw. However, fish was available only in small quantities, and would only be obtained either once or twice a year, prior to harvesting, when the water level was low. Majority of the rice paddies (81.03%) was flooded during the rainy season in the months of August and September. Mean inundation duration of rice paddies, from irrigation to draining, was 17.76 weeks. Irrigation ponds were present in the paddies of 68.97% of the farmers interviewed. Fish were also found in 86.67% of these ponds. In the ponds with fish, the fish were introduced into 48.15% of the ponds during flooding, which connected the ponds with adjacent rivers or the reservoir, and in 51.85% of the ponds, fish were introduced via stocking. Fish were available in smaller quantities in ponds where fish were introduced during flooding as compared to the active stocking of fish by farmers. Fish caught in the ponds were consumed raw by 25.93% of the farmers. Surface elevation of rice paddies ranged from 162.60m to 209.40m with a mean of 188.02m. During the main-season cultivation, rainfed cultivation was employed by 51.72% of the farmers and irrigation by 48.28% of the farmers. Of the farmers who employed irrigation, 65.38% obtained their water from the reservoir and the remaining farmers obtained the water from the rivers (Figure 6.7).

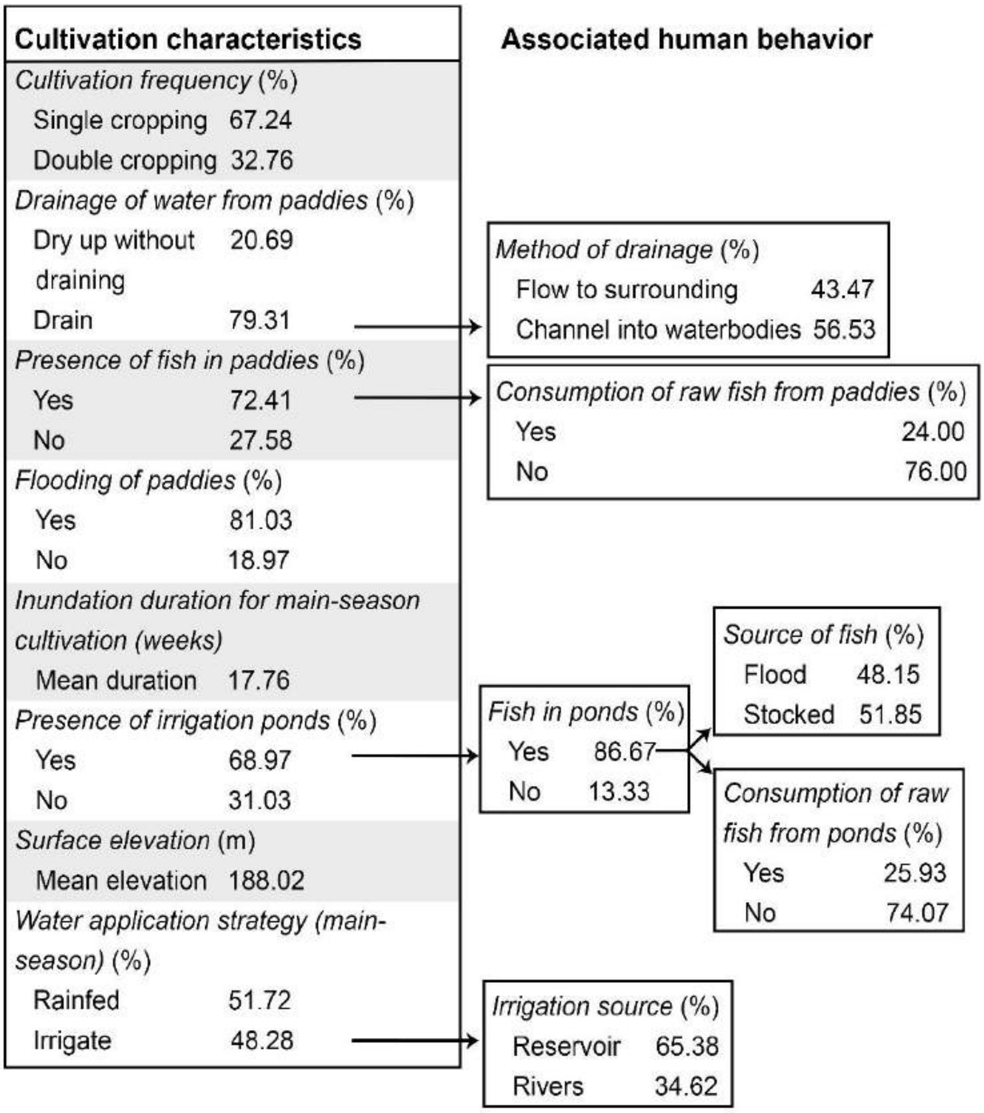


Figure 6.7: Summary of rice cultivation variables collected to examine the uneven contribution of rice paddies to *O. viverrini* transmission. Cultivation characteristics were listed on the table to the left, while the additional information collected were listed on the right.

The underlying structures among cultivation characteristics were visualized using MCA. Together, dimension 1 and 2 explained 36.20% of the variances (Figure 6.8). Cultivation frequency was clustered with the presence of irrigation ponds, surface elevation, and water application strategy on dimension 1. Specifically, single cropping was clustered with rainfed cultivation and highest elevation as opposed to double cropping which was clusterd with irrigation. Presence of fish in rice paddies was clustered with flooding of rice paddies, drainage of water from paddies, inundation duration, and surface elevation on dimension 2. Specifically, the absence of fish in rice paddies was found with the absence of floods in rice paddies, rice paddies drying without draining, and longer inundation duration, as opposed to the presence of fish in paddies, draining of paddies, and shorter inundation duration. Similarly, drainage of water from paddies was clustered with fish in paddies, flooding of paddies, and inundation duration on dimension 2, while presence of irrigation ponds was positioned close to the center of the plot and exhibited no obvious clustering with any groups of variables (Figure 6.8).

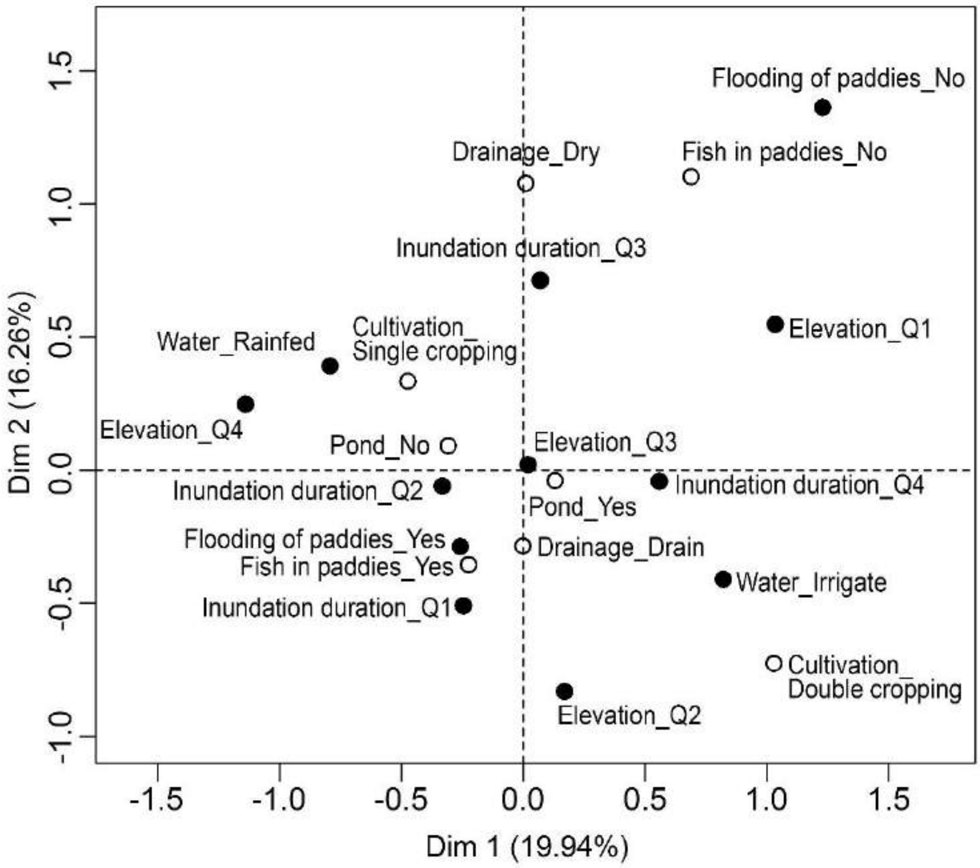


Figure 6.8: MCA plot showing the underlying structure of the data. The four cultivation characteristics with increased likelihood of *O. viverrini* transmission are represented by empty circles while the remaining characteristics by filled circles. Cultivation frequency was labelled as "cultivation", drainage of water from paddies as "drainage", presence of irrigation ponds as "pond", presence of fish in paddies as "fish in paddies", surface elevation as "elevation", and water application strategy as "water".

Fisher's exact test was performed on cultivation frequency and presence of irrigation ponds, and on cultivation frequency and water application strategy. due to small sample sizes (<10) in some cells of the contengency tables used for statistical analyses. *T*-test was performed on cultivation frequency and surface elevation (Table 6.2). Each pair of variables was tested individually instead of being tested together using a logistic regression model as the association between independent variables, surface elevation and water application strategy may render their contribution in explaining the dependent variable inaccurate. Cultivation frequency was significantly associated with surface elevation and water application strategy but not with presence of irrigation ponds. Double cropping occurred in fields with lower elevation (t=4.12,df=39.77, p-value<0.01), and was practiced on only irrigated rice paddies (Table 6.2).

Table 6.2: Relationship between presence of irrigation pond, surface elevation, and water application strategy with cultivation frequency using Fisher's exact test and *t*-test.

Cultivation frequency	**Presence of irrigation ponds**		**Surface elevation**	**Water application strategy**	
	Yes (%)	**No (%)**	**Mean (sd)**	**Irrigate (%)**	**Rainfed (%)**
Single cropping	46.43	65.00	191.42 (9.68)	34.48	100
Double cropping	53.57	35.00	181.05 (8.62)	65.52	0
***p*-value**	0.76		<0.01	<0.01	

Note: sd = standard deviation.

An additional test conducted between surface elevation and water application strategy showed that surface elevation of irrigated paddies was also found to be significantly lower than rainfed paddies (t=-2.93,df=22.33, p-value<0.01). Spatially, double cropping was practiced only on rice paddies located at the dam outlet or close to the river inlets of the reservoir, while single cropping was practiced on all of the rice paddies located further from the reservoir (Figure 6.9).

Table 6.3: Relationship between water application strategy and surface elevation using *t*-test.

Water application strategy	Mean surface elevation (sd)
Rainfed	193.68 (8.62)
Irrigate	181.97 (8.89)
***p*-value**	<0.01

Note: sd = standard deviation.

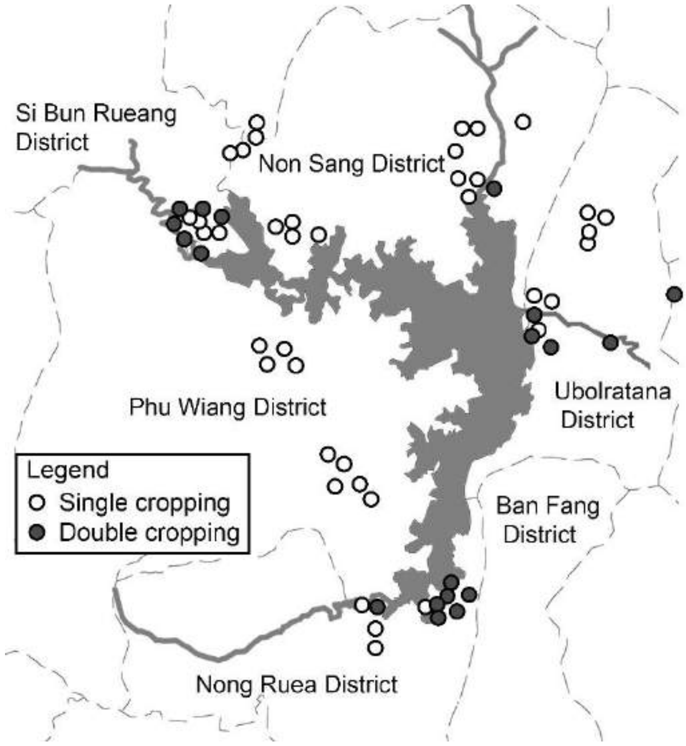


Figure 6.9: Distribution of cultivation frequency. Double cropping was practiced only on rice paddies located close to the river inlets of the reservoir or located close to the dam outlet.

Logistic regression was employed to test if flooding of paddies, inundation duration, and surface elevation could predict fish in paddies. Fish in paddies was associated with flooding of paddies but not with inundation duration and surface elevation. Specifically, the odds of fish found in paddies was 15.24 times higher in paddies that flood than paddies that did not flood (Table 6.3).

Although fish were present in most of the paddies, they were absent in all of the paddies located close to the dam outlet (Figure 6.10).

Table 6.4: Logistic regression analysis of fish in rice paddies. The odds of fish found in paddies was significantly higher in paddies that flood than paddies that do not flood.

Independent variable	**Adjusted OR**	**95% CI**	***p*-value**
Flooding of paddies			
Yes	15.24	3.52-84.29	<0.01
No	1		

Note: OR = odds ratio and CI, confidence interval. Only significant variable was shown.

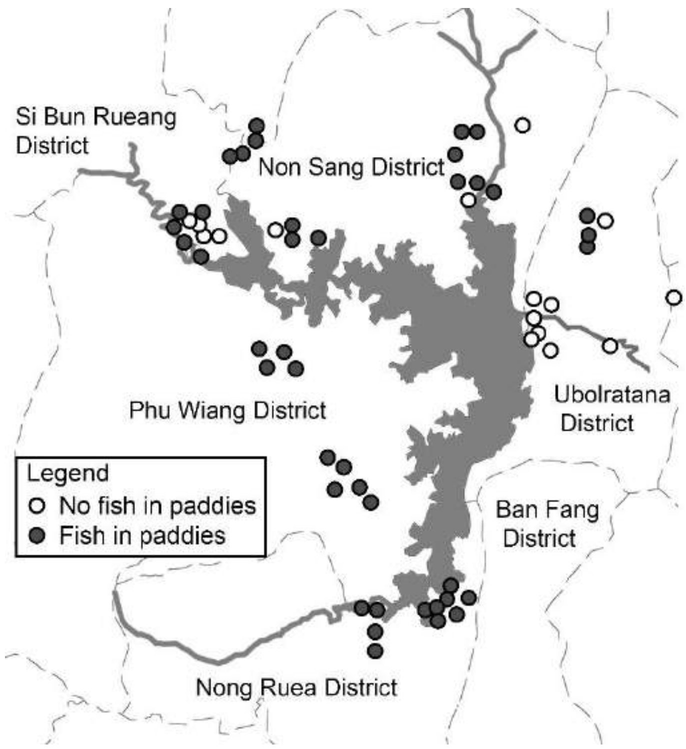


Figure 6.10: Distribution of the presence of fish in paddies. Fish were absent in the rice paddies located at the dam outlet.

Investigation of the association of flooding of paddies and inundation duration with drainage method using logistic regression revealed that the odds of paddies that were drained was 4.76 times higher in paddies that flood than paddies that did not flood. Majority of the rice paddies flooded during the late rainy season (Table 6.5). Most of the paddies will be drained prior to harvesting. The paddies that are allowed to dry up without draining are those located at the dam outlet, and paddies located further from the reservoir (Figure 6.11). All, except for one, of the rice paddies that are allowed to dry up without draining are single cropped rice paddies (Figures 6.9 and 6.11).

Table 6.5: Logistic regression analysis of drainage method. Odds of paddies that were drained was significantly higher in paddies that flood than paddies that do not flood.

Independent variable	**Adjusted OR**	**95% CI**	***p*-value**
Flooding of paddies			
Yes	4.76	1.12-20.62	0.03
No	1		

Note: OR = odds ratio and CI, confidence interval. Only significant variable was shown.

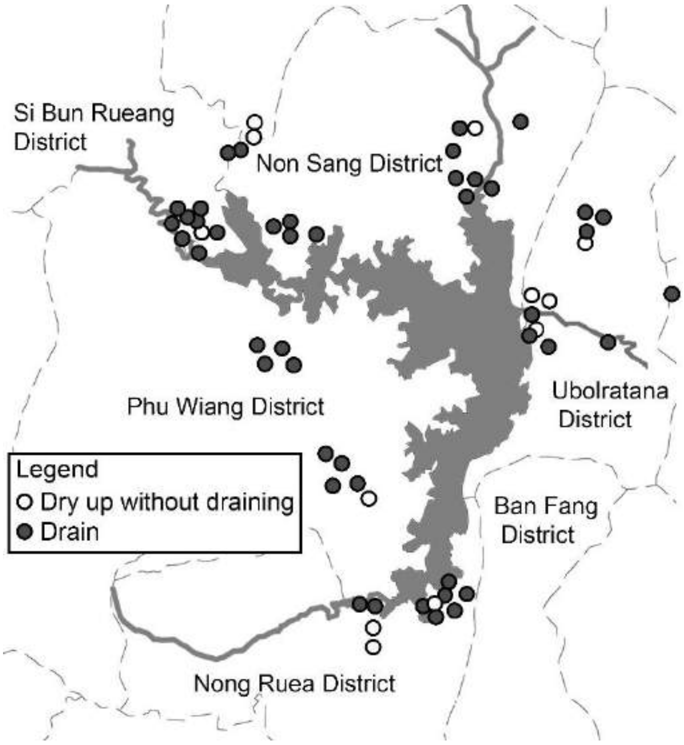


Figure 6.11: Distribution of drainage of water from paddies. Most of the paddies will be drained prior to harvesting, except for some of the paddies located at the dam outlet, and some of the paddies located further from the reservoir.

The presence of irrigation ponds was not found to be associated or clustered with any variables (Figure 6.10), suggesting that the decision to construct irrigation ponds was not affected by any physical conditions or cultivation process examined. No obvious spatial patterns were observed from the distribution map of rice paddies with irrigation ponds except for the rice paddies located towards the south of the reservoir, which were all found with irrigation ponds (Figure 6.12). As the presence of irrigation ponds (68.97%) was a more common occurrence than the absence of irrigation ponds (31.03%) among the rice paddies examined in this study, it is unclear if the presence of irrigation ponds in the rice paddies towards the south of the reservoir was a coincidence or due to underlying reasons not uncovered by the variables collected in this study.

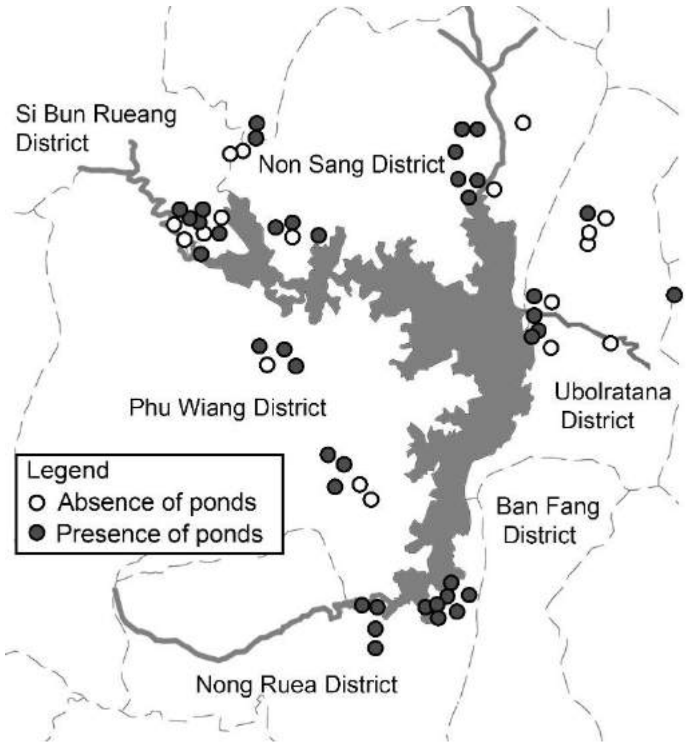


Figure 6.12: Distribution of the presence of irrigation ponds in paddies. No obvious spatial patterns were observed from the distribution map of rice paddies with irrigation ponds.

6.4 Discussion

6.4.1 How rice cultivation process and surface water modification shape the temporal variation of *O. viverrini* transmission

In this study area, the rice paddies are kept inundated for almost the whole cultivation duration except during sowing and harvesting, thus serving as semi-permanent water body for *Bithynia* snails. As with paddy rice cultivation in other parts of the world (International Rice Research Institute, 2016), cultivation methods at the Ubolratana reservoir are heavily dependent on water availability. Large amount of water is required during paddy rice cultivation as the rice paddies are flooded for long durations, where much of the water is lost through evapotranspiration and soil percolation (Bouman, 2009). Approximately 2500L of water is needed to produce 1kg of rice (Bouman, 2009), and almost half of all freshwater supply in Asia is dedicated to rice irrigation (Price et al., 2013).

The changes in water use of rice paddies across a year makes for a temporally dynamic landscape. While studies have examined the temporal change in *Bithynia* snail population (Lohachit 2004-2005; Kiatsopit et al., 2012; Wang et al., 2015) and the number of cercariae shed across seasons (Kiatsopit et al., 2014; Laoprom et al., 2016), little is known about the facilitation in *O. viverrini* transmission through water use and inundation duration during rice cultivation. Yet, rice cultivation process has direct influence on the biological communities associated with paddy rice cultivation, which vary according to the cultivation stages and water availability of rice paddies (Bambaradeniya and Edirisinghe, 2008; Natuhara, 2013).

Figure 6.13 is an interpretation of changes in water use derived from the cropping calendars (Figures 6.5 and 6.6). Rice paddies function as the equivalence of shallow ponds, floodplains (Elphick 2000; Natuhara, 2013), or dry land/ground across a year depending on water use during the cultivation process. For single cropping, some of the farmers began introducing water into the rice paddies in May, and by June, most or all of the farmers would have inundated their rice paddies. While inundated, the rice paddies function as shallow ponds, serving as habitats for freshwater organisms, including *Bithynia* snails.

Heavy rain in the months of August and September causes flooding of rice paddies, which connects the rice paddies to surrounding water bodies, including rivers and reservoir. Rice paddies thus function as floodplains, allowing *Bithynia* snails, snail-shed cercariae and cyprinid fish to come into contact with each other. Fish, including cyprinid fish, can also feed in the rice paddies (Chapman and Fernando, 1994) which allow these fish to come into contact with *Bithynia* snail-shed cercariae. Water begins drying up or is drained from the paddies by the end of October or November. Some paddies however, do not need to be drained but rather, are allowed to dry up naturally, thereby breaking the connectivity of cercariae and fish in surrounding water body.

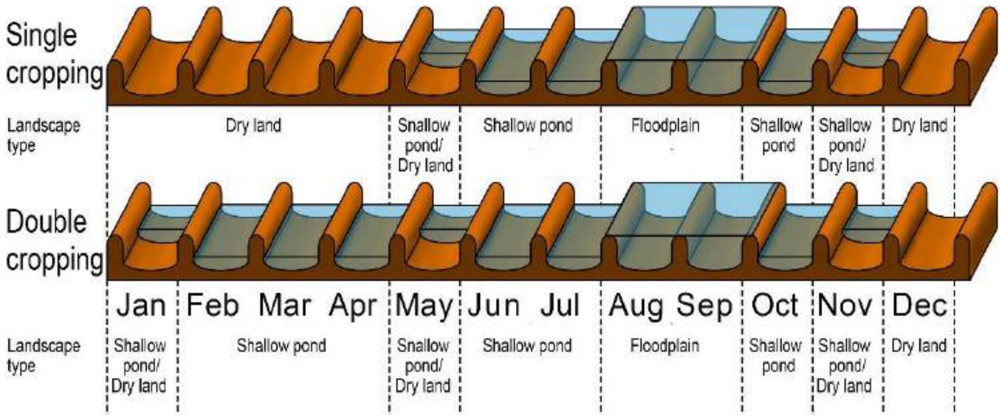


Figure 6.13: Variation in water level of rice paddies across a year as depicted by the diagram of a rice paddy with bunds. The rice paddy is dynamic landscape type functioning as a shallow pond, floodplain, or dry land/ground across a year depending on water use during the cultivation process.

Given that the water level variation between the rainy and dry season in northeast Thailand is the greatest as compared to any other regions in Southeast Asia, biological organisms have adapted to such extreme alternating rainy /dry environment (Caspers, 1979). However, farmers, in introducing water supply during the dry season, can have a great impact on the biological organisms. While rapid reproduction of *Bithynia* snails occurs during the summer monsoon season when water levels are high, there exists high variability in peak *Bithynia* snail density in rice paddies even within the same province (Lohachit, 2004-2005), suggesting that *Bithynia* snail reproduction and fish infection is no longer directly related to seasons, but also to the influence by human water management behavior, including irrigation (Petney et al., 2012). Peak *O. viverrini* metacercariae in fish also occurs during different seasons in different study sites (Sithithaworn et al., 1997; Maninvong et al., 2009), suggesting the importance of conditions other than natural seasonal changes in shaping infection transmission.

In addition, reservoir, ponds, and inundated paddies serve as refuge for *Bithynia* snails which continue to feed, reproduce, and shed cercariae during the dry season (Ziegler et al., 2013). The impact of *Bithynia* snails on *O. viverrini* transmission is exacerbated by the elevated amount of cercariae shed by each *Bithynia* snail during the hot-dry season, from March to May (Kiatsopit et al., 2014; Laoprom et al., 2016). The elevated amount of cercariae shed during the hot-dry season may be due to the higher temperature accompanying the hot-dry season, especially in shallow water bodies, inducing higher rate of *O. viverrini* infection by *Bithynia* snails (Prasopdee et al., 2015). Double cropped rice paddies thus facilitate the infection of *Bithynia* snails and the transmission of cercariae during the dry season.

6.4.2 The role of cultivation characteristics and associated human behavior in the spatial variation of rice paddies transmission risks

Rice paddies are ideal habitat for *Bithynia* snails as they thrive in slow-moving, shallow water with high turbidity (Lohachit 2004-2005; Petney et al., 2012). In fact, *Bithynia* snails are found in higher abundance in the rice paddies as compared to the rivers, ponds (Wang et al., 2015) or the Ubolratana reservoir (Kittivorachate, 2004), and *Bithynia* snails obtained from the rice paddies may shed more *O. viverrini* cercariae than snails obtained from other water body types (Kiatsopit et al., 2012).

As observed from the clustering of the cultivation characteristics from the MCA plot (Figure 6.10), there exists two main categories of rice cultivation characteristics determining *O. viverrini* transmisison risks—a cultivation duration group and a water connectivity group. Within each category, there exist a high risk group and a low risk group. Paddies belonging to the cultivation duration high risk group include paddies that are double cropped, irrigated, and with lower surface elevation. Paddies belonging to the cultivation duration low risk group include paddies that are single cropped, rainfed, and with higher surface elevation. Paddies belonging to the cultivation duration high risk group are found to be located close to the river inlets of the reservoir or close to the dam outlet (Figure 6.9).

Statistical analyses confirmed the association among variables in the cultivation duration group (Tables 6.2 and 6.3). Lower elevation facilitates irrigation, as depicted in Table 6.3 and by the accounts of farmers who cited elevation as a challenge in irrigation for rice paddies. Irrigation is necessary for double cropping. Due to insufficient water during the dry season, all farmers who practice rainfed cultivation are only able to cultivate once a year (Table 6.2), during the rainy season. As observed by Wang et al., (2013) there is a general trend of higher human infection prevalence in villages with a lower elevation and in farmlands with a higher water content, which suggests the likelihood of association between rice cultivation water use and human infection. As such, the heavy reliance of rice cultivation on water availability shapes the location where such cultivation duration high risk group can be found—close to the river inlets of the reservoir and dam outlet where irrigation sources are available.

Increased cultivation duration facilitates transmission by increasing the duration of rice paddies to function as snail habitats. When water in the rice paddies is drained, *Bithynia* snails in the rice paddies undergo aestivation which stops them from feeding, reproducing, and shedding of cercariae until the next cultivation season. *Bithynia* snails have a mortality rate of approximately 5.3% per month during aestivation (Brockelman et al., 1986). In this study, the rice paddies that are being cultivated twice a year are inundated from approximately May/June to October/November and from January to April/May. Such paddies are dry for approximately two to three months a year (estimated mortality of 10% to 15%), serving as *Bithynia* snail habitats for the rest of the year, as compared to single cropped paddies, which are dry for approximately six months (estimated mortality of 28%). As such, double cropping can prolong the survival of *Bithynia* snails which may not survive the long duration of six months of aestivation in the dry rice paddies.

The water connectivity high risk group includes rice paddies that flood, paddies that are drained prior to harvesting, and have fish in paddies, while water connectivity low risk group includes rice paddies that do not flood, paddies that are allowed to dry up, and the absence of fish in paddies. Statistical analyses showed that fish are more likely to be found in paddies that flood (Table 6.3), reinforcing the accounts of farmers who attributed the source of fish to flooding from surrounding waterbodies. Paddies that are required to be drained are also often paddies that flood (Table 6.4). It is likely that flooding and drainage of paddies share the same contingent causal factor of increased water availability. Water availability enables inundation of rice paddies late into the cultivation season, thus necessitating the drainage of paddies rather than drying for removal of water from the rice paddies prior to harvesting.

Most of the paddies examined in this study belong to the water connectivity high risk group, except for the paddies located at the dam outlet, which are not found with fish, as they seldom experience flooding. All of the rice paddies that are allowed to dry without draining are single cropped rice paddies but the reverse relationship does not hold as there are many single cropped rice paddies that are required to be drained. The decision to drain or dry the paddies may be governed by both physical limitation, such as limited water availability, and personal decisions, such as a trade-off in stopping irrigation earlier during cultivation to save on the cost of purchasing water from the dam outlet, or fuel for their personal water pumps (Figures 6.10 and 6.11).

Increased water connectivity facilitates transmission by encouraging cercariae connectivity between the *Bithynia* snails and cyprinid fish hosts. *Bithynia* snails shed cercariae into the water which will actively locate and burrow into cyprinid fish (Hass et al., 1990) to form metacercariae, the infective stage of *O. viverrini*. Numerous cercariae, more than a thousand of them, can be shed from one *Bithynia* snail in a day (Phongsasakulchot et al., 2005; Kiatsopit et al., 2014). As water in the rice paddies come into contact with fish habitats during drainage or flooding, or with fish that are introduced into the rice paddies, the cercariae, if present in the water, will thus be able to infect the fish. In this study, 79.31% of the farmers drain their paddies, potentially facilitating the transmission of cercariae to fish habitats. Conversely, flooding is an unusual occurrence for the farmers with rice paddies that are located at the dam outlet of the reservoir. Consequently, those paddies are not found with fish. Rice paddies belonging to the water connectivity low risk group thus impede *O. viverrini* connectivity as compared to the other paddies.

In addition to spatial variation of rice cultivation characteristics, human behavior shapes transmission risks. In this study, irrigation ponds are available in 68.97% of the rice paddies and of which, 86.67% are found with fish. Irrigation ponds put *Bithynia* snails from the rice paddies or ponds in close proximity with the fish in ponds, unlike paddies with no irrigation ponds, or those without fish. Fish are introduced by either stocking or via floods. Of ponds found with fish, 25.93% of the farmers consume those fish raw, which is similar to the 24.00% who reported consuming fish from their paddies raw (Figure 6.6). The outcome of infection transmission from fish to humans via irrigation ponds is thus dependent on the presence of the following conditions: raw fish consumption by humans and fish stocking or flooding to introduce fish into those ponds.

Bithynia snails in rice paddies are infected with *O. viverrini* via the consumption of *O. viverrini* eggs found in fecal matter (Centers for Disease Control and Prevention, 2012). Open defecation was convenient for the 31.11% of farmers interviewed who had no toilets in the paddies, and who resided too far from their rice paddies (Table 6.1). Additionally, cats and dogs as the reservoir hosts, will introduce fecal matter into the environment (Enes et al., 2010; Aunpromma et al., 2012). After a rain or seasonal flooding, such fecal matter can be washed into the rice paddies. Even defecation into the toilets, a septic tank system, can contribute to fecal contamination and potentially associated *O. viverrini* infection as documented by Kaewkes et al., (2012) and Wang et al., (2016), likely due to flooding or leakage of septic tanks.

Rice paddies located upriver of the dam, which are close to the river inlets of the reservoir, thus have the highest transmission risk, which can be exacerbated by high risk human behavior including the stocking of fish into irrigation ponds, open defecation, and raw fish consumption. Rice paddies located upriver of the dam but further away from the reservoir also facilitate infection transmission via flooding. Rice paddies located at the dam outlet are likely to have lower risk of transmission as connectivity between fish and snail habitats is broken by the absence of flooding. It can thus be valuable to classify rice paddies according to their transmission risks in the study of rice paddies-associated diseases as the spatial distribution of rice paddies even within a small geographic area can harbor very different infection transmission risks.

Notably, extremely low water levels in many of the reservoirs in Thailand, including the Ubolratana reservoir since the mid of 2015 to 2016 (Nathanri and Singha, 2016) triggered irrigation restrictions and prevented off-season cultivation for many farmers (Prasertsri, 2015). The rice subsidy program, which paid farmers above market rate for their rice, used to facilitate the expansion of rice cultivation (Sim, 2015). The drought situation coupled with the removal of rice subsidy program after the coming into power of the military government, however, has adversely affected the incomes of rice farmers, prompting some of them to turn to other occupations or cultivate only once a year (The Issan Record, 2015). The reduction in cultivation duration and/or spatial extent of rice cultivation by rice farmers shows that while rice cultivation is a highly specific process that is closely associated with the local environmental conditions, rice cultivation is also inevitably influenced by the larger political situation.

6.5 Conclusion

The mechanisms facilitating spatial and temporal variation of rice paddies in *O. viverrini* transmission were examined and discussed in this chapter. Temporal variation in water level during rice cultivation essentially converted rice paddies to the equivalence of different habitat types. Rice paddies can function as shallow ponds for harboring *Bithynia* snails when inundated, as floodplains to facilitate connectivity between snail and fish hosts during monsoon season, or as dry lands, which prevent *O. viverrini* transmission, when uncultivated. Double cropped rice paddies are inundated for almost the entire year, thus functioning as *Bithynia* snail habitats for a much longer duration than single cropped rice paddies.

Rice cultivation characteristics via MCA showed that they are clustered into two main groups, the cultivation duration group and the water connectivity group. The cultivation duration high risk group consisted of paddies that are double cropped, irrigated, and are on lower surface elevation. The water connectivity high risk group consisted of paddies that flood, with fish in the paddies, and are drained prior to harvesting. Rice paddies belonging to the cultivation duration high risk group tend to be found close to the river inlets of the reservoir and dam outlet where irrigation is possible. Most of the rice paddies examined in this chapter belonged to the water connectivity high risk group, except for most of the rice paddies located close to the dam outlet, which belonged to the water connectivity low risk group.

Rice paddies that are of the highest transmission risks are those located close to the river inlets of the reservoir or other irrigation sources, especially during monsoon season with increased connectivity, and hot-dry season where increased cercariae is shed, as such paddies belonging to both cultivation duration high risk group and water connectivity high risk group. This is followed by rice paddies located further from the reservoir or other irrigation sources as they belong to the cultivation duration low risk group but water connectivity high risk group via flooding and/or drainage. Rice paddies located at the dam outlet, which belonging to cultivation duration high risk group but water connectivity low risk group, have the lowest transmission risk, given that the cercariae in rice paddies have reduced likelihood of coming into contact with fish.

Human behavior interacts with rice cultivation transmission risks to facilitate infection transmission through human activities such as open defecation, stocking of fish into irrigation ponds, and raw fish consumption behavior. Human land use variation, including dam construction, affects spatial distribution of rice paddies of differing physical characteristics even within a small geographical area, thus emphasizing the importance of small-scale land use variation in shaping geographical unevenness of disease. Recognizing the mechanisms that are shaping the spatial and temporal variation in rice paddies is necessary for the study and control of *O. viverrini* and other waterborne or rice cultivation-associated diseases, including malaria, cholera, and schistosomiasis, as they share similar contingent causal factors, including surface water modification and/or water connectivity.

Chapter 7 Synthesizing factors contributing to the uneven distribution of *O. viverrini* and recommendations for disease control and research

7.1 Summary of research findings

This study has identified the environmental, social, and healthcare factors underlying the pathogenic landscape of *O. viverrini* (Figure 7.1-7.3). The dam construction and subsequent reservoir creation altered fish species assemblages, leading to a considerable increase in fish infection. The presence in the study area of a large reservoir also increased the accessibility of fish procurement, thus increasing human infection, especially for villages located close to the reservoir (Figure 7.1). In addition to affecting fish and increasing human infection, dam construction facilitated the transmission of *O. viverrini* during rice cultivation as the availability of irrigation water kept the paddies inundated for longer periods. Irrigation enabled double cropping, which increased the length of the period during which rice paddies could function as habitat for aquatic snails, and prolonged the duration for cercariae shedding. Additionally, flooding and temporal variation in water use facilitated the transmission of *O. viverrini* during rice cultivation. Flooding introduced fish into the paddies and enhanced the connectivity between cercariae and fish hosts. Drainage from rice paddies to surrounding fish habitats prior to harvesting also enhanced the connectivity between cercariae and fish. Temporal variation in water use during rice cultivation determined when cercariae transmission occurred (Figure 7.3). While landscape factors enhance the likelihood of infection, human behavior and social factors determine the ultimate outcome of infection through differences in exposure to (i.e. consumption of) raw fish and in the prevention and treatment priorities of local health centers. Given the close connections between health centers and the local communities, health centers can play pivotal roles in health outcomes by reducing existing infections via chemotherapy and potentially reducing future infection through effective health campaigns (Figure 7.2). Factors contributing to the uneven distribution of *O. viverrini* were thus identified, namely the uneven contribution of waterscapes to fish infection and consequently to human infection, the heterogeneous characteristics of rice cultivation, and the uneven focus by health centers on *O. viverrini* control (Figure 7.3).

Research questions	Research questions answered
 • How does dam construction affect *O. viverrini* infection of fish hosts?	**The reservoir and its river inlets harbored different fish species of varied *O. viverrini* infection densities, with the overall fish infection higher in the reservoir than the rivers.** --*H. siamensis* consistently dominated the rivers across the seasons, and was found in greater proportion in the rivers than in the reservoir. *C. armatus, H. siamensis, L. leptocheilus,* and *P. falcifer* were commonly found in the reservoir, although the most dominant species varied across seasons. --Fish overall *O. viverrini* infection density was found to be statistically significantly higher in the reservoir than in the rivers, and is consistently higher in all sampling seasons. --Two of the most dominant species in the reservoir, *L. leptocheilus* (97.20 cyst/kg) and *C. armatus* (17.14 cyst/kg) were found with the high infection density, while the most dominant species in the river, *H. siamensis* (0.06 cyst/kg), was found with low infection density.
 • What is the ensuing impact of dam construction on *O. viverrini* infection of the human hosts?	**The reservoir supplied fish to both villages close to the reservoir and those beyond the bordering area of the reservoir. The pattern of human infection intensities matched that of fish infection, with higher infection being found in villages closer to the reservoir. The reservoir has implications of increasing human *O. viverrini* infection likelihood through the change of fish species assemblages, the higher *O. viverrini* infection densities of the fish found, and the accessibility for villagers to procure fish.** --*H. siamensis, H. dispar,* and *C. armatus* were commonly used for making koi pla and *H. siamensis* and *P. brevis* for pla som. Several fish species could be used together for raw fish dishes, and this could include species with relatively high *O. viverrini* infection densities. --Among the various water body types, the reservoir supplies fish to a larger number and a broader spatial extent of villages beyond the area that is immediately boarding the reservoir. --Higher infection intensity was found in participants from villages located closer to the reservoir (N-reservoir and S-reservoir village) as compared to villages located closer to the rivers (N-river and S-river village).

Figure 7.1: Research outcomes for the first and second research questions raised in Chapter 1. Summaries of the research findings are in bold and the elaborations in bullet points.

Research questions	Research questions answered
 • What roles do human behavior play in opisthorchiasis and control?	**Socializing behavior can promote raw fish consumption. Males and also older adults, who tend to have higher infection are also significantly more likely to cite eating with friends as the reason for consumption. While *O. viverrini* awareness does not seem to alter infection levels, the proportion of people consuming raw fish is lower in people who are aware of *O. viverrini.*** --Infection prevalence was significantly higher among villagers in the south, increased with age, and was the greatest in males and in those who consumed raw fish. --Higher infection prevalence in males than in females accords with findings from some previous studies. Fishermen and/or farmers, were found to have higher infection prevalence in some studies, but were not found with higher infection in this study.
 • How do health centers influence opisthorchiasis in the communities they are providing service for?	**Infection prevalence is significantly lower in villages where chemotherapy is readily available. Health focus can affect human infection prevalence through chemotherapy and guide villagers' risk perceptions and awareness through the choices of health campaigns or monitoring programs. Chemotherapy is only palliative, with re-infections quickly occurring if the underlying factors that expose humans to infection are not dealt with.** --The pattern of villagers residing in the south of the reservoir being much more likely to be infected than villagers in the north may reflect inter-provincial differences in treatment efforts, as the use of praziquantel to treat infections can result in a sharp decline in prevalence. --Limited funding was made available for opisthorchiasis control efforts in the S-river and S-reservoir villages where the Isarn Agenda was not implemented. --The low sensitivity of the test employed in the S-reservoir village to test for infection may have led to erroneous results in the form of low prevalence data, leading to the lower prioritization of opisthorchiasis control by health centers. Villagers who consume raw fish may be lulled into a false sense of security when any tests for *O. viverrini* infection generate negative results, despite the frequent consumption of raw fish, as mentioned by several villagers interviewed. --Despite the increased focus on opisthorchiasis and CCA after the implementation of Isarn Agenda, there was no significant differences in awareness of the risks of O. viverrini infection, and the proportion of participants who reported eating raw fish remained high in villages where Isarn agenda was implemented.

Figure 7.2: Research findings for the third and fourth research questions raised in Chapter 1. Summaries of the research findings are in bold and the elaborations in bullet points.

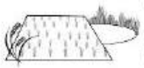
Research questions	Research questions answered
 • How does rice cultivation influence water connectivity facilitating *O. viverrini* transmission to fish habitats?	**Rice paddies vary spatially and temporally. Rice cultivation practices determine the variation in cultivation duration and water connectivity, which in turn, determine transmission risks. Paddies with the highest transmission risks are those located close to the reservoir and to the river inlets followed by rice paddies located further from the reservoir or other irrigation sources, followed by those located at the dam outlet. Distinctions should be made between paddies of vastly different infection transmission potential when examining rice cultivation-associated diseases.** --Rice paddies function as the equivalence of shallow ponds, floodplains, or dry land across a year depending on water use during the cultivation process. --The main-season cultivation occurs during the rainy season, while the off-season cultivation occurs during the dry season. Off-season cultivation is possible only for rice paddies that can be irrigated. This includes paddies located near the reservoir or rivers, or those at the dam outlet. --The underlying structure of rice cultivation characteristics can be grouped into two groups—a cultivation duration group and a water connectivity group. Paddies belonging to the cultivation duration high risk group include paddies that are double cropped, irrigated, and with lower surface elevation. The water connectivity high risk group includes rice paddies that flood, paddies that are drained prior to harvesting, and have fish in paddies, --Paddies belonging to the cultivation duration high risk group are found to be located close to the river inlets of the reservoir or close to the dam outlet, while most of the paddies belong to the water connectivity group, except for paddies located at the dam outlet, where flooding is uncommon.
 • How do the above factors (environmental, healthcare, and behavioral) explain the resistance to existing disease control efforts and influence uneven distribution of disease?	**The uneven distribution of *O. viverrini* is shaped by the varying waterscapes, including reservoir and rice paddies, created by human land use; varying human behavior; and varying chemotherapy efforts and health center focus. While chemotherapy and health campaigns have been implemented for disease control and elimination, the multitude of underlying factors predisposing people to infection are acting antagonistically with exisiting control efforts, leading to repeated re-infection. A holistic approach helps to pinpoint aspects to be focused on in policies and research, allowing for integrated disease control, which cannot be easily achieved when examining the risk factors individually. The factors shaping the pathogenic landscape of *O. viverrini* and suggestions for policy and research are discussed in this chapter (Chapter 6).**

Figure 7.3: Research outcomes for the fifth and sixth research questions raised in Chapter 1. Summaries of the research findings are in bold and the elaborations in bullet points.

7.2 The uneven distribution of *O. viverrini*

The uneven contribution of waterscapes to infections, the heterogeneous characteristics of rice cultivation facilitating disease distribution, and the uneven health centers' control efforts are of relevance to many pathogenic diseases. In the context of discussion on pathogenic diseases with complex life cycle, including opisthorchiasis, public health interventions need to be considered alongside behavior and risk perceptions of the local communities, and environmental risk factors.

Studies of interactions between humans and environments have predominantly been human-centric or environment-centric depending on the type of relationship studied or the perspective brought by the investigators (Harden, 2012). Research on the physical-human geographic influences on liver flukes is limited. The uneven contribution of the reservoir and rivers to infections was brought about by the disruption of biological processes through dam construction, leading to the modification of fish assemblage, increased infection risk of fish, and subsequently, disease risk of humans. This echoes the concepts of EcoHealth and One Health, highlighting how environmental and animal health can be consequential to human disease risks, and that well intentioned dam constructions can produce unintentional epidemiological impacts when the interactions between humans and environment are overlooked. A holistic approach is thus valuable in teasing apart the different contributing factors to diseases allowing for more well-tailored disease control measures.

The risk of disease transmission is increased for the rice paddies located close to the Ubolratana reservoir due to the increased cultivation duration and water connectivity brought about by the ease of irrigation and flooding. Aquaculture and water retention duration in double cropped rice paddies and irrigation ponds can potentially facilitate transmission, while seasonal variation in water levels determines the transmission risks at different time periods. Consequently, the impact of rice cultivation should be understood not just from the perspective of the anthropogenic modification of land use involving converting lowland forests to rice paddies. It should also be understood from the perspective of variations among rice paddies patches, as some rice paddies patches, such as those located close to the reservoir or other irrigation sources, have more risk of facilitating *O. viverrini* than other rice paddies patches, such as those located at the dam outlet. Spatial variation in rice paddies in this study is governed by two main groups of factors—those relating to the duration of cultivation, which includes cultivation characteristics double cropping, irrigation, and lower surface elevation, and those relating to water transmission. The latter includes cultivation characteristics that are associated with water transmission, such as drainage of water from paddies, flooding of paddies, and presence of fish in paddies. In the study of diseases associated with rice cultivation, distinctions should be made between paddies of "high risks" and those of "low risks" of pathogen transmission potential, as lumping rice paddies of such varied transmission potentials can mask the impacts of rice paddies' contribution to infections and the impact of rice paddies in shaping the distribution of *O. viverrini* cercariae.

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Although existing health education interventions seem to be having an impact on long-held practices (Sripa et al., 2015), there is great geographical unevenness in terms of the application and sustainability of these programs. Community-based interventions in particular districts and villages that focus on behavior modification through school curricula, social activities relating to prevention of diseases, and food preparation and cooking knowledge, are effective in certain locales (Kaewpitoon et al., 2008; Duangsong et al., 2013). There are problems of uneven public health outreach, underfunding, and changes in primary health campaigns over time, while environmental influences on disease distribution are often overlooked. Consequently, tackling the problems of opisthorchiasis has been effective in some places but not in others and lacks sustainability.

Moreover, chemotherapeutic treatment is not equally applied throughout northeast Thailand, as each province has the autonomy to decide on health priorities locally. While chemotherapy is highly effective in reducing infection prevalence, re-infections often occur upon the cessation of chemotherapy. For long-term sustainability, control strategies need to identify and target the wide range of factors underlying the pathogenic landscape of *O. viverrini* such as the influence of land use modification, including dam construction and rice cultivation variation, human behavior and livelihoods, and health center priorities. Although the implementation of integrated programs, such as the Lawa project in Thailand (Sripa et al., 2015), is critical in controlling the disease situation, such integrated programs need to lead to a long-term reduction of pathogens across their transmission pathways even after the termination of a program or funding. The measure of success for disease mitigation programs should not be based on temporary infection suppression through chemotherapy and other forms of short-term active controls, but on the level of re-infection in humans and infection statuses of other intermediate hosts.

7.3 The recalcitrance to healthcare control and the importance a holistic approach

Given the findings from this study, the research framework created in Chapter 1 (Figure 1.4) was expanded to include new relationships. The relative contributions to *O. viverrini* infection are presented as odds ratios, which are derived from either the logistic regression models or are converted from percentages (Figure 7.4). The factors in Figure 7.4 are referred to as risk factors, protective factors, or neutral factors. Risk factors are factors that will bring about an increase in infection. In a closed system, the control of risk factors will only lead to an increase in infection at a decreasing rate while protective factors can reduce infection. Neutral factors contribute neither to further increase nor decrease in infection.

The pivotal roles of health centers in *O. viverrini* control is highlighted in Figure 7.4. Health center efforts in chemotherapy and awareness are the only protective factors identified in this study, which can contribute to a reduction in human infection, while all the remaining factors are risk factors or neutral factors. In practice however, health center efforts alone have proven to be insufficient for infection control as other risk factors, such as land use modification and human behavior and livelihoods, continue to predispose humans to repeated re-infection.

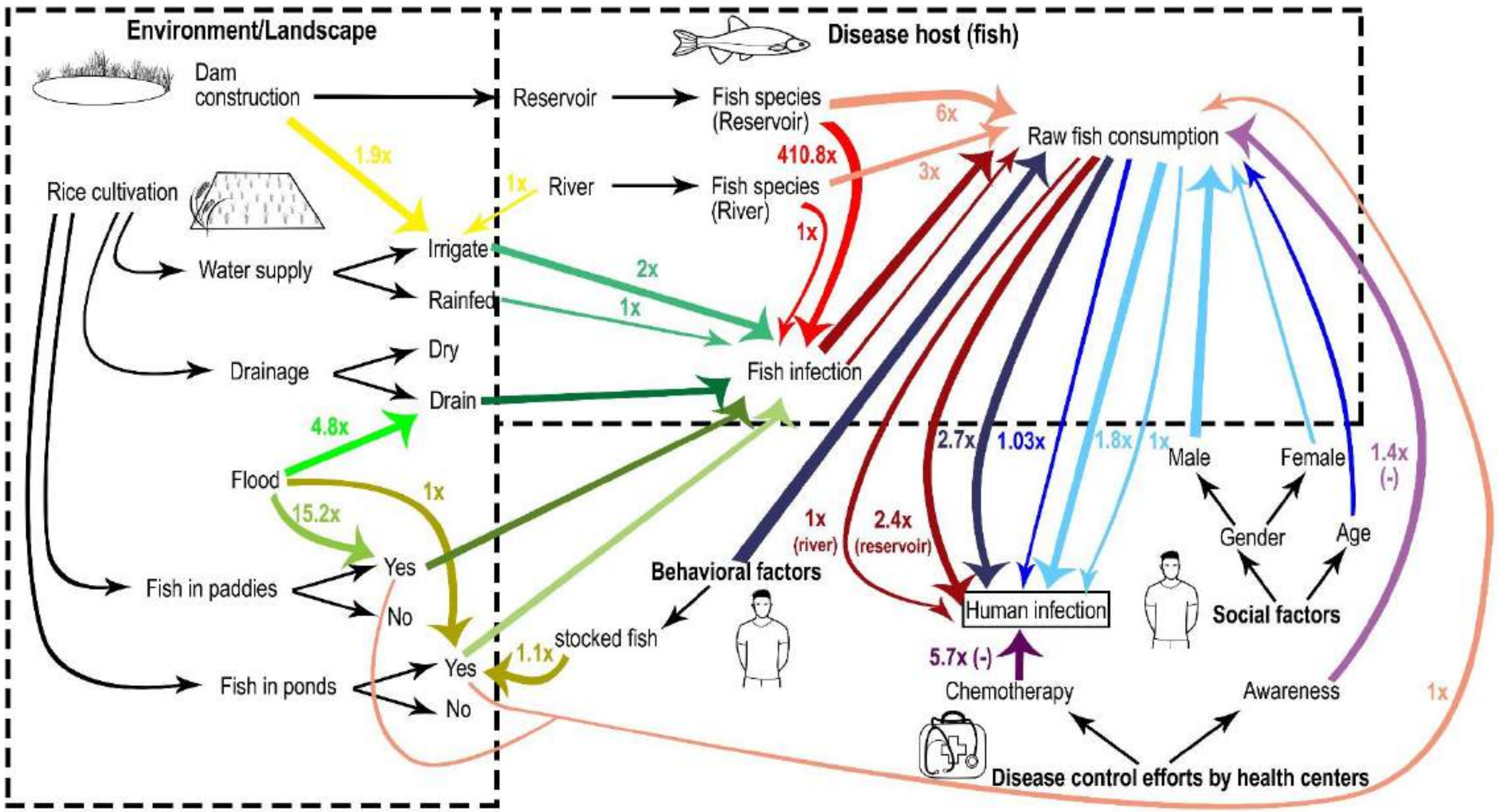

Figure 7.4: The relationships among factors shaping the pathogenic landscape of *O. viverrini*, which are examined in this study. Red arrows show the results for research aim 1, pink and brown arrows for aim 2, various shades of blue arrows for aim 3, shades of purple arrows for aim 4, and shades of green and yellow arrows for aim 5. The values beside these arrows represent their relative contributions to *O. viverrini* infection. They are derived from analyses in the earlier chapters including odds ratios from the logistic regression models or converted from percentages. The values are meant for comparisons between arrows of the same color but not across different colors.

The complete elimination of a risk factor central to the transmission pathway can serve as a protective factor if it can lead to a break in the transmission cycle of *O. viverrini* thus preventing human infection. Risk factors central to the transmission pathway of *O. viverrini* include the intermediate hosts, snail and fish, and raw fish consumption behavior. It is however challenging to eliminate any of the risk factors central to *O. viverrini* transmission. While molluscicide has been shown to be effective against *Bithynia* snails in the laboratory, *Bithynia* snail behavior, which can burrow into the mud, makes complete elimination of snails impossible, especially in the rice paddies (Thammasiri et al., 2010). The fish intermediate hosts are important sources of animal protein and livelihood in the region (Grundy-Warr et al., 2012), and the elimination of raw fish consumption behavior in humans has been met with limited and unequal success (Sithithaworn et al., 2014).

Except for risk factors central to the transmission pathway, the elimination of most of the other risk factors will convert them into neutral factors. Infection pathways are not broken by the elimination of such factors because other risk factors exist to support the continuation of *O. viverrini* life cycle. Environment/landscape factors are such risk factors. For instance, the presence of fish in paddies and irrigation ponds are risk factors while the absence of fish in those habitat types are neutral factors (Figure 6.4). Even the complete elimination of dams or rice paddies can only function as neutral factors as *O. viverrini* can persist in the absence of such human land use, albeit likely to be at a far lower prevalence and in fewer geographical locations. According to Petney et al. (2013), the original hosts of *O. viverrini* are likely to be fish-eating mammals found in the region including the fishing cat, leopard cat, crab-eating mongoose, otters, and even crab-eating macaque. These mammals maintain the *O. viverrini* transmission cycle, but prevalence and intensity of infection are likely to be substantially lower than that maintained in a human-modified environment. The presence of human-modified landscapes, including dam construction and rice paddy creation, exacerbates the abundance and range of the parasite, as they create positive feedback/reinforcing loops (labelled R1 and R2 in Figure 7.5) with the transmission cycle of *O. viverrini*.

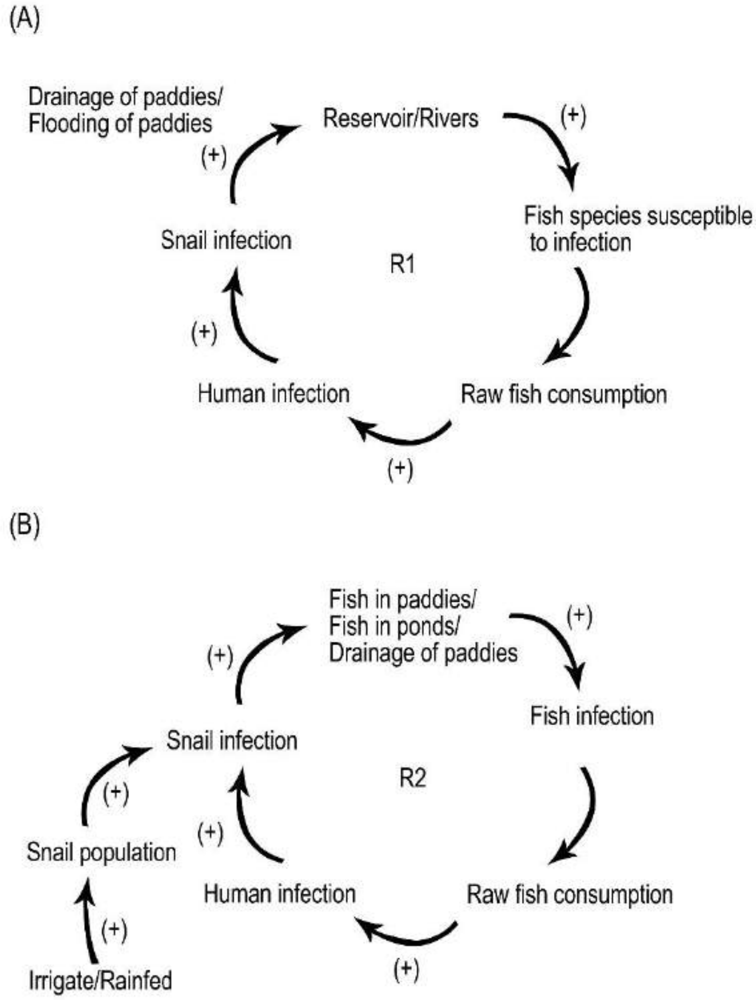

Figure 7.5: Positive feedback/reinforcing loops derived from Figure 7.2 (A) Relationship between reservoir or rivers with *O. viverrini* life cycle, labelled R1. Transmission of cercariae from snails to fish in the rivers or reservoir is facilitated by drainage or flooding of rice paddies. (B) Relationship between rice cultivation practices and *O. viverrini* life cycle, labelled R2. Rice cultivation practices facilitates transmission of cercariae from rice paddies to the major fish habitats, rivers and reservoir, and serve as minor fish habitats through flooding of paddies and irrigation ponds. Rice paddies, especially irrigated paddies, also increase snail population, which potentially increase snail-shed cercariae.

As shown in Figure 7.5A, the reservoir and rivers harbor fish species that are susceptible to *O. viverrini* infection (Chapter 4), when the fish species susceptible to infection are consumed, it increases the likelihood of human infection (Chapter 4 and 5). With improper sanitation, human infection increases snail infection, which can then increase cercariae transmitted to rivers or the reservoir via drainage or flooding (Chapter 6), creating a reinforcing loop (R1) (Figure 7.5A). As shown in Figure 6.5B, fish in paddies, fish in ponds, and the drainage of paddies can lead to fish being infected with *O. viverrini* cercariae in the rice paddies (Chapter 6). Consumption of infected raw fish increases human infection. Increase in human infection increases *O. viverrini* eggs produced by humans thus increases the likelihood of snails getting infected (Centers for Disease Control and Prevention, 2012). At the same time, the increased in snail population by rice cultivation allows more snails to be infected with eggs from infected humans to produce more cercariae. This is exacerbated by the life strategy of *O. viverrini* in snails, which undergo asexual reproduction capable of producing over a thousand cercariae from a single snail in one day (Phongsasakulchot et al., 2005; Kiatsopit et al., 2014). Increase in snail infection can then increase infection of fish in paddies or ponds (Centers for Disease Control and Prevention, 2012). It can also increase the number of cercariae present in the inundated rice paddies and introduced to the rivers or reservoir during drainage of paddies (Chapter 6). This creates a reinforcing loop (R2) (Figure 7.5B).

Conversely, health center control efforts and the consumption of raw fish can form negative feedback/ balancing loops (labelled B1 and B2) (Figure 7.6). As derived from the data collected for this study, provincial funding is dedicated to *O. viverrini* control in regions where *O. viverrini* infection prevalence is high (Chapter 6), and will likely to be channeled to other diseases when infection prevalence appears to be kept under control, creating a balancing loop (B1). Low infection prevalence and chemotherapy can lull people into a false sense of security, potentially reducing risk avoidance behavior, while high infection or death of loved ones from CCA can reduce raw fish consumption (Chapter 6), creating a balancing loop (B2).

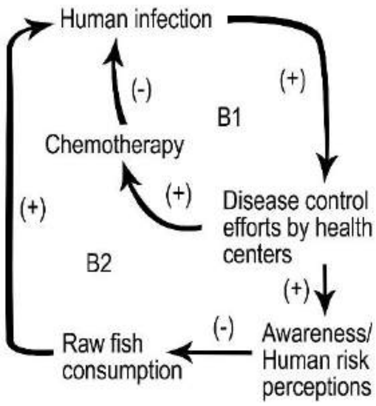

Figure 7.6: Negative feedback/reinforcing loop of chemotherapy efforts, labelled B1, and human awareness/risk perceptions which can lead to risk avoidance behavior, labelled B2.

Such antagonistic feedback mechanisms between the physical environment and healthcare and social environment supported the persistence of *O. viverrini* infection in humans. *O. viverrini* population persists in the environment as it is governed by reinforcing loops, allowing *O. viverrini* population to increase exponentially upon the termination of chemotherapeutic treatment if the underlying conditions continue to support the proliferation of *O. viverrini*. At the same time, the identification of such opposing feedback mechanisms points to the solution for *O. viverrini* control. Given the difficulty in the complete elimination any of the risk factors central to *O. viverrini* transmission, implementation of an integrated control strategy involving the reduction of environmental and social risk factors contribution, and the concomitant efforts of health centers in re-infection prevention is necessary. Such an integrated control strategy can reduce the overall parasite present in the landscape, either to levels that are sufficiently low to avoid health risks in the majority of the population, or to reduce the parasite population to below their basic reproduction numbers (Holme and Masuda, 2015), allowing for infection to die out in the long run. Given the likelihood of resistance development to chemotherapeutic drugs (Prichard et al., 2012) and the carcinogenic effect of prolonged treatment of infection (Pinlaor et al., 2008; Yongvanit et al., 2012), chemotherapeutic treatment should be used as a short-term measure instead of a long-term solution in disease control. Similar integrated control strategy, consisting of environmental modification, water management, snail control, education, and routine chemotherapeutic treatment of humans, is being applied to targeting schistosomiasis, where complete elimination of risk factors central to disease transmission is also challenging (Yang et al., 2016).

Designing a holistic integrated control strategy requires the recognition of features of parasitism that are shaping the pathogenic landscape, as identified in this study. The antagonistic relationship of health center control efforts with other environmental and social risk factors highlights the need for a holistic approach in the control of such disease with a close association with complex underlying factors. A holistic approach helps to pinpoint aspects to be focused on in policies and research, allowing for integrated disease control, which cannot be easily achieved when examining the risk factors individually.

7.4 Recommendations for disease control and future studies

Based on the findings of this study, recommendations for integrated control and potential for future research are discussed and summarized in Figure 7.7.

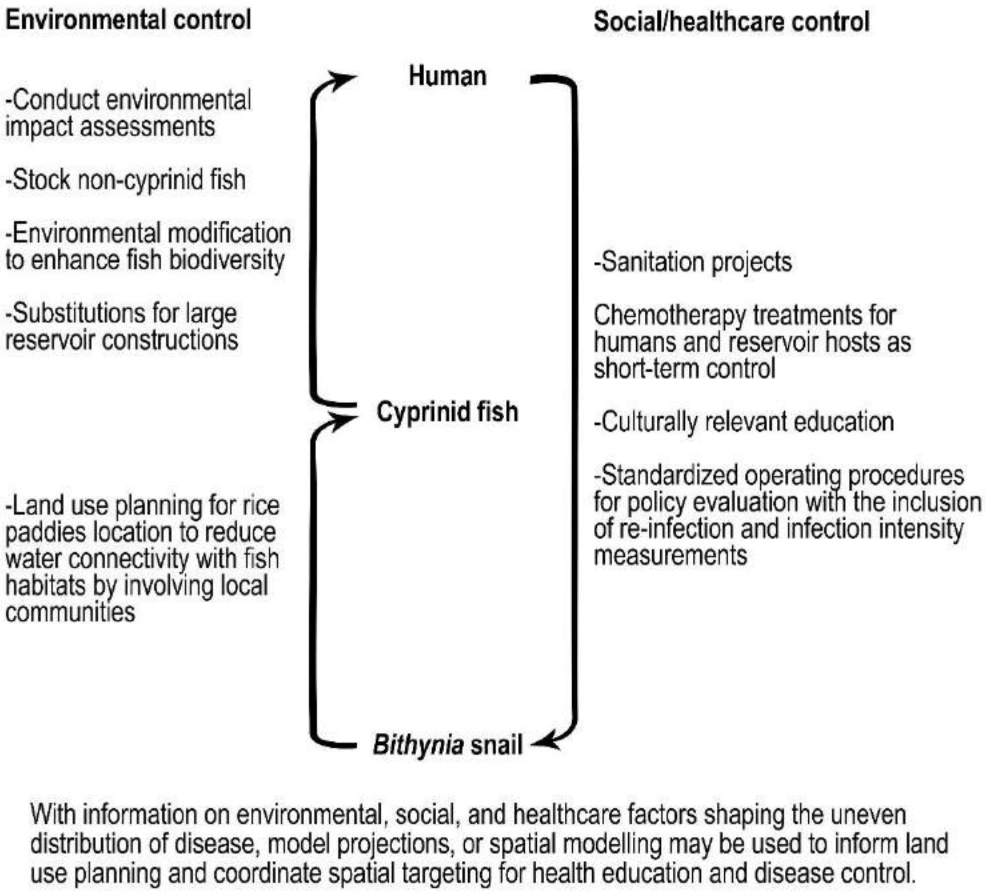

Figure 7.7: Summary of recommendations for an integrated control strategy. The control measures need to be implemented as a package to control *O. viverrini* transmission from different angles, so as to achieve long-term sustainability, even after the cessation of chemotherapy efforts.

Given the divergent and contradictory impact of dams and irrigation projects, environmental impact assessments not only control emergence and spread of opisthorchiasis and other water-associated diseases, but also mitigate conflicts between national and local interests in promoting economic development while maintaining integrity of the environment, livelihoods, and health of the local population (Sneddon, 2002; Keskinen et al., 2012). Conflicts between government and local population on the impact of dams and irrigation construction have been well documented in Southeast Asia, ranging from loss of livelihoods and homes after the development of Pak Mun Dam (Foran and

Manorom, 2009), loss of sedimentation nutrients for agriculture in the lower reaches of the Mekong River Basin (Kuenzer et al., 2012), to severe industrial pollution of the Nam Pong River Basin (Sneddon, 2002), where the Ubolratana dam is located. While epidemiological consequences have been taken into account in dams constructed decades after the Ubolratana dam, since the establishment of mandatory environmental impact assessments in 1981 (Office of Natural Resources and Environmental Policy and Planning, 2012), epidemiological consequences, including *O. viverrini* infection, has been mitigated through chemotherapy efforts instead of preventive measures through environmental control (Amornsakchai et al., 2000). Results from this study, however, highlighted possible environmental control measures that can be taken in future dam construction projects. Possible mitigation measures include positioning of rice paddies at the dam outlet instead of around the dam, environmental modification to enhance fish biodiversity, or the stocking of fish species not susceptible or less susceptible to *O. viverrini* infection.

Another avenue for fruitful research would be to examine the *O. viverrini* infection susceptibility of the common species that are being stocked in reservoirs, such as farmed fingerlings and juveniles, including exotic species (Welcomme and Vidthayanon 2003). Instead of stocking cyprinid fish in the Ubolratana reservoir (Kakkaeo et al., 2004), other species often consumed by locals, including Nile tilapia (Little et al., 1996), may be considered. However, fish ecology and trophic relationships must be taken in account when deciding on the fish species to stock. Alternatively, cyprinid fish with low likelihood of infection, including *H. siamensis*, as observed in this study and other studies

(Wang, 2012; Touch et al., 2013), may be stocked. Similarly, farmers may be encouraged to stock non-cyprinid fish into their irrigation ponds, which can prevent transmission of *O. viverrini*.

Mitigation measures such as enhancing fish biodiversity might be valuable in providing a dilution effect on the parasites. This may be achieved through habitat modification/rehabilitation, including the introduction of fish pass and restoration of the hydrological function and fish habitats in rivers and floodplains (Food and Agriculture Organization of the United Nations, 2015). While not without faults, run-of-the-river hydropower or small reservoirs instead of big reservoirs to reduce damage to the environment and fish diversity may be explored (Csiki and Rhoads, 2010; Premalatha et al., 2014; Anderson et al., 2015).

Given that the rice paddies found with the lowest infection transmission risks in this study are those located at the dam outlet, in terms of land use planning, to prevent flooding of rice paddies facilitating habitat connectivity, rice paddies may be situated at the dam outlet where flooding is uncommon. Areas upriver of the dam, which are adjacent to the reservoir and prone to flooding, can be used for other forms of cultivation or recreational activities. Local communities should be involved as one of the stakeholders in deciding the most suitable land use.

While molluscicide is widely used for snail control to prevent schistosomiasis (King and Bertsch, 2015), the costs of using of molluscicide for *O. viverrini* control may outweigh the benefits, given that the highest abundances of *Bithynia* snails are found in rice paddies where snails are able to avoid the molluscicide by burrowing. Application of the molluscicide can be harmful to other organisms including fish and other species of snails, thus reducing biodiversity (Isenring, 2010), which may lead to an increase in population levels of intermediate hosts through reduced interspecific competition (Haruay et al., 2008). Instead, preventative sanitation projects, involving increased availability of latrines and improved septic tank construction to prevent leakage, may be more effective in limiting snail infection in the long-term (Yang et al., 2016).

A promising area of behavioral modification that is currently being explored is changes in social behavior targeting children (Sirpa et al. 2015). Inculcating health-enhancing habits through school-based activities with family and community support may be effective in changing behaviors (Jepson et al., 2010). The cultural relevance of human behavior can also determine the effectiveness of health messages and campaigns. This includes the deep-seated raw food consumption culture (Grundy-Warr et al., 2012), and the perceptions and reasoning of risk avoidance behavior of locals shaped by their concept of health, life, and death in a largely Buddhist society. Samiphak (2014) argued that locals' risk avoidance behavior may be influenced by the concept of *karma*, and they may have less incentive for active risk avoidance behavior, as good health and longevity are considered as the rewards for good deeds, and the converse, diseases and sufferings as punishment for bad deeds. As such, health campaigns need to be culturally relevant and health messages need to be in line with the perspectives of locals.

In terms of health center policies, it is important to include infection intensity in addition to the commonly used infection prevalence for disease monitoring given the role of infection intensity in intensity-dependent mortality and providing different insights into the features of parasite transmission. Infection intensity and re-infection should be used as a measure of policy success in addition to infection prevalence. Chemotherapeutic treatments should be recognized as a short-term control aimed at lowering initial infection levels and it is necessary to adopt additional environmental control and behavioral modification measures to prevent re-infection. In addition, chemotherapeutic treatments need to be applied on reservoir hosts, especially cats, which are found with high *O. viverrini* infection prevalence and intensity (Enes et al., 2010; Anupromma et al., 2012). Standard protocol to carry out measurements and sampling techniques should be established to enable comparison across localities and prevent misinformation. For instance, as observed in this study, the use of the less sensitive fecal sampling method for infection determination—direct smear—lulls health center workers and local communities into a false sense of security, which can reduce risk-avoidance behavior. Such infection results are also not useful or may even be misleading for large scale spatial analyses. In the study of diseases with high geographical focality, such as many helminthiases, pooling data without taking into account of such variation produce results that are not very informative or even misleading, and should be avoided.

Given the high geographical focality of opisthorchiasis, geostatistical modelling, which relied upon detailed environmental, social, and healthcare information, such as those collected in this study, can be highly effective in informing land use planning and coordinate spatial targeting for health education and disease control (Lai et al., 2015; Kraemer et al., 2016). Along with data from the current study, future dam impact studies on fish species change, flooding and irrigation of rice paddies, will yield data for model projections on the potential impacts of dams to help in future land use planning. In cases where dam construction is unavoidable, such models can facilitate the implementation of mitigation measures prior to dam construction (Wang et al., 2016a). Taking landscape connectivity of rice paddies with surrounding water bodies into account could control the source of *Bithynia* snails, while using remote sensing models developed for identification of paddy rice cultivation growth stages (Xiao et al., 2006; Torbick et al., 2011) can allow for patches of rice paddies of varied risk characteristics to be identified. In terms of managing human disease incidence, recent efforts to provide more systematic digital health screening, using ultrasound and other tests, in numerous districts across several provinces of northeast Thailand (i.e. Cholangiocarcinoma Screening and Care Program 2015) will provide digital mapping data relating specifically to incidence of opisthorchiasis and CCA. This promises potential for coordinating spatial targeting for health education and disease prevention campaigns.

7.5 Conclusion

This study identified the environmental, social, and healthcare factors underlying the pathogenic landscape of *O. viverrini*, and highlighted the importance of a holistic approach in the study of other helminth diseases, and in fact, most pathogenic diseases. Well intentioned dam constructions can produce unintentional epidemiological impacts that could not be ascertained in studies that examine risk factors in isolation. Dam construction impact on fish infection through alteration of fish species assemblages and consequently affect overall fish infection status. The interactions between dam construction and human fish procurement behavior, and their preference for specific species of fish to be consumed raw, shape the distribution of human infection. Dam construction also enhances water connectivity, which facilitates infection from snail to fish through irrigation. Irrigation allows for double cropping, which increases the duration of rice paddies to function as snail habitats, and the source for snail-shed cercariae.

The importance of interacting risk factors in shaping the distribution of diseases in seemingly similar land use type is exemplified in the flood occurrence variation between rice paddies located upriver or downriver of the dam. Flooding, which connects rice paddies with the reservoir, facilitates fish infection at sites upriver of the dam, but is prevented in sites downriver of the dam.

Disease control efforts by health centers, including chemotherapy treatment, and education campaigns are protective factors important in disease control efforts, but are unevenly applied spatially and temporally. Moreover, chemotherapy is only palliative, with re-infections quickly occurring if the underlying factors that expose humans to infection are not dealt with, while repeated chemotherapeutic treatments can increase the carcinogenicity of opisthorchiasis. The importance of waterscapes in determining human infection is easily overlooked when only infection prevalence is used as a measure of success for disease control efforts, as infection intensity, which follows similar patterns as fish infection in this study can enable a very different understanding of the disease transmission. There is thus a need to include re-infection and intensity measurements in evaluating the success of control programs. Opisthorchiasis and other helminthiases exhibit high geographic focality due to the close association with the environment. A holistic approach to target disease treatment from different angles is necessary for sustainable disease control efforts, and inform the geographies of human pathogenic diseases. While this study focuses the control of opisthorchiasis, recognizing the features of parasitism shaping the pathogenic landscape and geographical focality of diseases is an important contribution to the framework of EcoHealth/One Health approach and to the control of other diseases sharing similar contingent causal factors, including other food/water borne diseases and soil-transmitted helminthiases. Integrated control programs should take into account of other communicable and non-communicable diseases, food security, and nutritional statuses etc., as the ultimate health and well-being of the population is determined by a series of interacting factors rather than a single pathogen.

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Appendix 1

List of publications from this thesis:

Ong, X., Wang, Y.-C., Sithithaworn, P., Grundy-Warr, C. & Pitaksakulrat, O. 2016. Dam influences on liver fluke transmission: Fish infection and human fish consumption behavior. *Annals of the American Association of Geographers*, 106**,** 755-772.

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Appendix 2

Participant Questionnaire
Landscape and human behavior factors determining fish to human transmission of liver fluke, *O. viverrini*, around the Ubolratana reservoir, northeast Thailand.

Instruction: Fill in the blanks. Please check the appropriate boxes with a ✓.

<u>Participant information</u>

1. What is your relationship to the head of the household? Please tick.

- ☐ Head of household
- ☐ Grandfather/Grandmother
- ☐ Father/Mother
- ☐ Uncle/Aunt
- ☐ Brother/Sister
- ☐ Cousins
- ☐ Son/Daughter
- ☐ Nephew/Niece
- ☐ Grandson/Granddaughter

2a. When was the last time you took the deworming drug for liver fluke? Please tick.

- ☐ Never **(skip 2b and 2c)**
- ☐ One year ago
- ☐ A few years ago
- ☐ More than 10 years ago

2b. Did you have a fecal examination to test for the parasite before taking the drug? (circle)
Yes/No

2c. Where did you get for medicine from? Please tick.

- ☐ From health center
- ☐ From hospital
- ☐ From friends/relatives
- ☐ Others:______________________________________

3. Occupation (may choose more than 1 option)

- ☐ Housewife
- ☐ Farmer
- ☐ Fisherman
- ☐ Contract worker
- ☐ Retiree
- ☐ Food seller
- ☐ Craftsman
- ☐ Office work
- ☐ Student
- ☐ Others:________

4. Education level. Please tick.

- ☐ Illiterate
- ☐ Secondary
- ☐ University
- ☐ Primary
- ☐ Diploma
- ☐ Others:____________

5. Personal average income/month

____

6. Household income/month

____

7. Number of people in household

____

<u>Fish consumption behaviour</u>

8. Do you eat "koi pla" , "pla som", "pla ra" or "mum"? How often?

Do not eat: **0**
Daily: **1**
Weekly: **2**
Monthly: **3**
Special occasions/ once a year: **4**

	Cooked frequency	Raw frequency
Koi pla		
Pla som		
Pla ra		
Mum		

*Indicate if the fish used are not cyprinids.

9. Do you know about *O.viverrini* parasite? Please tick.

- ☐ Yes
- ☐ No

If respondent eats raw koi pla, pla som or mum (exclude pla ra):
10a. Why do you choose to eat raw fish (koi pla/ pla som/mum)?
(may choose more than 1 option)

- ☐ Delicious
- ☐ Take medicine
- ☐ Eat with friends
- ☐ Habit
- ☐ Others:_______________

If respondent do not eat raw koi pla or pla som (exclude pla ra):
10b. Why do you not eat raw fish (koi pla/ pla som/mum)? Please tick.

- ☐ Avoid OV
- ☐ Avoid raw fish due to other health reasons eg. Stomachache
- ☐ Do not like raw fish
- ☐ Others:__________________

11. What are your most commonly eaten types of fish (cooked and raw)? How often do you eat them?

Do not eat: **0**
Daily: **1**
Weekly: **2**
Monthly: **3**
Special occasions/ once a year: **4**

	Consumption frequency
Cyprinid fish	
Catfish	
Snakehead	
Others:_________________	

……………………………..

End of Questionnaire

Thank You

แบบสอบถามการเคลื่อนย้ายและพฤติกรรมการกินปลาของประชาชน

คำชี้แจง เติมข้อความลงในช่องว่างให้สมบูรณ์ และทำเครื่องหมายในช่องว่าง ✓ หน้าคำตอบที่ท่านต้องการ

ส่วนที่ ข้อมูลทั่วไป 1

1. ท่านมีความสัมพันธ์ใดกับเจ้าของบ้าน กรุณาเลือก

☐ เจ้าของบ้าน

☐ พี่/ น้อง

☐ สามี/ภรรยา

☐ ปู่ตา/ ย่ายาย

☐ พ่อ/ แม่

☐ ลูกพี่ลูกน้อง

☐ ลูกชาย/ ลูกสาว

☐ ลุง/ ป้า

☐ หลายชาย/ หลานสาว (เป็นลูกของพี่หรือน้อง)

☐ หลายชาย/ หลานสาว (เป็นลูกของลูก)

2. ครั้งสุดท้ายที่ท่านกินยาถ่ายพยาธิสำหรับรักษาโรคพยาธิใบ้ตับ

☐ ไม่เคย (ข้ามข้อ 2ข และ 2ค)

☐ น้อยกว่า 1 ปี

☐ มากกว่า 1 ปี

☐ มากกว่า 10 ปี

2ข. ท่านได้ตรวจอุจจาระก่อนกินยาถ่ายพยาธิหรือไม่ (วงกลมคำตอบ)

ใช่ / ไม่ใช่

2ค. ท่านได้รับยาถ่ายพยาธิจากที่ใด

☐ จากสถานีอนามัย

☐ จากโรงพยาบาล

☐ จากเพื่อน/ญาติพี่น้อง

☐ อื่นๆ

3. อาชีพ (ตอบได้มากกว่า 1ข้อ)

- ☐ 1.แม่บ้าน
- ☐ 2.ทำไร่/ทำนา
- ☐ 3.ชาวประมง
- ☐ 4.ลูกจ้าง
- ☐ 5.ไม่ได้ทำงาน
- ☐ 6.ขายอาหาร
- ☐ 7.ช่างฝีมือ
- ☐ 8.ข้าราชการ/รัฐวิสาหกิจ
- ☐ 9.นักเรียน
- ☐ 10.อื่นๆ

4. จบการศึกษาระดับใด

- ☐ 1. อ่านไม่ออก
- ☐ 2. ประถมศึกษา
- ☐ 3. มัธยมศึกษา
- ☐ 4. ปวช/ปวส
- ☐ 5.ปริญญาตรี
- ☐ 6.อื่นๆ

5. รายได้เฉลี่ยต่อเดือน ……….......บาท

6. รายได้ของครอบครัวเฉลี่ยต่อเดือน ..…………..บาท

7. จำนวนสมาชิกในครัวเรือน …..……….คน

ส่วนที่ 2 พฤติกรรมการกินปลา

8. ท่านเคยกินอาหารต่อไปนี้หรือไม่ อย่างไร

ไม่เคยกิน	ใส่ =0	กินทุกวัน	ใส่ =1
กินบ่อยๆทุกสัปดาห์	ใส่ =2	กินบางครั้งทุกเดือน	ใส่ =3

นานๆครั้ง เฉพาะโอกาสสำคัญๆของปี ใส่=4

	ปรุงสุก	กินดิบ หรือสุกๆดิบๆ
ก้อยปลา		
ปลาส้ม/ส้มปลา		
ปลาร้า		
หม่ำ		

*ระบุชนิดปลาด้วย ถ้าไม่ใช่ปลาขาวปลาตะเพียน Indicate if the fish used are not cyprinids.

9. ท่านรู้จักพยาธิใบไม้ตับหรือไม่

- ☐ 1.รู้จัก
- ☐ 2. ไม่รู้จัก

10 คำถามเกี่ยวกับการกินก้อยปลาดิบ ปลาส้มดิบ หรือหม่ำดิบ

10a. ถ้าอาสาสมัครตอบว่าเคยกินก้อยปลาดิบ ปลาส้มดิบ หรือหม่ำดิบ (ไม่รวมปลาร้าดิบ)

ทำไมท่านจึงกินก้อยปลาดิบ ปลาส้มดิบ หรือหม่ำดิบ (เลือกได้มากกว่า 1 คำตอบ)

- ☐ 1.อร่อย
- ☐ 2.กินยารักษาพยาธิแล้ว
- ☐ 3.กินกับเพื่อนๆ
- ☐ 4.เป็นความเคยชิน
- ☐ 5.อื่นๆ ______________

10b. ถ้าอาสาสมัครตอบว่าไม่เคยกินก้อยปลาดิบ ปลาส้มดิบ หรือหม่ำดิบ (ไม่รวมปลาร้าดิบ)

ทำไมท่านจึงไม่กินก้อยปลาดิบ ปลาส้มดิบ หรือหม่ำดิบ

- ☐ หลีกเลี่ยงการติดเชื้อพยาธิใบไม้ตับ
- ☐ หลีกเลี่ยงจากปัญหาด้านสุขภาพ เช่น ปวดท้อง
- ☐ ไม่ชอบกินปลาดิบ
- ☐ อื่นๆ _________________

11. ปลาชนิดใดต่อไปนี้ ที่ท่านกิน (ทั้งปรุงสุกและกินดิบ) และบ่อยครั้งเพียงใด

ไม่เคยกิน	ใส่ =0
กินทุกวัน	ใส่ =1
กินบ่อยๆทุกสัปดาห์	ใส่ =2
กินบางครั้งทุกเดือน	ใส่ =3
นานๆครั้ง เฉพาะโอกาสสำคัญๆของปี	ใส่ =4

	ความถี่ของการกิน
11a.ปลาขาวปลาตะเพียน	
11b. ปลาดุก	
11c. ปลาช่อน	
11d. อื่นๆ	

....