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Determinants for WSD outbreaks in Indonesian smallholder shrimp ponds – a pilot study of locality factors, WSSV genotype distributions and pond factors

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1 Acknowledgments

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We are especially grateful also to the participating farmers for their cooperation and to the study field team, ably led by Mr Moch. Natsir Amin (BBAP, Takalar) and assisted by Mr Mohd. Syaichudin (BBAP, Takalar).

2 Executive summary

This study focused on events in a representative, 50-pond Indonesian shrimp farming system in Pangkep district, South Sulawesi. Ponds were stocked with *Penaeus monodon* and the study extended across a single cropping period between May and October 2010. It was designed to improve our understanding of the main causal pathways for white spot disease (WSD), the most serious cause of production loss in these systems. The longitudinal observational study focused on recording the occurrence of different genotypes of the causal infectious agent, white spot syndrome virus (WSSV), in selected components of the system across time, and looking for relationships with pond outcomes, particularly WSD occurrence. We expected that findings would (a) enable relevant Indonesian agencies, and ultimately farmers themselves, to better identify localities suitable for smallholder shrimp farming using better management practice (BMP) programs and (b) inform modification and simplification of these programs, thereby improving both profitability and adoption rates.

Objectives were:

1. To determine the stability of WSD outbreak-associated WSSV genotypes when passaged through WSSV-free *Litopenaeus vannamei*, WSSV PCR test-negative *P. monodon* and selected other WSSV PCR test-negative, non-penaeid hosts;
2. To identify, using locality-specific environmental data, pond environmental data and data on WSSV genotype distribution and dynamics, the likely determinants for WSD outbreaks at a suitable, broadly representative locality in South Sulawesi.

Our transmission trials showed that genotypy variations did not occur during three sequential passages (four including the preparation of the stock inoculums) in *L. vannamei* or alternatively in other crustacean hosts. This indicates the genotypes are likely to be sufficiently stable for use in local epidemiological studies during disease outbreaks in shrimp ponds.

WSD outbreaks were recorded in 11 of the study ponds. Outbreaks were attributed to WSSV genotype TRS5 in seven ponds and to genotypes TRS4 and TRS6 in two ponds each. Data analysis showed that:

- WSSV was ubiquitous at the site. Strategies to prevent WSD outbreaks are probably more useful than trying to prevent WSSV infection in endemic areas.
- Stocking with WSSV PCR test-negative postlarvae (PLs) supports maintenance of an outbreak-free pond for at least the first two months of production.
- Once PLs have been excluded as a risk factor for WSSV infection, biosecurity (minimising risk of heavy WSSV exposure post stocking) and environmental factors (management of risk factors for WSD outbreaks) become more important.
- Water released from WSD outbreak ponds was likely to be an important initiator of disease outbreaks in upstream and downstream ponds;
- Crabs, wild shrimp, zooplankton and/or polychaetes were not important sources of WSSV infection for WSD outbreaks in farmed shrimp.

These findings suggest that stocking PCR test-negative PLs, coupled with careful management of water intake during nearby WSD outbreaks are the two most useful practice changes farmers can adopt to reduce the risk of outbreaks in their ponds. Establishment of an active, well-resourced and trained extension service, committed to fostering cohesive, informed farmer groups, is the key to enabling these inexpensive practice changes.

3 Introduction

Over 100,000 smallholders across Indonesia use traditional methods to produce *Penaeus monodon* ('monodon') shrimp in more than 200,000 ha of brackishwater ponds, often in polyculture with milkfish and seaweed. Up until the mid-1990s, these farmers were major contributors to the total national farmed shrimp production. However, since then, white spot syndrome virus (WSSV) has spread widely across Indonesia, and recurrent crop failures due to white spot disease (WSD) have progressively reduced the smallholder sector's contribution to its present level of approximately 5%. Evidence suggests this decline is continuing, as more smallholders cut their losses and avoid stocking shrimp altogether.

Other findings, derived mainly from ACIAR projects FIS/2000/061, FIS/2005/075, FIS/2005/076 and FIS/2005/169, strongly suggest that successful smallholder shrimp farming at any locality in Indonesia now requires particular farmer characteristics (individual and collective), skilful management of diverse biological and environmental factors, an effective extension service, suitable market conditions and supportive policies by governments at district, provincial and national levels.

Currently, the most promising way smallholder farmers can return to full production is by adopting better management practice (BMP) programs. These programs currently comprise 'packages' of individual BMPs which, individually or in various combinations, reduce the risk of WSD by blocking each of the known or potential causal pathways. Because WSSV is so widespread in the farming environment and has many potential pathways whereby it can enter a pond and kill the shrimp, the BMP 'packages' are necessarily quite complex and therefore difficult, and sometimes expensive, for many farmers to implement successfully.

Importantly, many of WSD's potential causal pathways addressed by the BMPs have been identified from laboratory studies or inferred from basic principles, and therefore may not be relevant in many field situations. By identifying the main causal pathways for WSD in smallholder shrimp ponds, we planned to simplify BMP programs and thereby make them easier and cheaper for smallholder farmers to adopt and adapt.

Site-specific factors affecting pond environments and/or biosecurity, appear to influence WSD outbreak occurrence. Recent BMP program implementations for monodon farmers in South Sulawesi, whether by ACIAR under FIS/2000/061 and FIS/2005/169, or by other agencies, have been almost uniformly unsuccessful, while those in Central and East Java have been generally successful. FIS/2002/076 showed that, in coastal aquaculture areas in South Sulawesi, problematic soils, notably acid sulfate soils (ASS) and sandy soils, together with hydrological problems arising from poor canal design and layout, are relatively common.

Adding further layers of complexity, FIS/2002/075, in a study of WSSV genotype distribution and transmission pathways in Indian smallholder ponds, suggested that coastal aquaculture environments, including those in Indonesia, are 'saturated' with various WSSV genotypes, carried in a diverse range of clinically normal, infected host populations. Furthermore, the Indian study findings suggested that pond outbreaks often follow spread of infection involving a limited number of genotypes between neighbouring ponds via shared canals.

Taken together, and putting social, market and policy factors aside, this evidence suggested that success of smallholder monodon farming at any locality in Indonesia depends on interactions between various site-specific physical and environmental features, the distribution and load of various WSSV genotypes in host populations, the virulence and/or competitive fitness of these genotypes, and the maintenance of suitable pond environments and biosecurity. This SRA, conducted in parallel with FIS2005/169, was designed to improve our understanding of these 'technical' interactions. We expected its findings would (a) enable relevant Indonesian agencies, and ultimately farmers themselves, to better identify localities suitable for smallholder shrimp farming using BMP programs and (b) inform modification and simplification of these programs, thereby improving both profitability and adoption rates.

We therefore set out to identify and track WSSV genotypes within and between shrimp ponds in a selected, representative farming area in South Sulawesi, using the tandem repeat sequence (TRS) number as a genotype marker. This approach required that the TRS number invariably remains stable whenever infection is transmitted between individuals of the same or different species. However, in a somewhat controversial study, Waikhom et al (2006) suggested that differential host passaging induces genomic variation, including in TRS number, in WSSV. We therefore proposed two components to the current study. The first involved transmission trials in aquaria to examine the stability of TRS number when specific WSSV genotypes are passaged between species. Findings would then be used to inform the second component, the field study.

Although we provided concurrent extension advice to farmers, this SRA was not designed to measure its influence.

Study objectives were as follows.

1. To determine the stability of white spot disease (WSD) outbreak-associated WSSV genotypes when passaged through WSSV-free *Penaeus vannamei*, WSSV PCR test-negative *P. monodon* and selected other WSSV PCR test-negative, non-penaeid hosts;
2. To identify, using locality-specific environmental data, pond environmental data and data on WSSV genotype distribution and dynamics, the likely determinants for WSD outbreaks at a suitable, broadly representative locality in South Sulawesi.

Prof. Richard Whittington, Faculty of Veterinary Science, University of Sydney led the study, thereby enabling close linkages with FIS/2005/169. Prof Bambang Sumiarto, Faculty of Veterinary Science, Gadjah Mada University, administered the project in Indonesia, particularly in relation to release of funds to Directorate General Aquaculture (DGA), the key Indonesian implementing agency.

Dr Richard Callinan (Senior Research Fellow; USydney) coordinated the project. Core technical teams were led initially by Mr Arief Taslihan (Project Epidemiologist, Indonesia; DGA) and later by Dr Murwantoko (Senior Research Scientist; GMU), Mr Nicholas Gudkovs (Microbiologist, AAHL), and Dr Akhmad Mustafa (Senior Researcher, RICA, Maros).

Dr Peter Walker (Senior Research Scientist; AAHL), Dr Jenny-Ann Toribio (Project Epidemiologist, USydney), Prof. Jesmond Sammut (Environmental Scientist - Aquaculture; UNSW), Prof. Bambang Sumiarto (GMU) and Prof. Kamiso (Senior Research Scientist, GMU) provided expert advice and assistance as necessary. In particular, Dr Toribio supervised the epidemiological analyses of field data by postgraduate student Ms Emma Rooke (Animal Health Australia).

4 White Spot Syndrome Virus Genotype Stability Study

4.1 Introduction

White spot syndrome virus (WSSV) is an important pathogen of farmed penaeid shrimp. It is a large double-stranded DNA (dsDNA) virus with a circular genome of approximately 300 kbp (van Hulten et al. 2001, Yang et al. 2001). Comparison of the complete genome sequences of WSSV isolates from China (WSSV-CN), Thailand (WSSV-TH) and Taiwan (WSSV-TW) has revealed >99% nucleotide sequence identity (Marks et al. 2004). However, although most genes are highly conserved, several loci have been identified in which there are variations including single nucleotide mutations, large insertions/deletions and variation in the number of repeat sequence units within intergenic homologous regions (hrs) and in non-hr direct repetitive sequences, most of which occur within open reading frames (Marks et al. 2004).

The three non-hr direct repeat regions displaying the largest variation between the prototype isolates are located in ORF75, ORF94 and ORF125 and these loci have been employed for WSSV molecular epizootiological studies (Pradeep et al. 2008). Variable number tandem repeat (VNTR) genetic markers have been used to demonstrate variations in WSSV genotypes in moribund shrimp collected from different ponds during disease outbreaks (Wongteerasupaya et al. 2003, Musthaq et al. 2006), distinguish WSSV genotypes in shrimp and wild crustaceans collected from the same pond (Hoa et al. 2005), identify multiple genotypes in individual diseased and healthy shrimp (Hoa et al. 2005). A recent study used a combination of all three VNTR markers in ORF75, ORF94 and ORF125 to study modes of transmission in semi-intensive and improved extensive farming systems and concluded that analysis based on the ORF94 VNTR only was a reasonable alternative to the triple marker approach (Hoa et al. 2011). However, it has also been reported that the number or repeat sequences in ORF94 can shift significantly during differential passaging through shrimp (*Penaeus monodon*), crabs (*Portunus sanguinolentus* and *P. pelagicus*) and freshwater prawns (*Macrobrachium rosenbergii*) (Waikhom et al. 2006) and it was concluded that VNTR variations may result from host selection rather than geographic isolation. If confirmed, this could have significant implications for the interpretation of molecular epizootiological studies employing VNTR markers.

This study was undertaken to re-examine this observation using isolates representing two different WSSV genotypes derived from moribund black tiger shrimp (*Penaeus monodon*) collected during disease outbreaks on farms in Indonesia. The isolates were amplified to produce working stocks in specific-pathogen-free (SFP) shrimp (*Litopenaeus vannamei*) and then sequentially passed through shrimp and freshwater prawns by feeding of infected tissues.

4.2 Materials and Methods

4.2.1 Field collection of WSSV samples

In order to obtain a range of WSSV genotypes representative of the viruses currently affecting Indonesian shrimp, samples of dead and moribund shrimp were collected over a 2 month period in late 2010 from suspect WSSV disease outbreaks in shrimp ponds in the area surrounding Kendal, Central Java, Indonesia. Additional samples of moribund shrimp

were also collected from production ponds in the project study area around Makassar (Table 1). In order to ensure viability and recovery of infectious virus, moribund animals were collected whole onto ice and frozen at -80°C on arrival in the laboratory.

Table 1: Field samples collected for preparation of WSSV stocks.

No.	Sample Code	Pond ID	BMP	Comment
1	B11	Zaim/Badri	Basic	
2	B7	Slamet	Basic	
3	B3	Adam	Basic	
4	B19	Hasim	Basic	
5	B5	Asori	Basic	
6	F12	Wahab	Full BMP	
7	B9	Azim	Basic	
8	B12	Maskur	Basic	
9	B14	Saidun	Basic	
0	B16	Gunadi	Basic	
11	F10	Azhar	Full BMP	
12	Swim Crab	Rajungan	Basic	Putrified
13	B1	H. Satori	Basic	
14	Cherax	Soekarno Hatta	Airport interception	
15	C10.52	Yunarto	Control	Old sample
16	C10.53	Yunarto	Control	Old sample
17-19	Group 1	Makassar		Pangkep study area
20-22	Group 2	Makassar		Pangkep study area

4.2.2 PCR testing of WSSV samples

DNA was isolated from 20-30 mg tissue from pleopods and gills (shrimp) or walking legs (crabs) using QIAGEN spin columns (DNeasy Blood and Tissue Kit Cat: 69504).

Prior to WSSV screening the suitability of DNA samples was confirmed using a 18S ribosomal RNA conventional PCR. The decapod PCR contained 20 mM Tris–HCl (pH 8.4), 50 mM KCl, 1.5 mM MgCl2, 200 µM each dNTP, with 0.2 µM of each primer (Deca-20a2: 5′-CTTCCCCCGGAACCC AAAGACT-3′ and Deca-20s9: 5′-GGGGGCATTCGTATTGCG A-3′) and 0.5 U Platinum Taq DNA polymerase (Invitrogen Cat: 10966–034) and used 2 µl of DNA in a reaction volume of 20 µl. Cycle conditions were: 1 cycle of 94 °C for 2 min, 40 cycles of 94°C for 30 s, 55°C for 30 s, 72°C for 30 s and a final extension of 72°C for 5 min. PCR products were analysed by electrophoresis in 1.5% (w/v) agarose gels (Fermentas Cat: R0491) in 0.5× TBE buffer (Promega Cat: V4251) using standard conditions. Samples which did not yield the expected 240 bp amplicon were diluted with PCR quality water and re-tested. Samples failing to amplify were re-extracted.

The presence of WSSV in the field samples was confirmed by one-step PCR using species specific primers WSSV-1a16 (5'-TTCCAGATATCTGGAGAGGAAATTCC-3') and WSSV-1s5 (5'-CACTCTGGCAGAATCAGACCAGACCCCTGAC-3') using the same conditions described above.

WSSV in samples from the differential passage experiment was confirmed using a WSSV ORF167-targeted Taqman assay (Walker et al., 2011). The primers Taq-WSSV-f: 5'-CCGACGCCAAGGGAACT-3', Taq-WSSV-r: 5'-TTCAGATTCGTTACCGTTTCCA-3' and probe Taq-WSSV-probe: 5'-6FAM-CGCTTCAGCCATGCCAGCCG-TAMRA-3' (Applied Biosystems, Foster City, CA, USA) were designed to target the same region as the OIE-recommended nested PCR (Lo et al., 1996; Lo et al., 1997) and have been validated against this assay. All Taqman assays were conducted in duplicate using 0.4 µM of primer and 0.2 µM of probe in Taqman Fast Universal PCR Master Mix 2X (Applied Biosystems Part No.: 4352042), in a final reaction volume of 20 µl using standard conditions. WSSV copy number was estimated using a standard curve constructed using standardised dilutions of a plasmid standard.

The WSSV VNTR genotypes were determined by PCR amplification of the ORF94 tandem repeat region, based on methods described by Walker et al. 2011. Two primer sets were used with QIAGEN HotStarTaq Master Mix (Cat: 203445). The master mixes and volumes were the same in each case, with 1.5 mM MgCl2 and 0.2 µM of each primer in a total volume of 25 µl. The thermal cycling parameters were optimised for each primer pair and are as follows: Geno-WS-f1 (5'-TATTGACCCCGACCACCGCTGC-3') and Geno-WS-r1 (5'-TCCGCCTCTGCCCACGCATTGA-3') were subjected to 1 cycle of 94°C for 15 min, 40 cycles of 94°C for 20 s, 66°C for 20 s, 72°C for 90 s and a final extension of 72°C for 10 min. The second primer set WS-94f (5′-CGA TGA CGA TGG AGG AAC T-3′) and WS-94r (5′-TCT TCA ACC ACC GTC ACT-3′) were cycled at 94°C for 15 min, 40 cycles of 94°C for 20 s, 62°C for 20 s, 72°C for 75 s, followed by a final extension of 72°C for 10 min. Following electrophoresis, the number of tandem repeat sequences (TRS) was calculated by estimating the size of PCR products relative to molecular size standards (100 bp DNA ladder Promega Cat: G2101) and comparing these to the expected size of amplicons obtained with each primer pair. The calculated TRS values for individual samples were verified by checking the consistency of calculated values for bands in gel photographs.

4.2.3 Preparation of WSSV inoculum

The primary inoculum used for WSSV passage consisted of healthy shrimp infected with WSSV derived from the frozen field sample. Following WSSV genotyping of the frozen field specimens, three samples which showed the presence of a distinct and unique TRS type were selected for preparation of inoculum.

The gill and muscle tissue from animals ID 12, 13 and 17 were aseptically dissected and placed on ice; all traces of cuticle were removed. The tissues were then macerated in disposable petri dishes, using sterile scalpel blades, until the tissue was finely divided. The tissue was diluted 1:10 w/v with sterile phosphate buffered saline and the suspension for each sample was divided between sterile microcentrifuge tubes and centrifuged at high speed (8,000 rpm) for 10 min. Each clarified supernatant was passed through a 0.20 µm membrane filter (Sartorius Cat: 16534) prior to inoculation. The preparation was then used to inoculate groups of 15 SPF shrimp (*L. vannamei*) by parenteral injection. Each animal was given 50 µl of clarified supernatant into the 2nd abdominal segment using a 1 ml tuberculin syringe and 18G hypodermic needle. The animals were then transferred to 20 litre glass aquaria, where they monitored every 3 or 4 h for signs of disease. Dead and moribund animals were removed from the tank, chopped into small pieces and frozen in batches at -80°C as a source of WSSV virus for the first phase of the differential passage experiment.

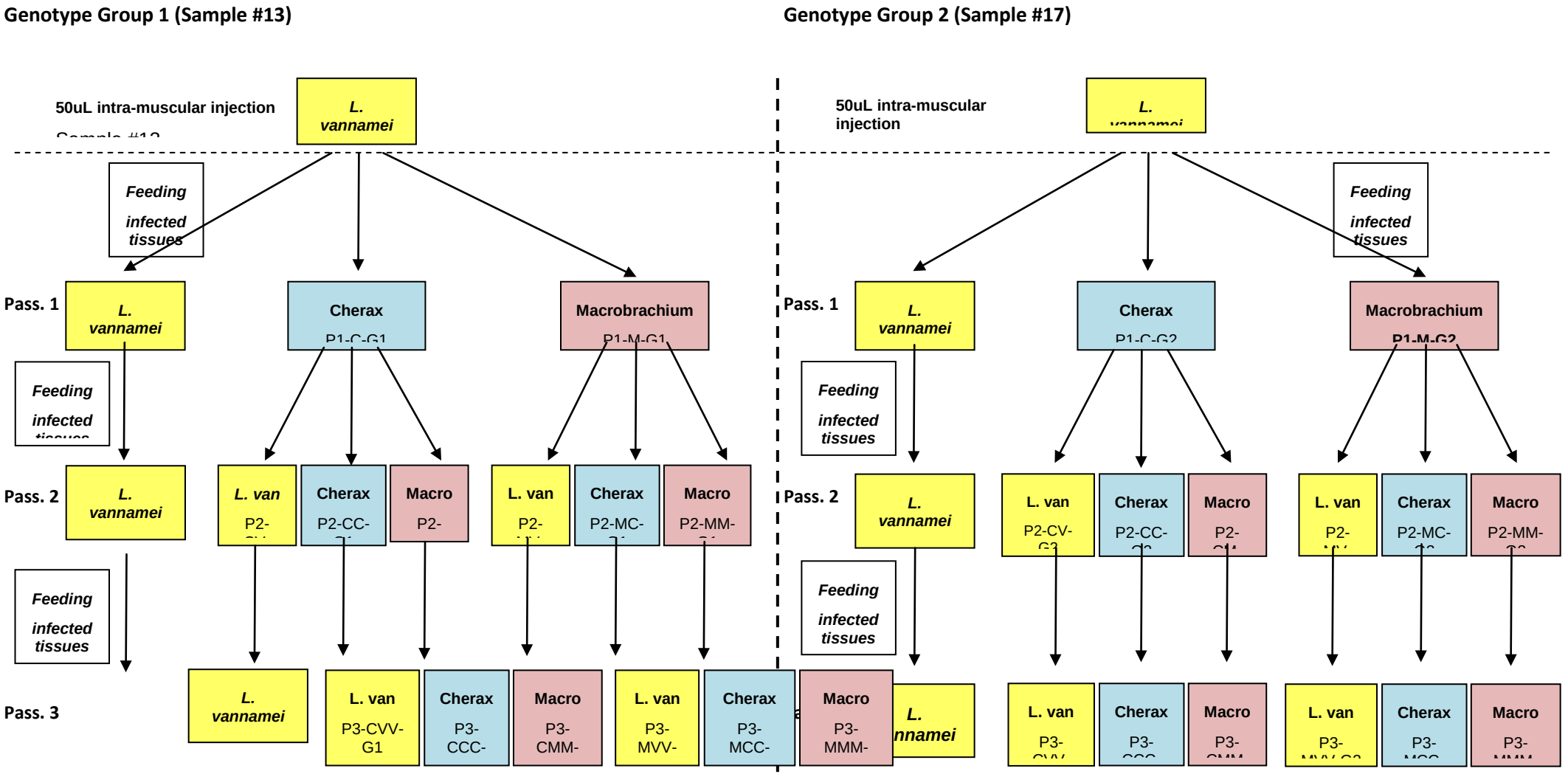
4.2.4 Differential Passage of WSSV in marine shrimp, freshwater shrimp (scampi) and crayfish

Litopenaeus vannamei, Macrobrachium rosenbergii and Cherax sp. were obtained from commercial producers in West Java. These animals appeared clinically normal and were set aside in 100 litre fibreglass holding tanks in the bio-secure aquarium facility in the Indonesian Center for Fish Disease and Environment Laboratory, Serang to acclimatise for 5 to 7 days prior to commencement of the feeding experiments. Samples were taken from each batch of animals to confirm freedom from WSSV. All animals were maintained in water from the same source.

The experimental design used for primary parenteral inoculation and sequential feeding regime is summarised in Figure 1. In order to provide sufficient material for subsequent feeding steps, 10 animals were used for Passage 1. For subsequent Passages 2 and 3, only three animals were used for each feeding group. The experimental tanks were emptied and cleaned between each group of animals. When required, animals were transferred from the large holding tanks to 20 litre glass aquaria for feeding. During the course of the trial, animals found dead or moribund or animals showing signs of disease where euthanized, a small sample of gill and pleopod was fixed in 80% v/v ethanol for WSSV analysis and genotyping, and the remaining tissues used for feeding in the next passage step.

Although WSSV is thought to infect most if not all decapod crustaceans, it was anticipated that the dose, speed of replication and onset of clinical signs would be different in each of the groups at various stages of passage. For this reason and in order to maintain a degree of synchronisation between groups, each of the three feeding passage steps was limited to 3 to 5 days, depending on the availability of aquaria.

Figure 1: Experimental design for feeding passage of 2 WSSV genotype groups



Sample Code

Passage Number – P1, P2 or P3
Species in passages – CMC = *Cherax* to *Macrobrachium* to *Cherax*
Genotype Group – either 1 or 2

4.3 Results

4.3.1 PCR testing of WSSV field samples and selection of genotypes

All 22 frozen samples collected in the field were PCR-positive for WSSV using the 1a16/ 1s5 one-step conventional PCR (Results for samples 1-16 are shown in Figure 2). A clear amplicon of approximately 200 bp after a single round of amplification suggested all the samples contained high levels of virus. Sample 12, a WSSV infected swimming crab (Rejungan-Jepara), was found to be putrid when thawed for DNA isolation but still produced a clear positive result by this test. This sample was included as all the other samples tested were derived from farmed P. mondon and it was considered likely that this sample would have been geographically isolated and subject to a different selective pressures, providing a different WSSV genotype to the shrimp.

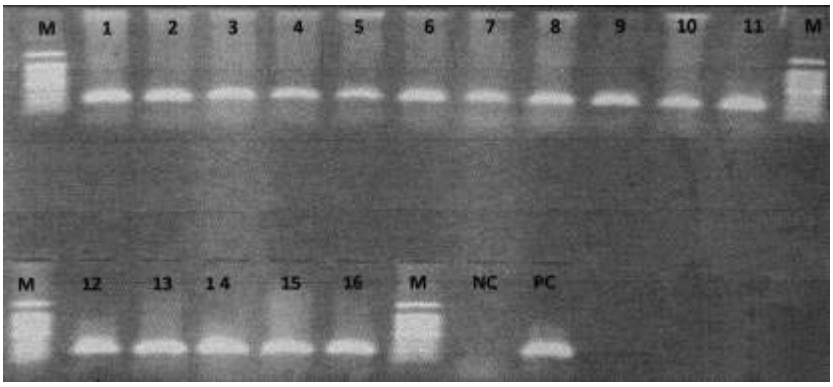
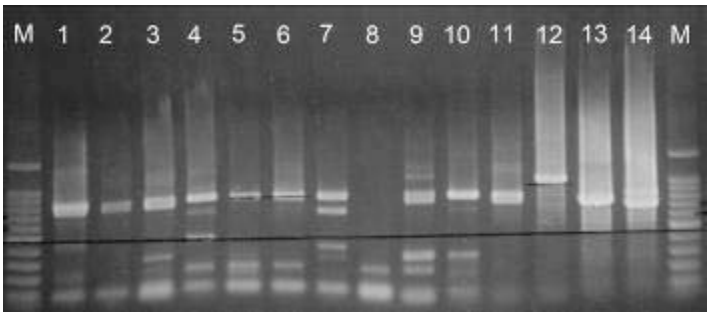


Figure 2: WSSV cPCR (1a16/1s5) of frozen field samples 1 to 16. Lane numbers correspond with sample ID numbers (Table 1). 1.5% agarose, 0.5x TBE

Sufficient WSSV DNA was present to run the WSSV genotyping PCRs with a single round of amplification. All 22 samples were screened using both sets of genotyping primers. The results obtained for all samples using primers Geno-WS-f1/ Geno-WS-r1 are shown in Figures 3A and 3B.

A



B

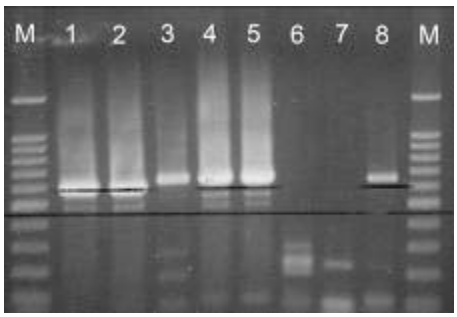


Figure 3. A: WSSV genotyping PCR (Geno-WS-f1/ Geno-WS-r1) of samples 1 to 14, lane numbers correspond to sample ID (Table 1). Samples 12 and 13 were selected for passage. B: Field samples 15 to 22 (lanes 1-8). Sample 17 (lane 3) was selected for passage.

After rounds of testing with both sets of genotyping primers, six samples (5, 6, 12, 13, 17 and 18) appeared to be possible candidates for passage, as they appeared to each contain a single WSSV genotype and displayed TRS profiles that could be readily differentiated from each other using the ORF94 directed PCR. The TRS profiles of samples 5, 6 and 13 appeared to be the same size, corresponding to a TRS of 7. Sample 12 from crabs was unique; the estimated TRS 11 was larger than that seen in any of the P. monodon samples. Samples 17 and 18 from P. monodon at the study area in Pangkep, Makassar appeared to have the same TRS 5 genotype. It was uncertain if samples 5 and 6 had a second genotype present, or if the low molecular size band observed was a PCR artefact or primer dimer. For this reason samples 5 and 6 were included in the final assessment and were tested again using the "inner" WS-94f / WS-94r primers (Figure 4). A second faint low molecular size band was still visible in this assay and so field samples 12 (TRS11), 13 (TRS7) and 17 (TRS5) were selected for passage (Figure 4, lanes 3, 4 and 5)

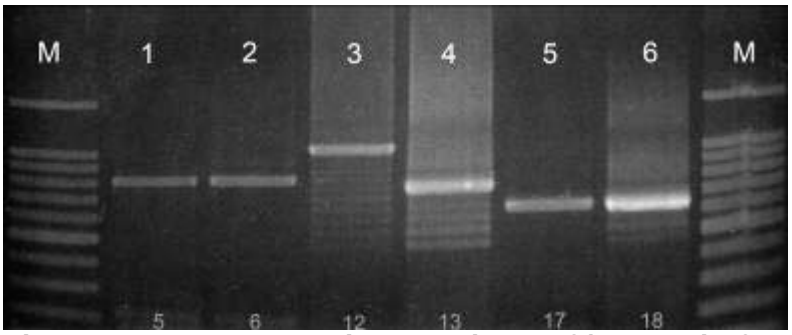


Figure 4: WSSV genotyping PCR (WS-94f / WS-94r) of candidate WSSV samples 5, 6, 12, 13, 17 and 18. Samples 12, 13 and 17 were clearly differentiated and were selected.

4.3.2 Preparation of WSSV stocks

Three separate batches of 15 shrimp (*L. vannamei*) were inoculated with clarified supernatants of samples 12, 13 and 17. Apart from the volume administered and diluent used, no attempt was made to standardise these inocula.

No shrimp in the group inoculated with Sample 12 (TRS 11; crab) showed any signs of disease and all shrimp were euthanized 5 days post-inoculation. Despite the clear demonstration of WSSV DNA by PCR, it was evident that this sample had undergone significant putrefaction before freezing. It was concluded that the virus in this sample was no longer viable.

All shrimp inoculated with sample 13 (TRS 7; P. monodon from Kendal) were severely affected post-inoculation and all these animals died or became moribund within 48 h. All shrimp in the group inoculated with sample 17 (TRS 5; P. monodon from Pangkep) were similarly affected. The onset of disease in these animals was slower but all animals were found dead or became moribund within 72 h post-inoculation. Shrimp tissues were collected from each of these two groups, cut into small pieces and pooled as stocks for the first transmission trial. Subsequent real-time PCR analysis verified that there were high WSSV genetic loads in each of these stocks (Figure 5).

4.3.3 WSSV passage in *L. vannamei*, Cherax sp. and *M. rosenbergii*

Using the two stocks, each containing a different ORF94 VNTR genotype, sequential feeding of the *L. vannamei*, Cherax sp. and *M. rosenbergii* was carried out according to the schedule summarised in Figure 1.

Over the course of the feeding trial, the *L. vannamei* appeared most susceptible to WSSV infection. In all 3 passages by feeding with each genotype, *L. vannamei* became moribund or died within 5 days and contained high WSSV genetic loads of in their tissues. In contrast, in Cherax sp. and Macrobrachium mortalities were only observed during Passage 1 when animals were fed with the *L. vannamei* stocks containing high levels of WSSV. Although WSSV was detected by PCR in these species in Passages 2 and 3, viral genetic loads were low and few moribund animals were observed; most animals were clinically normal.

4.3.4 WSSV genotyping and genotype stability

WSSV genotyping was conducted on samples from each passage group. Those samples showing the highest levels of virus were tested preferentially. As additional animals were available, the Group B (TRS 5) passage in Macrobrachium was performed in duplicate. The results obtained using the genotyping PCRs are summarised in Figure 5. We observed no variations in either the TRS5 genotype or the TRS7 genotype during sequential passage in any of the hosts examined.

4.4 Summary and conclusions

- Dead and moribund shrimp and a swimming crab collected over a 2 month period in late 2010 from suspect WSSV disease outbreaks in shrimp ponds in the area surrounding Kendal, Central Java, and from shrimp collected from production ponds in the project study area around Makassar. An additional crayfish sample (Cherax sp.) was obtained from an interception by Quarantine at Jakarta airport.
- All 22 of the samples tested positive by PCR for WSSV DNA and all were screened using genotyping primers for the VNTR in ORF94.
- Six samples (5, 6, 12, 13, 17 and 18) appeared to be possible candidates for preparation of stocks for experimental transmission as they appeared to each contain a single WSSV genotype and displayed TRS profiles that could be readily differentiated. From these, samples 12 (TRS11), 13 (TRS7) and 17 (TRS5) were each injected into separate batches of 15 shrimp (*L. vannamei*) to prepare inoculums for feeding experiments.
- For sample 12 (from a putrified crab), there were no signs of disease in the infected shrimp and it was assumed that the virus was no longer viable. All shrimp infected with samples 13 and 17 displayed signs of white spot disease. Tissues were collected from each of these two groups and pooled as stocks for transmission trials. WSSV real-time PCR testing verified that there were high WSSV genetic loads in each of these stocks.
- Batches of marine shrimp (*L. vannamei*), freshwater shrimp (Macrobrachium rosenbergii) and Cherax sp. were obtained from commercial producers in West Java. All appeared clinically normal. They were held in 100 litre fibreglass holding tanks in the

bio-secure aquarium facility in the Indonesian Center for Fish Disease and Environment Laboratory, Serang to acclimatise for 5 to 7 days prior to commencement of the feeding experiments. Samples were taken from each batch of animals to confirm freedom from WSSV by real-time PCR.

- Each of the WSSV stocks prepared from samples 13 (TRS7) and 17 (TRS5) was used to infect the *L. vannamei*, Macrobrachium rosenbergii and Cherax sp. sequentially through three passages by feeding. At each passage level, samples derived from all previously infected groups were used to infect all three host species, allowing analysis of all combinations of alternative passaging in different hosts. As additional animals were available, the TRS5 passage in Macrobrachium was performed in duplicate.
- In all 3 passages by feeding with each genotype, *L. vannamei* became moribund or died within 5 days and contained high WSSV genetic loads. In contrast, in Cherax sp. and Macrobrachium mortalities were only observed during the first passage when animals were fed with the *L. vannamei* stocks containing high levels of WSSV. Although WSSV was detected by PCR in these species in passages 2 and 3, viral genetic loads were low and most animals were clinically normal.
- WSSV genotyping was conducted on samples from each passage group. Those samples showing the highest levels of virus were tested preferentially. No variations were observed in either the TRS5 genotype or the TRS7 genotype during sequential passage in any of the hosts examined.
- It was concluded that, although the very existence of various TRS genotypes in the ORF94 VNTR indicates that the genotypes are naturally variable, variations did not occur during three sequential passages (four including the preparation of the stock inoculums) in *L. vannamei* or alternatively in other crustacean hosts. This indicates the genotypes are likely to be sufficiently stable for use in local epidemiological studies during disease outbreaks in shrimp ponds.

Figure 5: Summary of WSSV Taqman Ct values and TRS genotypes in Transmission Experiment

Geno Group A

Field ID #13 TRS-7

Code	C_t	TRS	Code	C_t	TRS	Code	C_t	TRS	Code	C_t	TRS	Code	C_t	TRS	Code	C_t	TRS	Code	C_t	TRS	Code	Code	Code
Stock-A	10.2	7																					
P1-V	17.5	7	P1-C	19.4	7							P1-M	23.6	7									
P2-VV	11.2	7	P2-CV	31.5		P2-CC	28.4		P2-CM	32.2		P2-MV	27.2		P2-MC	27.6		P2-MM	31.3	7			
P3-VVV	10.8	7	P3-CVV	23.2	7	P3-CCC	19.9	7	P3-CMM	26.5		P3-MVV	24.3	7	P3-MCC	30.8		P3-MMM	25.3					

Geno Group B

Field ID #17 TRS-5

Code	Ct	TRS	Code	Ct	TRS	Code	Ct	TRS	Code	Ct	TRS	Code	Ct	TRS	Code	Ct	TRS	Code	Ct	TRS	Code	Ct	TRS	Code	Ct	TRS	Code	Ct	TRS
Stock-B	12.6	5																											
P1-V	14.0	5	P1-C	27.1	5							P1-M	12.2	5							P1-M	25.5	5						
P2-VV	10.9	5	P2-CV	11.2	5	P2-CC	12.1	5	P2-CM	29.6		P2-MV	27.6		P2-MC	28.2		P2-MM	28.0		P2-MV	34.4		P2-MC	20.3	5	P2-MM	22.6	5
P3-VVV	11.9	5	P3-CVV	12.8	5	P3-CCC	24.4	5	P3-CMM	25.9	5	P3-MVV	26.8		P3-MCC	26.2		P3-MMM	27.3		P3-MVV	30.3		P3-MCC	30.6		P3-MMM	28.4	

5 Soil mapping and canal function studies at a representative smallholder shrimp farming site in South Sulawesi

After careful consultation, we selected a 50-pond study site (Figure 6) at Gentung village, Pangkep district, approximately 60km north of the provincial capital, Makassar. The site is representative of smallholder shrimp farming areas and practices in South Sulawesi and farmers agreed to participate in the study. This site has a history of serious, recurrent shrimp crop losses attributed to WSD. Other site selection criteria are listed in the following section.

Staff from Research Institute for Coastal Aquaculture (RICA), Maros agreed, using standard methods established under FIS2002/076, to do the following.

- Produce a soil map for the SRA site prior to the cropping period. This was to comprise complete soil analyses for the six BMP and three control ponds only, plus soil analyses for texture (clay/sand/silt), acidity and organic matter for the remaining ~ 40 ponds;
- Measure canal flow rates and function for the site prior to and during the crop.

In brief, soil samples were taken from all BMP and control ponds (ponds 13, 33, 37, 45-50) and from surveillance ponds 16, 17 and 18. Twenty three samples were taken from each of these ponds across both the plateau and trench part of the pond. Soil samples were taken at a depth of 0-0.25m and 0.25-0.5m from the surface. Fourteen other points outside the study area were sampled to assist in interpolation of the data across the non-sampled ponds. The Kriging method in ArcView 3.2 was used to interpolate and produce soil maps across the study area for each soil variable studied.

In the event, the Maros group produced a rather different and much less useful report than that requested. Many findings were based on methods of questionable validity and led to the following uncertainties and limitations.

- The canal classification and flow data presented were rudimentary and, contrary to our original agreement, did not provide information on flow velocity and direction at representative times during the cropping period;
- The soil study was difficult to analyse due to the availability of only summary data across the whole study site for each variable, and the subjectiveness of using graded colour on printed maps to determine levels of a soil variable. As noted above, there were some reservations about the methods. Although the methods seemed robust for the ponds sampled (23 samples from each BMP and control pond), interpolation of data across the rest of the study site based on 14 single point samples outside the study area was not detailed enough to be able to usefully determine soil variables as risk factors for individual ponds.

Further, the Maros group exceeded their remit and, in their report, erroneously attempted to link WSD outbreaks (based on incorrect data) to soil types in ponds at the site.

The original Maros report, in Bahasa Indonesia, is presented in Appendix 1 (Mustafa and Hasnawi, 2010). Aspects of the report deemed useful for epidemiological analysis are included in the following section.

6 WSD outbreak studies at a representative smallholder shrimp farming site in South Sulawesi

6.1 Methods

6.1.1 Study type

A longitudinal observational study was undertaken involving 50 smallholder ponds stocked with *Penaeus monodon* across a single cropping period between May and October 2010. The outcome of interest was WSD outbreak status.

6.1.2 Study site and study pond selection

A study site in South Sulawesi was sought that would be broadly representative of traditional coastal smallholder shrimp farming in Indonesia. The eligibility criteria included (Callinan, 2009):

- having a history of widespread and recurrent WSD outbreaks
- farmers being generally naïve in relation to BMP or related implementations
- having farmer cooperation and consent to the study objectives
- having ponds ready to be stocked around the beginning of the cropping period
- having a moderately to well-flushed canal system which ponds share and directly intake and expel water
- having low to moderate soil porosity
- having crabs and wild shrimp commonly inhabiting ponds
- accessibility for the research team and other logistical issues

Gentung village, Labakkang locality, Pangkep district, South Sulawesi[1] was selected (Figure 6) then mapped to identify key physical, hydrological, biological and infrastructure features. Of particular interest were the canal layout, pond water intake and release pathways, and soil types.

Fifty ponds were enrolled at this locality (Figure 7). The sample size was determined by available resources for pond visits and data collection.

[1] Location of study site - Gentung village, Labakkang locality, Pangkajene archipelago district, South Sulawesi

Figure 6. Pangkep district showing location of the study site (inset = Figure 7)

Figure 7: Study ponds, Gentung village, Labakkang locality, Pangkep district, South Sulawesi

Key: blue – full BMP ponds; light blue – biofilters for full BMP ponds; purple – basic BMP ponds; orange – control ponds; green – surveillance ponds.

Nine of the 50 ponds were involved in a small BMP study to describe the effect of BMPs and pond conditions on pond performance. Of these, three ponds had full BMP implementation (ponds 45, 46 and 47), three ponds had basic BMP implementation (ponds 48, 49 and 50) and three were control ponds (ponds 13, 33 and 37). Extension advice by senior project staff was provided to farmers of the six BMP ponds only. Extension advice to remaining farmers was provided, on request, by District Dinas staff trained under FIS2005/169. For detail on the BMPs implemented in full and basic BMP ponds see Table 2.

Table 2: Summary of full and basic BMP implementation programs compared with control and surveillance ponds

BMP advocated	Program (number of ponds)		
	Full BMP (3)	**Basic BMP (3)**	**Control (3) Surveillance (41)**
Biofilter/reservoir	+	-	No advice
Ponds prepared correctly (includes correcting pH, removing black sediment)	+	- (assumes pond not dried out)	No advice
Good quality PLs, PCR test negative, properly transported and acclimated	+	+	No advice
Stocking density	0.5 – 2 shrimp per square metre		No advice
Water quality monitored	+	+	Control ponds only
Pond bottom and macroalgal abundance managed	+	+	No advice
Biosecurity measures applied	+	Partly (e.g. intake restricted during notified outbreaks)	No advice
Apply emergency harvest decision tree	+	+	No advice
Monitor shrimp health	+	+	No advice
Extension advice	+ (from project staff)	+ (from project staff)	- (only from project-independent staff if requested)

The six ponds for BMP implementation were purposively selected on the basis of perceived biosecurity characteristics that would allow full or basic BMP implementation. In addition, the three full BMP ponds were selected based on the requirement for a shrimp free reservoir (biofilter pond) which could hold water for 7 days before introduction to the grow-out pond. The three control ponds were randomly selected from the other 44 enrolled ponds.

The remaining 41 study ponds were surveillance ponds that were investigated at disease outbreak and/or crop exit.

One of each of the 3 full BMP, 3 basic BMP and 3 control ponds, was randomly selected for weekly monitoring of WSSV levels and genotypes over the cropping period (ponds 37, 45 and 48).

6.1.3 Analysis of soil at study site

As noted above, the Maros group's report provided summary data for each soil variable across the study site and maps relating to the range of values for each soil variable observed across the study site; light orange for the lowest and dark orange for the highest value for that soil variable. Soil variables of interest to this study are listed in Appendix 3.

6.1.4 Stocking of ponds

Test-negative PLs for full and basic BMP ponds were sourced from the Indonesian Government's Center for Brackishwater Aquaculture Development in Takalar. Non-BMP pond farmers (control and surveillance ponds) were given money to buy PLs from whatever source they chose.

For BMP ponds, PLs were required to undergo a selection procedure before being considered suitable for stocking. Initially, PLs underwent a visual assessment then a stress test to determine quality, health and vigour. Post larvae were sequentially tested for WSSV, first by WSSV i-screen test[2] then by WSSV nested PCR. The PLs were also examined microscopically to rule out monodon bacilovirus and hepatopancreatic parvovirus.

If the PLs failed any stage of the selection process the tank from which they came was rejected for pond stocking.

BMP ponds were stocked on 23 May 2010 at between 0.5 and 2 shrimp per square metre. Control and surveillance ponds were stocked on 4 May 2010 at a stocking rate determined by the farmer.

6.1.5 Pond data collection

A variety of data on pond characteristics and management, and data and samples from ponds were collected in this study. Data on pond characteristics and management was collected by technical field officers during interviews with the farmers at pond enrolment, pond stocking, and from the 9 ponds enrolled in the BMP study also during cropping, using purpose designed questionnaires.

Water quality and pond health measurements, and collection of farmed shrimp and pond fauna samples for virus detection, were performed by technical field officers in conjunction with the farmers. The technical field officers were trained for these data and sample collection activities by senior researchers including the project epidemiologist, project coordinator, and laboratory personnel.

For all 50 study ponds, data were collected on stocking and pond characteristics. At pond exit, days of production (DOP), type of exit (normal harvest, emergency harvest or crop

[2] *GeneReach Biotechnology Corp., Taiwan*

failure), WSSV PCR status and productivity data was collected. Samples of farmed shrimp and pond fauna (crabs, mysids, plankton and polychaete worms) were taken at pond exit for quantitative WSSV PCR and genotyping.

For the 9 ponds in the small BMP study, data were collected on pond preparation, water quality, pond bottom health and pond profitability.

For the 3 ponds monitored weekly for quantitative WSSV PCR and genotyping, samples of pond fauna and up to 10 samples of farmed shrimp were collected throughout the grow-out period.

Standard operating procedures (SOPs) were developed for use by technical field officers and farmers for sampling and to guide management of the 9 ponds in the BMP study. Of particular relevance were SOPs for disease outbreak investigation and an emergency harvest decision tree.

Pond exit occurred when a pond had a normal harvest, an emergency harvest or where there was pond failure (i.e. no harvest possible). Crop success was considered to be a crop harvested at >=90 days.

6.1.6 Disease outbreak investigation

During the grow-out period the farmer notified the technical field officer if there was:

- an unusual change in feed consumption and/or
- an increasing number of sick shrimp, or
- an increasing number of dead shrimp, or
- the farmer was planning an emergency harvest.

In this case, the technical field officer visited the pond and initiated a disease outbreak investigation. At this time, the field officer collected data into a pond outbreak form, conducted pond-side Shrimple tests[3] and collected samples, and submitted the samples, pond outbreak form and results of pond side tests to the Takalar laboratory.

When the farmer released untreated water from the pond up to 7 days after the mortality event, this was recorded.

A WSD outbreak was confirmed when:

- >=5 shrimp found moribund or dead and
- Shrimple test positive for >= 3 out of 5 shrimp tested and
- Nested WSSV PCR positive

Histology was attempted for definitive diagnosis in cases where results were equivocal.

[3] *EnBioTec Laboratories Co., Ltd., Tokyo, Japan*

6.1.7 Laboratory testing for WSSV at pond exit

The Takalar laboratory performed WSSV testing at pond exit for all ponds using IQ 2000™ WSSV Detection and Prevention System[4], a nested PCR certified by the OIE and validated as fit for the purpose of diagnosing WSD[5].

Gill samples were collected from 60 shrimp through systematic random sampling of the shrimp at harvest, placed in 70% ethanol and tested in 6 pools of 10 shrimp each. Sample size was determined to give a 95% confidence that infection is present at <5% assuming a perfect test.

6.1.8 Quantitative WSSV PCR and genotyping

Quantitative WSSV PCR and genotyping was performed at the Australian Animal Health Laboratory (AAHL) on samples collected at pond exit for all ponds, at outbreak investigation for ponds that experienced a disease outbreak event and weekly for the 3 weekly monitored ponds in the BMP study.

Taqman® qPCR was used to determine viral load and PCR amplification of the variable tandem repeat region on WSSV ORF 94 was used to determine genotype (Walker 2011b).

6.1.9 Data management

All data recorded on questionnaires and field record sheets were entered into spreadsheets in Microsoft Excel. This data was reviewed and compiled by the Australian project coordinator to prepare a summary in Excel by enrolled pond.

An Excel workbook containing summary data of pond variables of interest by enrolled pond was provided by the Australian project coordinator for the purpose of analysis. Also provided were the raw data workbooks in Bahasa Indonesia (one per enrolled pond) and blank workbooks in English.

Columns in the Excel workbook were given simple names for easy identification during analysis. Data was scanned for data entry errors and, where data was the result of calculations, checked for calculation errors and mistakes corrected.

Summary data was provided on quantitative WSSV PCR and genotyping by AAHL. The Excel worksheets provided mean viral copy numbers per 2µL DNA sample and genotype where available.

Results from quantitative WSSV PCR were categorised according to their mean viral copy number (Table 3).

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[4] *GeneReach Biotechnology Corp., Taiwan*

[5] *http://www.oie.int/our-scientific-expertise/certification-of-diagnostic-tests/the-register-of-diagnostic-tests/*

Table 3: Categorisation of mean viral copy number per 2µL DNA sample

Mean viral copy number	Description	Infection
0	Zero	No detectable
1-20	Negligible	Negligible
21-200	Low	Light
201-2000	Medium	Moderate
2001+	High	Heavy

6.1.10 Data analysis

Pond variables data was exported from the Excel workbook summary and analysed in Genstat (13th edition VSN International). Data from AAHL on quantitative PCR and WSSV genotyping was analysed in Excel.

Pond variables data

An initial descriptive evaluation of the data was undertaken. Frequency tables and bar charts were created for categorical variables and summary statistics, box and whisker plots and histograms created for continuous variables.

For continuous variables, outliers and extreme outliers were identified and assessed as to whether they should remain in the dataset or not. Outliers were defined as those between 1.5 and 3 times the interquartile range beyond the quartiles and far outliers as those >3times the interquartile range beyond the quartiles (Genstat 13th edition, VSN International).

Continuous variables were assessed for normality through visual means, comparison of median and mean values, and the skewness calculation in Genstat. Variables with a skewed distribution were log transformed then reassessed for normality.

Descriptive analysis of each variable against the outcome (WSD outbreak) was undertaken. Contingency tables were created for categorical variables and box plots with summary statistics by outcome status created for continuous variables.

Explanatory variables were investigated using univariable logistic regression for their association with the outcome. P-values, odds ratios and their confidence intervals were calculated. A p-value of <0.05 was considered to be significant.

For non-explanatory continuous variables, t-tests were performed to test the null hypothesis that there was no difference between the WSD outbreak group and the non-outbreak group and/or between BMP and non-BMP ponds.

A total of 20 variables were investigated as potential risk factors for WSD outbreak in the 50 study ponds. An additional 21 explanatory variables were investigated in the 9 ponds involved in the BMP study. For each water quality variable, minimum, maximum, average and range for each variable was entered into univariable logistic regression, as were summary statistics for fluctuations in these variables over the cropping period.

For detail on variables investigated, see Appendix 2.

Quantitative WSSV PCR and genotyping

Descriptive analysis for mean copy viral numbers and genotype in shrimp and other pond fauna was performed. The relationship between outbreak and non-outbreak ponds, temporospatial factors, WSSV levels and genotype was assessed.

6.2 Results

6.2.1 Description of study ponds

Enrolled ponds were clustered with the river running east-west roughly through the centre. Approximately half the ponds (26 ponds) were located south of the primary canal (the river) with the other half (24 ponds) located north of the river. Some study ponds extend away from the cluster on the northern, eastern and south western side of the cluster (Figure 7).

The primary source of water for the ponds is the river which supplies all ponds either directly, via secondary canals entering the river or tertiary canals entering secondary canals. All ponds took and released water directly from primary (20 ponds), secondary (18 ponds) or tertiary (9 ponds) canals with the exception of three full BMP ponds which took water from their corresponding biofilter reservoir (Figure 8 and Figure 9).

The predominant soil type is sandy loam with some areas of argillaceous sand (i.e. sand with a high clay content) (Mustafa and Hasnawi, 2010).

Of the 50 enrolled ponds, four ponds experienced crop failure shortly after stocking (ponds 20, 21, 37 and 38). No shrimp were seen post-stocking and it is likely that the PLs failed to acclimatise and died. These ponds were excluded from the analysis because they were not in production long enough to have the opportunity to develop the outcome (WSD outbreak). All results presented for the pond variables dataset are based on the remaining 46 study ponds.

The average pond area was 0.95 hectares with a minimum area of 0.15ha and a maximum of 3.8ha.

All batches of PLs from both BMP and non-BMP ponds tested negative for WSSV before stocking.

All ponds used a nursery to acclimatise PLs from the hatchery before releasing into grow out ponds.

6.2.2 WSD outbreaks

Of forty six ponds, eighteen ponds (39%) experienced mortality events during the cropping period. Of these, eleven ponds (61%) had WSD outbreaks (ponds 5, 6, 7, 13, 15, 16, 17, 18, 22, 28 and 47), four ponds had mortality events of unknown cause (ponds 8, 14, 23 and 48) and one pond experienced complete crop failure due to poisoning by pesticide use (pond 31). Two ponds had minor mortalities and then progressed to normal harvest (ponds 19 and 24).

For the 11 WSD outbreak ponds all demonstrated mortalities, were Shrimple test positive, and all but two ponds which had missing results (ponds 5 and 13) were PCR positive at pond exit. [Ponds with missing data were later found to be PCR positive through the genotyping work.]

Thirty ponds (65%) recorded a normal harvest, including the two with minor mortalities (ponds 19 and 24).

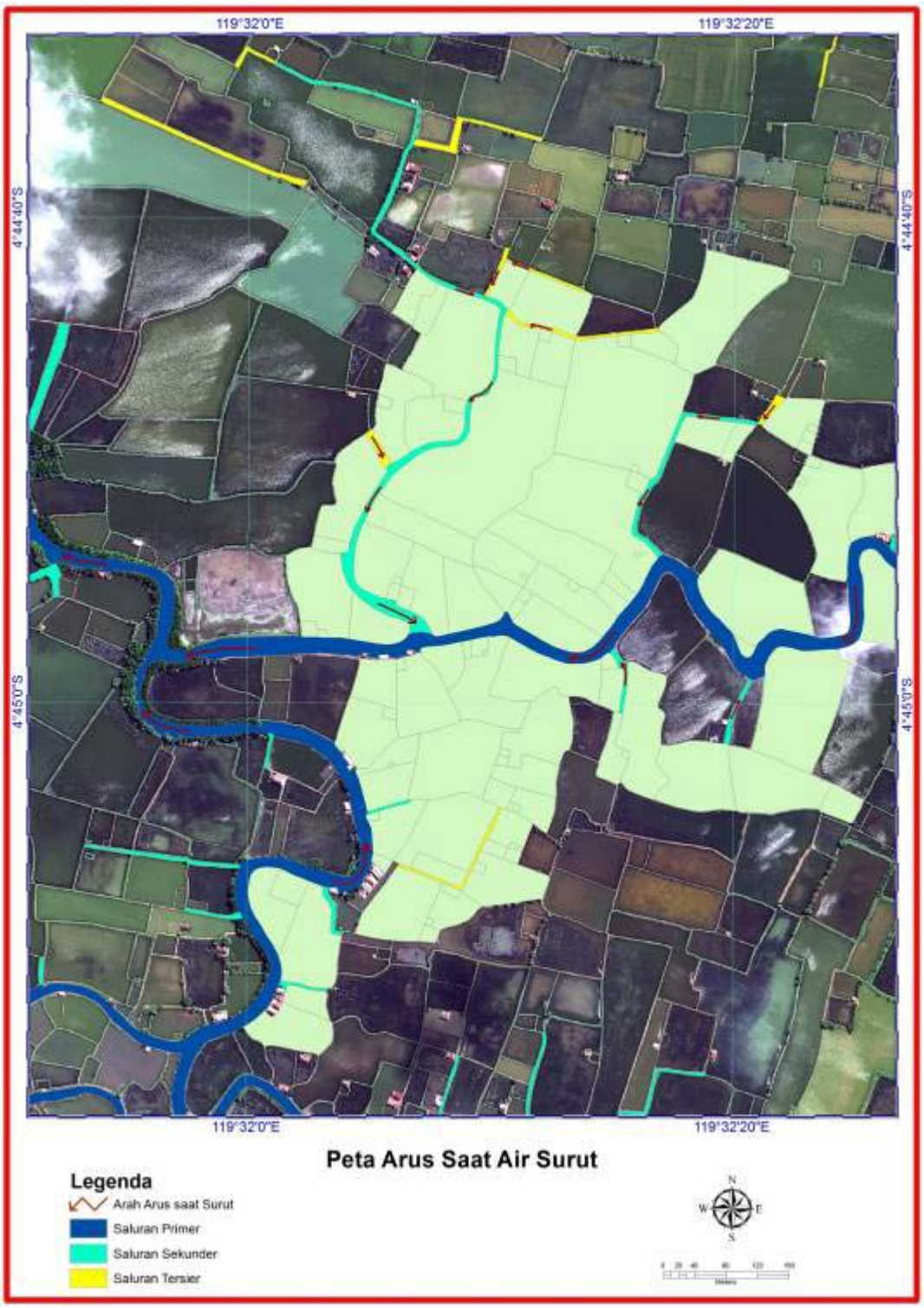

Figure 8: Map of canal structure at study site including water flow at low tide

Key: dark blue – primary canals; light blue – secondary canals; yellow – tertiary canals; light green – study site; red arrows – water flow at low tide.

Nine of the eleven outbreak ponds (82%) were located in a cluster south of the river and 2 ponds north of the river. In all cases WSD ponds were contiguous with other WSD ponds although the 2 ponds north of the river have only a small part of shared embankment (Figure 9).

Figure 9: Map of WSD outbreak ponds across the study site

Key: red – WSD outbreak ponds.

6.2.3 WSSV infection

Forty two of forty six ponds had results for WSSV nested PCR on farmed shrimp at pond exit. Of these, forty one ponds (98%) were PCR positive (pond 27 was PCR negative).

6.2.4 Evaluation of pond variables for WSD outbreak status

Twenty potential pond risk factors were evaluated at univariable level. Following is a detailed description of some potential risk factors investigated.

BMP status

There was no significant difference between BMP status and WSD outbreak status even when full and basic BMP categories were collapsed into a single BMP variable.

Days of production

A mean of 123.8 days of production (DOP) was observed. Outbreak ponds, on average, exhibited shorter cropping periods (104.2 days) than non-outbreak ponds (130 days). This difference was statistically significant (P = 0.018, OR 0.966, CI 0.937, 0.995).

Table 4 displays DOP (categorised by month; 1 month = 30days) by WSD outbreak. None of the ponds exited production in the first 2 months of cropping. The first two WSD outbreaks occurred at 78 days. The next 8 outbreaks occurred between 90 and 122 days and constituted 40% of pond exits (8/20) during this period. During this time, an additional 4/20 exited due to non-WSD mortalities. The last WSD outbreak occurred at day 147 and was the only BMP pond to succumb to WSD by the case definition.

Forty of forty six ponds (87%) achieved the 90 day crop success period. Most ponds exited production in the 4th and 5th months of production. Eleven ponds were in production for more than 5 months (150+ days).

Table 4: Days of production (categorised by month; 1 month = 30 days) by WSD outbreak (0 = no WSD; 1 = WSD).

DOP (month) / WSD	<30 (1)	<60 (2)	<90 (3)	<120 (4)	<150 (5)	150+ (5+)	Total
0	0	0	4 (1*)	8 (3)	12 (1)	11	35
1	0	0	2	7	2	0	11
Total	0	0	6	15	14	11	46

* number in brackets indicates a subset of non-WSD ponds which exited due to non-WSD mortality events.

Table 5 displays DOP (categorised by month; 1 month = 30 days) by BMP status. All BMP ponds (full and basic) were in production for >120 days whereas over half non-BMP ponds (52.5%) exited before 120 days.

Table 5: DOP by BMP status

DOP (month) / BMP	<30 (1)	<60 (2)	<90 (3)	<120 (4)	<150 (5)	150+ (5+)	Total
Full	0	0	0	0	1	2	3
Basic	0	0	0	0	1	2	3
None	0	0	6	15	10	9	40
Total	0	0	6	15	12	13	46

The categories of full and basic BMP were combined to create a binary BMP variable, i.e. BMP and non-BMP, to investigate any potential differences in DOP between these two groups. The association was non-significant at a significance level of 0.05 (p=0.103, CI -46.71, 4.48) (Table 6).

Table 6: Days of production against BMP binary (BMP vs. non-BMP ponds)

	P value	difference in means	SE of difference	lower CI	upper CI
DOP	0.103	-21.12	12.7	-46.71	4.476

Soil types and pond embankments

Results of the soil analysis have been reported elsewhere (Mustafa and Hasnawi, 2010). A summary is provided in Appendix 3.

No soil parameter was found to be statistically significant against the outcome. However, a subjective visual assessment was made by using soil maps to compare soil types between outbreak and non-outbreak ponds.

Based on soil type maps, in comparison to non-outbreak ponds, outbreak ponds tended to have a lower redox value than non-outbreak ponds. The southern side of the river where nine of the eleven outbreaks occurred tended to have higher soil pyrite levels than north of the river. Iron and aluminium levels varied across the site and did not appear to show differences between outbreak or non-outbreak ponds. In general the outbreak ponds were low in clay with moderate to high sand constitution.

Eighteen ponds shared an embankment with a previously affected WSD outbreak pond. Of these, 3 shared a high sand embankment. There was no statistical difference between ponds sharing embankments of any kind and WSD outbreak.

Water flow

All ponds that succumbed to WSD outbreak released untreated water into the canal system within 7 days of the outbreak.

Most ponds (41 of 46) took water from the same primary canal (or its dependant secondary and tertiary canals) into which a WSD outbreak pond released water within 7 days of an outbreak. Only 10 did the same for secondary canals (and its dependent

tertiary canal) and 4 ponds took water from tertiary canals into which WSD outbreak water had been discharged. There was a marginal difference between those that did take water from the primary canal system and those that didn't with those that did possibly less likely to succumb to WSD outbreak (p=0.066, OR 0.162, CI 0.02, 1.19). However, the likelihood ratio chi-squared test for overall significance for this regression equation was also only marginally significant (p=0.065).

The average distance from any given pond and its closest WSD outbreak pond (measured via the canal system) was 354.5m. There was a marginally significant difference in the distance to a WSD outbreak pond between outbreak and non-outbreak ponds (P = 0.056, OR 0.207, CI 0.039, 1.092).

Lunar cycle

Of the eleven ponds that experienced WSD outbreaks, three (27%) experienced outbreaks in the new moon phase, 5 (45%) in the first quarter phase, 1 pond (9%) on the cusp between the first quarter moon and full moon phase, 1 pond in the full moon phase and 1 pond during the last quarter moon phase.

New moon → First quarter → Full moon → Last quarter → New moon

Figure 10: Moon phase cycle

6.2.5 Productivity variables

The mean total harvest was 71.16kg and the median 42.8kg. The mean total harvest for outbreak and non-outbreak ponds was 56.8kg and 41.4kg respectively.

Mean productivity (total harvest/pond area) was 86.6kg and median, 60.7kg. The mean productivity for outbreak ponds was 69.6kg and for non-outbreak ponds, 56.1kg.

Mean survival of shrimp was 23.7% with little difference between outbreak and non-outbreak ponds (23.3% and 25% respectively).

There was no statistically significant difference for productivity variables between WSD outbreak ponds and non-outbreak ponds (Table 7). However there was a statistical difference between productivity and BMP status (Table 8). BMP ponds had a higher total harvest than non-BMP ponds (p=0.003, CI 0.196, 0.930).

Table 7: Results of t-tests for productivity against WSD

	P value	difference in means	SE of difference	lower CI	upper CI
Productivity (log 10 (x+1))	0.380	-0.092	0.103	-0.300	0.117
Total harvest (log 10 (x+1))	0.397	-0.135	0.158	-0.454	0.183
Survival	0.619	-1.740	3.474	-8.748	5.269

Table 8: Results of t-tests for productivity against BMP binary (BMP or not BMP)

	P value	difference in means	SE of difference	lower CI	lower CI
Productivity (log 10 (x+1))	0.698	0.077	0.197	-0.320	0.474
Total harvest (log 10 (x+1))	0.003	0.563	0.182	0.196	0.930
Survival	0.762	2.050	6.723	-11.5	15.60

6.2.6 Intensively monitored ponds

Of the 9 ponds studied for pond preparation, water quality, pond bottom and profitability variables, one pond (pond 37) was excluded from analysis due to crop failure shortly after stocking.

Of the 8 remaining ponds, WSD outbreak was recognised in two ponds – pond 13 and pond 47.

Univariable logistic regression did not identify any statistically significant associations between WSD outbreak and pond preparation, pond bottom condition, water quality variables (minimum, maximum, average and range) or fluctuations in water quality variables (minimum, maximum, average and range).

6.2.7 Profitability

No significant difference was found between WSD and non-outbreak ponds with regard to profitability variables (Table 9).

Table 9: Profitability against WSD outbreak status

	P value	difference in means	SE of difference	lower CI	upper CI
Total prod cost (family cost not included)	0.728	-1159700	3200098	-8726732	6407332
Family labour cost	0.334	199250	191943	-254622	653122
Total revenue	0.991	89503	7645436	-17989088	18168093
Revenue shrimp_log10	0.630	-0.312	0.618	-1.773	1.150
Revenue milkfish_log 10	0.473	0.204	0.269	-0.4328	0.8411
Revenue tilapia	0.999	1905	1024705	-2421138	2424947
Profit_log 10	0.944	0.029	0.402	-0.9202	0.9790
Profit per ha	0.388	1831588	1989232	-2872200	6535377

However, significant differences were found in 5/8 profitability variables for BMP status (Table 10). Increased production and labour costs were offset by increased revenue in BMP ponds. Importantly, total profit (log10) was significantly increased in BMP ponds (p=0.026, CI 0.111, 1.257).

Table 10: Profitability against BMP status (BMP vs. non-BMP ponds)

	P value	difference in means	SE of difference	lower CI	lower CI
Total prod cost (family cost not included)	0.005 *	5822767	1235888	2647413	8998120
Family labour cost	0.003 *	384083	76005	192909	575258
Total revenue	0.007	14579316	3885729	5391024	23767608
Revenue shrimp_log10	0.111	0.834	0.457	-0.2457	1.913
Revenue milkfish_log 10	0.021	0.488	0.164	0.09984	0.8769
Revenue tilapia #	-	-	-	-	-
Profit_log 10	0.026	1.257	0.242	0.1108	1.257
Profit per ha	0.514	1236004	1797833	-3015197	5487205

6.2.8 Quantitative WSSV PCR

From the fifty study ponds, 720 samples were taken on 99 sampling days from 43 ponds. One sampling day was defined as all the samples taken in one pond on a particular day; 2 ponds sampled on the same day would constitute 2 sampling days. Samples were taken for quantitative Taqman WSSV PCR and genotyping. No results are available for ponds 1, 2, 20, 21, 36, 38, and 44.

The three weekly sampled ponds were sampled 13, 18 and 18 times each for ponds 37, 45 and 48 respectively. The other ponds were sampled 1-3 times each (an average of 1.25 times each).

Of the 720 samples, 211 had no detectable DNA, 218 had negligible DNA, 203 were determined to have light infections and 36 and 52 samples had moderate to heavy infections respectively (Table 11).

Of 227 samples taken from pond fauna (excluding farmed shrimp), 60 crab samples were taken, 76 mysid samples, 90 plankton samples and 1 polychaete sample. Quantitative PCR revealed 47 samples with no detectable infection, 58 with negligible infection, 93 with light infection and 26 and 3 samples with moderate and heavy infection respectively.

Table 11: Number of samples for each mean viral copy number category for each of the sampled pond fauna (including farmed shrimp)

Mean WSSV viral copy number	Crabs	Mysids	Plankton	Polychaete	Sub Total (pond fauna)	Shrimp	Grand total
0	16	18	13	0	47	164	211
1-20	17	31	10	0	58	160	218
21-200	25	24	43	1	93	110	203
201-2000	2	2	22	0	26	10	36
2001+	0	1	2	0	3	49	52
Total	60	76	90	1	227	493	720

On average, plankton carried a consistently higher viral load that other non-farmed pond fauna (Table 12).

Table 12: Comparison of mean viral copy number of plankton against other pond fauna

Species	Viral copy number		
	Zero and negligible	Low	Medium and high
Crab	33	25	2
Mysid	49	24	3
Polychaete	0	1	0
Plankton	23	43	24

Of the 11 outbreak ponds, 10 ponds showed heavy infections with high viral copy numbers recorded in shrimp at pond exit and in 2 ponds also in plankton. The remaining outbreak pond (pond 7) showed negligible infection in the 1 shrimp sample.

Of the remaining 32 non-outbreak ponds sampled, 23 showed light infections in pond fauna and farmed shrimp, 3 showed light to moderate infections, 3 showed light to heavy infections and one pond showed moderate to heavy infection. 2 ponds had no detectable WSSV DNA in any samples although the findings were based on 1 sample per pond so may not be representative.

Table 13 shows a comparison of viral load of samples between outbreak and non-outbreak ponds. 92.2% of samples from non-outbreak ponds have a viral copy number of <200 (i.e. zero, negligible or low) compared with 49.4% in outbreak ponds.

This table again shows that plankton tend to have higher viral loads than other pond fauna in non-outbreak ponds. 25.3% of plankton have viral copy numbers >200 in non-outbreak ponds compared with 3.8%, 3% and 5.7% in crabs, mysid and shrimp respectively. In outbreak ponds, only farmed shrimp demonstrate higher viral loads.

Table 13: Comparison of viral load in farmed shrimp and pond fauna between WSD outbreak and non-outbreak ponds.

Viral copy number category		0		1-20		21-200		201-2000		2001+		Total	
WSD status	**Species**	**No.**	**%**	**No.**	**%**	**No.**	**%**	**No.**	**%**	**No.**	**%**	**No.**	**%**
Non-outbreak pond	Crab	16	30.2	16	30.2	19	35.8	2	3.8	0	0	53	100
	Mysid	18	26.9	28	41.8	19	28.4	2	3	0	0	67	100
	Plankton	13	16.5	9	11.4	37	46.8	20	25.3	0	0	79	100
	Polychaete	0	0	0	0	1	100	0	0	0	0	1	100
	Shrimp	160	36.1	156	35.2	102	23	6	1.4	19	4.3	443	100
	Total	207	32.2	209	32.5	178	27.2	30	4.7	19	3.0	643	100
Outbreak pond	Crab	0	0	1	14.3	6	85.7	0	0	0	0	7	100
	Mysid	0	0	3	33.3	5	55.6	0	0	1	11.1	9	100
	Plankton	0	0	1	9.1	6	54.5	2	18.2	2	18.2	11	100
	Shrimp	4	8	4	8	8	16	4	8	30	60	50	100
	Total	4	5.2	9	11.7	25	32.5	6	7.8	33	42.9	77	100
	Total	211	29.3	218	30.3	203	28.2	36	5	52	7.2	720	100

Ponds sampled weekly

Pond 37

Fifty two samples were collected from pond 37 on 13 sampling days from 14 May–29 July 2010 (Table 14). Very few shrimp were seen after stocking with the first shrimp sample collected on 18 June 2010. Only negligible infections were detected in shrimp.

Crab, mysid and plankton samples were collected every week except 23 July 2010. Samples up to and including 1 July 2010 showed negligible infection but the viral load increased over the last 4 sampling days to reveal negligible, low and moderate infections.

In this pond, one polychaete worm was sampled which had a light infection.

Table 14: Average viral copy numbers for different species from pond 37

Date	May			June					July				
Species	**14**	**21**	**28**	**4**	**11**	**18**	**23**	**24**	**1**	**8**	**15**	**22**	**29**
Crab	0	0	0	0	0	0	-	5	5	0	16	41	11
Mysid	3	0	1	0	0	0	-	0	0	8	8	54	0
Plankton	3	0	0	4	3	0	-	2	0	181	0	76	225
Polychaete	56	-	-	-	-	-	-	-	-	-	-	-	-
Shrimp *	-	-	-	-	-	0	0	0	0	12	9	6	12
No. shrimp	0	0	0	0	0	2	1	2	2	2	2	2	2

Pond 45

Two hundred and twenty three samples were collected from pond 45 on 18 sampling days from 28 May–24 Sept 2010 (Table 15). Nine to ten shrimp were collected on each sampling day. Shrimp samples up to and including 24 June showed negligible levels of virus after which time negligible to low levels of virus were detected at fluctuating levels across the shrimp sample. On the last sampling day one shrimp was detected with heavy infection and the rest with zero to low levels of virus. The pond recorded a normal harvest at this time.

A total of 11, 15 and 18, crab, mysid and plankton samples were taken over the sampling period. Samples up to and including 1 July 2010 showed zero to negligible infection. For the remaining 12 sampling days plankton displayed consistently higher viral copy numbers than other pond fauna including shrimp with the exception of the one shrimp with heavy infection in the last sample.

Table 15: Average viral copy numbers for different species from pond 45

Date	May	June				July					August				September			
Species	**28**	**4**	**11**	**18**	**24**	**1**	**8**	**15**	**22**	**29**	**5**	**12**	**19**	**26**	**4**	**9**	**16**	**24**
Crab	6	0	2	0	0	0	**63**	12	6	9	**22**	-	-	-	-	-	-	-
Mysid	-	-	-	0	0	0	8	0	14	0	9	**294**	8	86	7	31	10	6
Plankton	0	0	0	0	0	0	**217**	129	111	199	395	**654**	309	54	239	423	372	81
Shrimp *	1	2	1	4	2	90	55	4	4	19	20	76	43	75	28	9	3	14612
No.	10	10	10	10	10	10	10	10	10	10	10	10	10	10	9	10	10	10

* average viral copy number for number of shrimp sampled

Pond 48

Two hundred and thirty samples were collected from pond 48 on 18 sampling days from 28 May–21 Sept 2010 (Table 16). Eight to ten shrimp were collected on each sampling day. For the first 5 weeks (up to and including 24 June), shrimp samples showed zero to negligible levels of virus. For the next 5 weeks, shrimp samples demonstrated variable levels of virus with negligible to low levels of virus detected. The ensuing 5 weeks saw increasing average viral copy number until shrimp were detected with heavy WSSV infection. On the 16th sampling day, 9 September 2010, all shrimp samples recorded zero to negligible infection. On the ensuing 2 sampling days, the sampled shrimp again demonstrated heavy infection although the average viral copy number was greatly reduced from that experienced during the previous wave of viral infection. This pond didn't record a harvest. The farmer reported that his shrimp had disappeared without evidence of mass mortality.

A total of 17, 17 and 18, crab, mysid and plankton samples were taken over the sampling period. Samples from 1 July 2010 onwards show viral copies increasing to low to moderate infections from previously zero to negligible infections. Plankton showed higher viral copy numbers than other pond life except for one mysid sample.

Table 16: Average viral copy numbers for different species from pond 48

Date	May	June				July					August				September			
Species	**28**	**4**	**11**	**18**	**24**	**1**	**8**	**15**	**22**	**29**	**5**	**12**	**19**	**26**	**4**	**9**	**17**	**21**
Crab	1	5	10	0	0	127	135	36	0	29	18	322	182	89	269	188	79	
Mysid	nc	6	3	2	0	215	0	9	8	15	16	11	23	4	29	18	31	40
Plankton	6	2	7	0	0	51	221	191	150	201	289	335	348	256	375	329	110	147
Shrimp *	<1	1	<1	<1	1	38	6	4	18	3	81	142	27	807449	411163	4	12718	1410
No.	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	9	10

* average viral copy number for number of shrimp sampled

Ponds with more than one sample

Of the 40 ponds that weren't sampled weekly, 8 ponds were sampled more than once providing additional temporal information on viral load in the ponds (Table 17).

Table 17: Comparison of average viral copy numbers in progressive samples of ponds sampled more than once

Pond	No. of samples	Type of exit *	Level of infection (average viral copy number)			Comment
9	2	nh	22	62		Consistent with nh
12	2	nh	9	37		Consistent with nh
18	2	eh (WSD)	838977	33		Harvest before 2nd sampling date; 2nd sample no shrimp
26	3	nh	0	278	12	Harvest before 3rd sampling date; consistent with nh
28	3	eh (WSD)	0	118041	911788	Consistent with WSD outbreak
29	2	nh	0	29		Consistent with nh
39	2	nh	8	35		Consistent with nh
49	2	nh	61	19		Consistent with nh

* nh – normal harvest; eh – emergency harvest.

Heavy WSSV infections are consistent with WSD outbreak. Zero to low or medium viral loads only are consistent with normal harvest. In 5/6 ponds with normal harvest, viral load increased over time.

6.2.9 Genotyping

Genotype information was available for 31 ponds. Twenty six ponds were infected with a single genotype of TRS-4, TRS-5 or TRS-6. The remaining 5 ponds had mixed infections of TRS-4/5 or TRS-4/6 (Table 18).

Table 18: Genotype combinations found in ponds across the study site by WSD outbreak status

WSD outbreak	4	5	6	4, 5	4, 6	Total
0	15	3	0	0	2	20
%	75%	15%	0%	0%	10%	100%
1	2	5	1	2	1	11
%	18%	45%	9%	18%	9%	100%
Total	17	8	1	2	3	31
	55%	26%	3%	6%	10%	100%

The predominant genotype was TRS-4 which infected 22 (71%) of the study ponds (Table 19). The next most common genotype was genotype TRS-5 found in 10 (32%) ponds, then genotype TRS-6 which was found in 4 (13%) ponds.

Table 19: Total numbers of a specific genotype found across the study site

WSD outbreak	4	5	6	Number of ponds
0	17	3	2	20
% *	85%	15%	10%	
1	5	7	2	11
%	45%	64%	22%	
Total	22	10	4	31
	71%	32%	13%	

* number of ponds in which genotype was found / number of ponds – total does not equal 100% due to some ponds being infected with multiple genotypes.

Of the ponds that did not experience an outbreak 85% were infected with genotype 4. However, in ponds that did experience an outbreak, this dropped to 45% with the predominant genotype being TRS-5 (64%).

In outbreak ponds with more than one circulating genotype, the genotype that caused the outbreak was presumed to be the one which caused heavy infection of shrimp (Table 20). Genotype TRS-5 is implicated as the cause in 64% of outbreak ponds and TRS-4 in only 18% (Table 21).

Table 20: Level of infection experienced by different species in ponds with multiple infections.

Pond	Genotype	Shrimp	Crab	Mysid	Plankton
6	4, 6	6	4		4
17	4, 5	5			4
24	6, 4	6			4
47	4, 5	4 / 5		5	5
50	4, 6	4		6	

Key: green – outbreak pond; yellow – light infection; orange – moderate infection; red – heavy infection.

Table 21: Genotype likely to have caused WSD outbreak across the site

outbreak genotype	4	5	6	
count	2	7	2	11
%	18%	64%	18%	100%

In 4/5 ponds with more than one circulating genotype the genotype circulating in pond life is different from the genotype circulating in shrimp (Table 20). In the remaining pond (pond 47) the genotype TRS-5 is infecting shrimp and pond life with 1/7 shrimp sampled showing a different genotype (TRS-4) at low levels.

Of the 9 outbreak ponds south of the river, TRS-5 appeared to be the cause of WSD in 6 ponds (ponds 13, 15, 16, 17, 18 and 22), TRS-6 for 2 ponds (ponds 5 and 6) and TRS-4 for pond 7 (Figure 10). This suggests that the cluster of outbreak ponds south of the river is not one single outbreak.

Of the two outbreaks north of the river, one appears to have been caused by genotype TRS-4 (pond 28) and the other by TRS-5 (pond 47).

Figure 10: Genotypes found in ponds across the study site

Key: red – outbreak ponds; black numbers – TRS genotype (e.g. 5 = genotype TRS-5)

The outbreaks in ponds north of the river were separated temporally by 85 days (Figure 11). The ponds did not share water sources with pond 28 taking water from the river and pond 47 from the tertiary canal via 2 biofilter ponds.

WSD outbreaks in ponds south of the river occurred between outbreak day 1 and 30. Ponds in the locally extensive TRS-5 outbreak were clustered but did not share the same water source (Table 22).

Table 22: Outbreak day (OD) and water source for pond in TRS-5 outbreak

Pond number	Outbreak day *	Water source
13	20	Secondary canal 1
15	7	Secondary canal 1
22	21	Secondary canal 1
16	26	Secondary canal 2
17	30	Primary canal
18	15	Primary canal

* day 1 = 1st WSD outbreak detected at the study site

Ponds 5 and 6 recorded WSD outbreaks associated with TRS-6 on outbreak day 1 and 28 respectively. Pond 7 recorded a TRS-4 WSD outbreak on days 21. These three ponds share a tertiary canal water source.

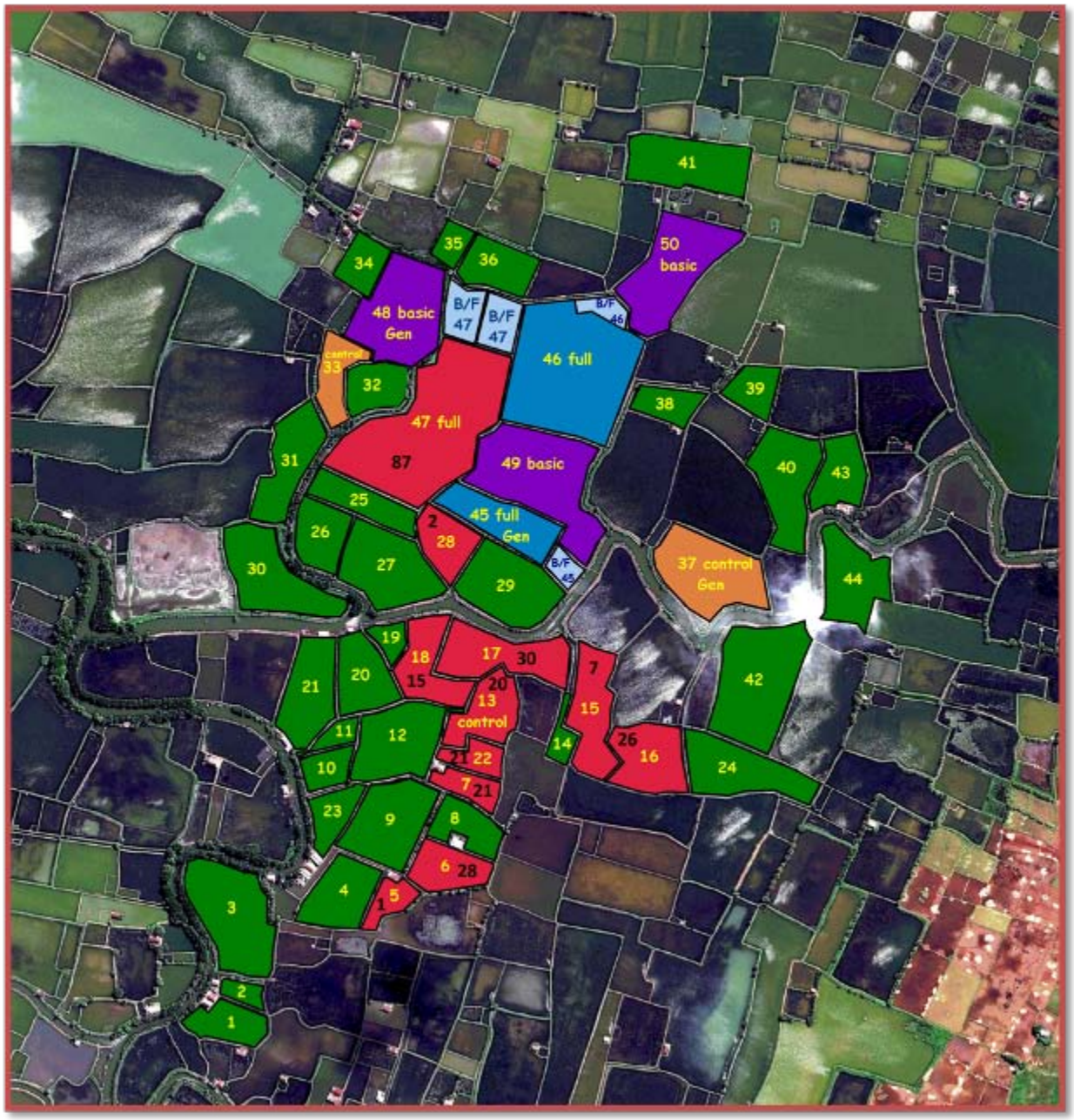

Figure 11: Outbreak map of study site with outbreak day (day 1 = 1st WSD outbreak detected at the site)

Key: red – outbreak pond; black number – day of outbreak

6.3 Discussion

The objective of this study was to contribute to an understanding of the interactions between risk factors and how they might influence the occurrence of white spot disease (WSD) outbreaks. It is clear from this study and others that occurrence of WSD outbreak is multifactorial and not just dependant on WSSV infection status.

6.3.1 Post larvae, WSSV infection, WSD outbreak and DOP

All ponds in this study stocked nested PCR negative PLs, yet all but 1 pond were PCR positive at pond exit. This indicates that WSSV is ubiquitous in this environment and that horizontal transmission of infection is common post stocking.

The first WSD outbreak was on DOP 78 and only 5 other ponds did not make the 90 day cut-off for crop success; 1 from WSD outbreak, 1 from a non-WSD mortality event and 3 with a normal harvest. This supports the findings of previous researchers who found that ponds stocked with WSSV test negative PLs were less likely to develop disease within the first 40-45 days of production and supports a threshold hypothesis for disease outbreak (see later in discussion) (Withyachumnarnkul, 1999).

It is not know why 3 farmers chose to initiate a normal harvest before what would normally be considered to be a successful cropping period.

6.3.2 Use of a Nursery

Of the 50 enrolled ponds, the only 4 ponds that didn't use a nursery to acclimatise PLs to grow out ponds are the same 4 that were excluded from the analysis due to crop failure at stocking. Salinity shock is highly suspected and reinforces the importance of using a nursery to acclimatise PLs from the high saline hatchery (approx 15ppm) to the variable salinity of the grow-out ponds.

6.3.3 Soils and pH

Acid sulfate soils are a risk to aquatic life through the potential for them to cause high water acidity and release ionic heavy metals (in particular iron and aluminium) which are toxic to aquatic life.

Pyrite, an essential indicator of acid sulphate soils, is present at the site. Post preparation soil pH for the nine BMP study ponds ranged between 5.1 and 6.8 but water pH never dropped below 7.1 during the cropping period. Soil pH measured in the field (pH_F) was fairly neutral (average 7.1, range 6.8-8.0) but, after forced oxidation with hydrogen peroxide (pH_{FOX}), showed a potential to achieve a pH down to 3.65 ($pH_F - pH_{FOX}$)

Acid sulphate soils are present at this site but perhaps post preparation soil acidity was buffered before stocking by the alkalinity of brackish canal water used to fill ponds, or by the application of lime, preventing water acidification.

Iron and aluminium concentrations were measured in soil but the information does not allow interpretation of their concentrations in pond water or for their potential to harm shrimp. Iron and aluminium concentrations in soil show there is potential for release of toxic forms should water acidify and dissolve the metals into solution.

Although a statistically significant association was not found between WSD outbreak ponds and sharing of embankments with ponds previously affected with WSD, it appears that outbreak ponds have a higher sand content than non-outbreak ponds. Researchers have speculated about porosity of embankments as a risk factor for WSD spread either directly through seepage of water (Withyachumnarnkul, 1999).

6.3.4 Lunar phase

Although there is an association between WSD outbreaks and the new and first quarter moon phases in this study, dates used to determine the lunar phase during which WSD outbreak occurred are those on which the disease was confirmed by Shrimple test. In some cases mortalities may have been observed days before a diagnosis of WSD was made. In addition, the incubation period for fulminating WSD is said to be 4-7 days so shrimp in this study could have succumb to clinical infection up to 2 weeks before diagnosis (Withyachumnarnkul, 1999).

A longitudinal study or retrospective study of data from previous studies (larger sample size) could provide more evidence for whether this association is real or just occurred by chance.

6.3.5 Productivity and profitability

Significant differences between productivity and profitability were not seen between outbreak and non-outbreak ponds but were seen between BMP and non-BMP ponds.

BMP ponds harvested more shrimp in total (total harvest) than non-BMP ponds but were not more productive (productivity = total harvest/pond area). This could be because 5/6 of the BMP ponds were the largest in the study. Larger ponds could be preferable environments in which to grow shrimp and this could be due to a potentially lower stocking rate, opportunity for shrimp to find a suitable microclimate across the pond or, conversely, a more stable pond environment due to the large body of water. Productivity is a more useful measure of pond success than total harvest if production costs are related to pond area.

BMP ponds were more profitable overall than non-BMP ponds but not per hectare and not in the revenue raised from farming shrimp. BMP ponds could be more productive and profitable due to provision of focussed extension, farmers monitoring their ponds more closely due to twice daily data collection and/or use of an emergency harvest decision tree to optimise harvest decision-making (Turnbull et al., 2002). However, it is possible that pond size has confounded the association between productivity and profitability, and BMP status.

6.3.6 Quantitative WSSV PCR and genotyping

The qPCR and genotyping work provided significant improvement to this epidemiological investigation over observation of risk factors alone. Additionally, investigations of the 3 ponds sampled weekly for qPCR and genotyping, and other ponds sampled on more than once, provided an opportunity to gather some longitudinal data within this study.

Ponds showed a generalised gradual increase in viral load over time and across the species sampled. Ponds infected with low to moderate levels of WSSV do not experience outbreaks but may develop high viral loads and subsequent outbreak over a number of days.

Data from pond 45 and 48 shows that viral load can increase from negligible/low to high within 7 days (Tables 14 and 15). This supports a threshold hypothesis in which WSSV infected ponds become outbreak ponds due to gradual increase in viral load up to a host-agent-environmental tipping point leading to exponential viral growth and outbreak.

6.3.7 Classification of cases/non-cases

The findings of the qPCR also allow for reflection on the accuracy of diagnosis of WSD outbreak as per the case definition. It is suspected that pond 8 and 48 have been misclassified as non-WSD ponds. Retrospective quantitative PCR analysis show very high viral load in shrimp collected at mortality events in these ponds.

Conversely, pond 7 shows only negligible to low viral load and could be misclassified as a WSD outbreak pond. Another possibility is that pond 7 was indeed an outbreak pond but the 1 shrimp sample was not representative and was carrying a non-outbreak genotype [TRS-4]. This situation occurred in pond 47 where some shrimp carried the outbreak genotype at high levels [TRS-5] and one shrimp sampled demonstrated low levels of infection for a different genotype [TRS-4]. If we assume that pond 7 was an outbreak pond (based on clinical signs) then it could be that outbreak shrimp not sampled were carrying a different outbreak genotype, likely TRS-6 (see below).

6.3.8 Outbreak clusters

A hypothesis of the likely progression of WSD events across the study site is presented here supported by the qPCR and genotyping work.

North of the river

Two outbreak ponds were identified north of the river during the study (ponds 28 and 47) and another in retrospect based on qPCR results (pond 48).

Pond 28 was infected with TRS-4 and became an outbreak pond on outbreak day 2.

Pond 47 and 48 were infected with TRS-5 and became outbreak ponds on day 64 and 87 respectively, 23 days apart. Pond 47 used biofilter ponds which take water from a tertiary canal which is fed by the secondary canal from which pond 48 takes and releases water (Figure 12). Both ponds exchanged water during the time in which pond 48 was displaying heavy viral infection in shrimp (Table 15). The distance between the inlet for pond 47's biofilter and the inlet/outlet for pond 48 is very close. It is not known whether pond water was stored for 7 days in the biofilter pond (as per protocol) before being released into pond 47. It is likely that outbreaks in ponds 47 and 48 are related probably through movement of infected water.

South of the river

In the outbreak(s) south of the river, there is strong temporal, spatial and genotypical evidence that outbreak ponds were part of 2 WSD outbreaks – a confined outbreak of TRS-6 and a locally extensive outbreak of TRS-5.

Genotype TRS-6

Pond 5 and 6 recorded an outbreak associated with TRS-6 on day 1 and 28 respectively. No genotype information was available from pond 8, which is suspected to have become

an outbreak pond on outbreak day 16. Pond 7 had clinical signs consistent with WSD on day 21. Pond 5, 6, 7 and 8 share water from the same tertiary canal.

In this outbreak cluster we could hypothesise that something triggered pond 5 to become an outbreak pond, then release of large volumes of infected water into the shared tertiary canal and intake by pond 6, 7 and 8 resulted in outbreaks in these ponds. This could be more likely in tertiary canals due to slower canal flows compared with larger canals or the river (Mustafa and Hasnawi, 2010).

Genotype TRS-5

An outbreak of genotype TRS-5 included six ponds (Table 21). All ponds were infected between outbreak day 7 and 30. The ponds do not share the same water source but three separate but related water sources. Other non-outbreak ponds that share water sources with these TRS-5 outbreak ponds didn't succumb to WSD outbreak. One of these non-outbreak ponds (pond 14) recorded an emergency harvest during this time but returned a 5/5 Shrimple negative result and negligible viral load on qPCR (based on 1 shrimp sample).

While it is possible, even probable, that ponds in this outbreak cluster that did share the same water source became diseased from each other, another mode of transmission could have been involved in the transmission of WSD outbreak between ponds that are not directly linked by waterways.

That said, if the presence of an outbreak genotype alone is not enough to cause disease, it is hard to imagine another means of transmission, apart from bulk movement of a massive load of virus in water (seepage through pond walls?) that could make such an impact in an already endemic site. Non-enrolled ponds at the site could have also experienced outbreaks contributing to viral saturation of the water to all ponds affected in this cluster.

It is known that the causes of WSD outbreak are multifactorial and it is likely that the predominant precipitating cause of WSD outbreak in any given pond is dependent on a combination of factors. For example, the TRS-6 cluster of infection south of the river is likely to have spread through low water flow rate in the tertiary canal, whereas the infection in the TRS-5 outbreak could have spread through a combination of movement of water and movement of infection through fomites or even seepage through pond walls.

In this study, pond fauna was not considered a risk factor for WSD outbreak (see below).

6.3.9 Genotypes

Despite genotype TRS-4 being the predominant genotype found across the study site, genotype TRS-5 is implicated in almost twice as many outbreak ponds than TRS-4 or TRS-6 combined. This could be due to real differences in pathogenicity between WSSV genotypes or be confounded by the relative size of the TRS-5 outbreak south of the river or other unidentified local factors.

6.3.10 Incubation period

This study supports previous work done on 'incubation periods' for WSSV. Withyachumnarnkul observed an 'incubation period' for WSD outbreak in ponds stocked with grossly normal test positive PLs of about 40-45 days (Withyachumnarnkul, 1999) whereas experimental work demonstrated that fulminating disease could be elicited within 3-7 days of exposure. The difference is probably related to dose and route of infection.

This study suggests an incubation period for pond involved in outbreak clusters of between 8 and 27 days with an average of 18 days.

South of the river, cases occurred 15-27 days after the index case in the TRS-6 cluster (Figure 12). In the TRS-5 cluster, subsequent cases occurred 8-23 days after the first (Table 21); in ponds located in the primary canal, subsequent cases occurred 15 days after the first, and in ponds located in the secondary canal, subsequent cases occurred 13 and 14 days after the first.

In the TRS-5 affected ponds north of the river, pond 47 was affected 23 days after pond 48.

It seems reasonable that incubation periods in a WSD outbreak cluster would be longer than in experimental infection but shorter than in the index pond. The time taken for the 1st pond in a cluster to become diseased would likely be related to a gradual increase of viral load in the pond over the cropping period up to a threshold or tipping point. Subsequent ponds would succumb more quickly due to overwhelming viral load from the index and other diseased ponds.

6.3.11 The role of pond fauna

It is difficult to make generalised assumptions from this data about the potential role of non-farmed pond fauna to cause WSD outbreak in farmed shrimp because most ponds only had samples of pond fauna taken at harvest. Ponds with mixed genotype infections are useful in this respect.

Where mixed infections existed, the genotype circulating in pond fauna is different from the genotype circulating in shrimp for 4/5 ponds (Table 19). This suggests that multiple genotypes can be circulating in a pond with only one causing an outbreak. In this limited sample, it seemed that pond fauna are not implicated in the epidemiology of WSD outbreaks.

While farmed shrimp demonstrate the ability to develop extremely high viral copy numbers, other pond fauna don't tend to have this quality, rarely reaching heavy infections even in outbreak ponds. Plankton, however, carried consistently higher viral loads that other pond fauna. It is not clear what the implication of this finding is but it could be that increasing plankton viral load (regardless of genotype) could be a potential indicator of threshold factors that might precipitate a WSD outbreak. There is not enough evidence in this study for this finding to be explored.

6.3.12 Study design

Although a risk factor study was explicit in the objectives as outlined in the ACIAR project proposal, this study is primarily designed for qPCR and genotype study.

Small sample sizes and lack of longitudinal data for many of the potential risk factors made it difficult to draw causal links or get sufficient power to detect statistical differences. In addition, variables with more than 2 categories often failed to have sufficient numbers of observations, particularly when comparing outbreak to non-outbreak ponds, and this has made some variables difficult to analyse.

The qPCR and genotyping work however turned out to be very powerful enabling conclusions to be drawn about temporal, spatial and epidemiological aspects of WSD outbreak across the site. The study could have been improved by collection of pond fauna

just before PL stocking in every pond. This would have enabled more information about the epidemiology of circulating genotypes in outbreak and non-outbreak ponds.

The soil study was difficult to analyse due to the availability of only summary data across the whole study site for each variable, and the subjectiveness of using graded colour on printed maps to determine levels of a soil variable. We also had some reservations about the methods. Although the methods seemed robust for the ponds sampled (23 samples from each BMP and control pond), interpolation of data across the rest of the study site based on 14 single point samples outside the study area was not detailed enough to be able to usefully determine soil variables as risk factors for individual ponds.

6.3.13 Bias

This study has intentional selection bias to an area where BMPs are not well known by farmers and where WSD is reportedly common.

Steps were taken to reduce non-response bias, loss to follow up and missing data by using technical field officers to support and work with farmers to guide them through to the end of the study and gather the required data.

Non-differential misclassification bias is likely in the study with regards to the outcome. Despite a clear case definition, it is apparent that some ponds were misclassified when viewed retrospectively with the additional qPCR data. In addition, misclassification of WSSV status may occur due to imperfect tests.

Recall bias has been minimised through the use of data recording sheets and a close working relationship between farmers and field officers.

Measurement error is likely in this study. Measures taken to reduce measurement bias include training of field officers to collect data and take samples, and using a small number of field officers to collect samples across all ponds. Transcription errors are likely given the large amount of data collected. Data was reviewed before analysis to detect obvious errors and inconsistencies. These were followed up and corrected where possible. In one case a value was removed from the dataset.

One confounder was identified in the study but there may be others. It is possible that the large number of ponds involved in the TRS-5 outbreak south of the river could have confounded the relationship between distance to outbreak and WSD outbreak.

Clustering was not considered in the analysis but could be a potential place for further analysis.

The external validity of this study is high in coastal smallholder shrimp farms in Indonesia because the site was specifically selected to be broadly representative of this type of production system. It may not be valid to extrapolate the results to other types of shrimp farming enterprise in Indonesia or to other countries where methods of farming may be different.

6.4 Key findings

The purpose of this study was to use epidemiology to identify risk factors for WSD outbreaks and use them to implement better management practices.

Specific findings related to previously posed questions are:

1. Water from WSD outbreak ponds was likely to be an important initiator of disease outbreaks in upstream and downstream ponds
2. Crabs, wild shrimp, zooplankton and/or polychaetes were not important sources of WSSV infection for WSD outbreaks in farmed shrimp
3. There was not enough evidence to determine whether adverse conditions in ponds trigger WSD outbreaks associated with sporadically occurring WSSV genotypes
4. Biofilter ponds were not effective in reducing the risk of WSSV infection in growout ponds. There was not enough evidence to determine whether they are useful for reducing the risk of WSD outbreaks in growout ponds

These findings suggest that management of water intake during an outbreak is the single most useful thing that farmers could do to manage the risk of WSD outbreak in their ponds. A cohesive farmer group could provide the coordination required to communicate disease reporting and support producers to implement other better management practices.

Other findings of this study are:

1. WSSV is ubiquitous. Strategies to prevent WSD outbreaks are probably more useful than trying to prevent WSSV infection in endemic areas.
2. Use of a nursery to acclimatise PLs from hatchery to grow-out pond is essential.
3. Stocking with negative PCR tested PLs supports maintenance of an outbreak-free pond for at least the first 2 months of production.
4. Once PLs have been excluded as a risk factor for WSSV infection, biosecurity (prevention of WSSV infection post stocking) and environmental factors (management of risk factors for WSD outbreak) become more important.
5. Non-farmed pond fauna were not a significant risk factor for WSD outbreak.
6. Plankton carry higher viral loads than other pond fauna (including farmed shrimp in non-outbreak ponds)
7. Pond fauna appear to be incapable of developing very high viral loads.
8. WSD outbreak water release may be a higher risk in tertiary canals possibly due to slower water flow rate.
9. Superior productivity and profitability of BMP ponds could suggest the value of provision of extension advice, engaging farmers in better pond management and use of an emergency harvest decision tree to support optimum harvest time for potentially affected ponds. [If not confounded by pond size].
10. Quantitative PCR and genotyping is an extremely useful tool for epidemiological studies.
11. Mixed genotype infections are useful in studying relationships between genotypes, pond life and WSD outbreaks.

12. A larger longitudinal study or a retrospective study of data from previous studies could shed more light on whether differences in lunar phase do indeed affect precipitation of WSD outbreaks.

Despite challenges, the use of epidemiology to determine risk factors and generate hypothesis for component causes of WSD outbreak is valuable. In endemic areas virus exclusion is probably not practical or cost effective. Hopefully this study can add to a body of knowledge about WSSV and WSD outbreak which will support increased productivity and profitability as a means to improving social and economic conditions in smallholder shrimp farmers.

7 Conclusions and recommendations

7.1 Conclusions

FIS2005/169 'Improving productivity and profitability of smallholder shrimp aquaculture and related agribusinesses in Indonesia', which ran in parallel with the current study, identified major constraints to successful BMP program adoption by smallholder shrimp farmers. These constraints were unforeseen at the start of that project and proved far beyond its resources to remediate. They included widespread, physically marginal farming sites, inadequate local infrastructure, farmers' limited resources and farmer group disunity. The project also highlighted the need for major capacity building and resourcing for (a) district-level extension service agencies and (b) government and university-based researchers involved in aquatic animal health management.

We expected that the current study, by improving our understanding of how some of these constraints interacted at a selected problematic site, would (a) enable relevant Indonesian agencies, and ultimately farmers themselves, to better identify localities suitable for smallholder shrimp farming using better management practice (BMP) programs and (b) inform modification and simplification of these programs, thereby improving both profitability and adoption rates.

Although a risk factor study was explicit in the objectives as outlined in the project proposal, this study was primarily designed for qPCR and genotype study. Transmission trials conducted under this SRA showed that, although the very existence of various TRS genotypes in the ORF94 VNTR indicates that the genotypes are naturally variable, variations did not occur during three sequential passages (four including the preparation of the stock inoculums) in *L. vannamei* or alternatively in other crustacean hosts. This indicates the genotypes are likely to be sufficiently stable for use in local epidemiological studies during disease outbreaks in shrimp ponds.

Small sample sizes and lack of longitudinal data for many of the potential risk factors made it difficult to draw causal links or get sufficient power to detect statistical differences. In addition, variables with more than two categories often failed to have sufficient numbers of observations, particularly when comparing outbreak to non-outbreak ponds, and this made some variables difficult to analyse.

Because of the questionable methods used, contrary to our instructions, findings of the externally-commissioned soil mapping and canal function study proved of very limited use in identifying related risk factors for WSD outbreaks.

The qPCR and genotyping work, however, turned out to be very powerful enabling conclusions to be drawn about temporal, spatial and epidemiological aspects of WSD outbreaks across the site. The study could have been improved by collection of pond fauna just before PL stocking in every pond. This would have enabled more information about the epidemiology of circulating genotypes in outbreak and non-outbreak ponds.

These limitations notwithstanding, findings of the genotype stability study and the longitudinal observational field study were most useful and strongly suggested the following.

(a) WSSV genotypes, as identified by TRS number, remain stable when passaged through hosts of the same or different species. Quantitative PCR, linked with genotyping, therefore remain important tools studies of WSSV dynamics in shrimp farming systems. Where mixed genotype infections occur, they are particularly useful in studying relationships between genotypes, pond biota and WSD outbreaks.

(b) Stocking WSSV PCR test-negative PLs, coupled with careful management of water intake during nearby WSD outbreaks are the two most useful practice changes farmers can adopt to reduce the risk of outbreaks in their ponds.

(c) Establishment of an active, well-resourced and trained extension service, committed to fostering cohesive, informed farmer groups, is the key to enabling these inexpensive practice changes. This conclusion is supported by the findings of a Student Research Team from Royal Veterinary College, London, whose members formally interviewed 27 farmers from the SRA site six months after the conclusion of the study, in April-May 2011. Their work, done in collaboration with SRA team member Dr Mardiana (UNHAS), found that formal dissemination of knowledge regarding BMPs was limited, with the majority of farmers unaware of the principles and guidelines. This points to a major failure of extension service delivery to non-BMP participating farmers during and following the SRA. The RVC report's abstract is presented in Appendix 4.

7.2 Recommendations

Over 100,000 smallholders across Indonesia use traditional methods to produce monodon shrimp in more than 200,000 ha of brackishwater ponds, often in polyculture with milkfish and seaweed.

Up until the mid 1990s, these farmers were major contributors to the total national farmed shrimp production. However, since then, WSSV has spread widely across Indonesia, and recurrent crop failures due to WSD have progressively reduced the smallholder sector's contribution to its present level of approximately 5%. Evidence suggests this decline is continuing, as more smallholders cut their losses and avoid stocking shrimp altogether.

The findings of this small study, whose validity probably extends beyond the time and place in which it was conducted, clearly point out the need for follow-up work.

There is an urgent need for further field trials testing the production effects of the two key, low-cost interventions identified in the longitudinal study, i.e., (a) stocking WSSV PCR test-negative PLs and (b) careful management of water intake during nearby WSD outbreaks.

Lasting changes at individual farmer and farmer group levels are unlikely without sustained input from an active, well-resourced and trained extension service, committed to fostering cohesive, informed farmer groups. This issue must be addressed if smallholder shrimp farming in Indonesia is to recover from its current crisis.

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8.2 List of publications produced by project

None to date.

9 Appendixes

9.1 Appendix 1: Maros report

HUBUNGAN KARAKTERISTIK LAHAN DAN PENYAKIT UDANG WINDU DI KAWASAN PERTAMBAKAN DESA GENTUNG, KECAMATAN LABAKKANG, KABUPATEN PANGKEP

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PENDAHULUAN

Di kawasan pesisir termasuk kawasan pesisir Kabupaten Pangkep, Provinsi Sulawesi Selatan dijumpai lahan rawa. Lahan rawa adalah lahan yang dipengaruhi oleh kondisi pasang surut laut atau sungai sekitarnya. Dalam kaitannya dengan sumberdaya alam, dikenal istilah lahan yang merupakan suatu lingkungan fisik yang terdiri atas tanah, topografi, hidrologi, vegetasi dan iklim dimana pada batas-batas tertentu mempengaruhi kemampuan penggunaan lahan (FAO, 1976; World Bank, 1995 *dalam* Rajitha *et al*., 2007). Tanah yang dapat dicirikan oleh karakteristik tanah adalah merupakan salah satu faktor lingkungan yang dapat mempengaruhi produktivitas tambak sebab dapat mempengaruhi kualitas air, proses biologi dan rekayasa tambak. Oleh karena itu, faktor kualitas tanah merupakan faktor yang dipertimbangkan dalam evaluasi kesesuaian lahan untuk budidaya tambak (Treece, 2000; Salam *et al*., 2003; Karthik *et al*., 2005; Mustafa *et al*., 2007). Setiap jenis tanah memiliki karakteristik tanah yang berbeda dan tentunya menuntut pengelolaan tanah yang berbeda pula.

Pengelolaan tanah yang tepat dapat meningkatkan produktivitas tanah termasuk tanah tambak dengan penggunaan masukan yang seminimum mungkin dan tidak

menyebabkan terjadinya degradasi lingkungan. Setiap jenis tanah memiliki karakteristik tersendiri sehingga pengelolaan tanahnya juga bersifat khas terhadap penggunaan tanah tersebut (Mustafa dan Rachmansyah, 2008).

Faktor lingkungan fisik lain dari lahan selain tanah adalah hidrologi. Seperti halnya dengan tanah, maka hidrologi adalah juga faktor yang dipertimbangkan dalam evaluasi kesesuaian lahan tambak yang tentunya sangat berpengaruh terhadap produktivitas dan keberlanjutan dari budidaya tambak.

Penyakit telah muncul sebagai penghambat utama terhadap keberlanjutan usaha budidaya udang. Serangan serius penyakit udang telah terjadi di banyak negara penghasil udang. Banyak penyakit terkait dengan kemunduran kualitas lingkungan dan tekanan yang berasosiasi dengan intensifikasi tambak udang. Leung *et al*. (2001) menyatakan bahwa usaha budidaya udang yang memiliki tambak yang lebih luas dengan lebih banyak limbah yang masuk kedalam saluran pemasukan dan membuang lumpur tanah dasar tambak lebih berpeluang untuk terserang penyakit. Tambak yang mengambil air laut melalui saluran berpeluang lebih kecil terserang penyakit (Leung *et al*., 2001) dan produktivitas tambak lebih tinggi (Mustafa *et al*., 2007). Pemecahan berbagai masalah pada budidaya udang windu telah ada misalnya kesadaran terhadap konsep *biosecurity*, penggunaan benih unggul, sistem budidaya terpadu dan BMP (Best Management Practices) yang berbasis pada penerapan budidaya ramah lingkungan. ACIAR (Australian Center for International Agricultural Research) telah melakukan kajian mengenai penerapan BMP di Desa Gentung, Kecamatan Labakkang. Dalam pelaksanaan penerapan BMP tersebut, dilakukan suatu kajian khusus untuk mengetahui karakteristik lahan terutama kualitas tanah dan hidrologi dalam upaya penentuan pengelolaan lahan dan hubungannya dengan penyakit pada udang windu di kawasan pertambakan Desa Gentung, Kecamatan Labakkang, Kabupaten Pangkep.

BAHAN DAN METODE

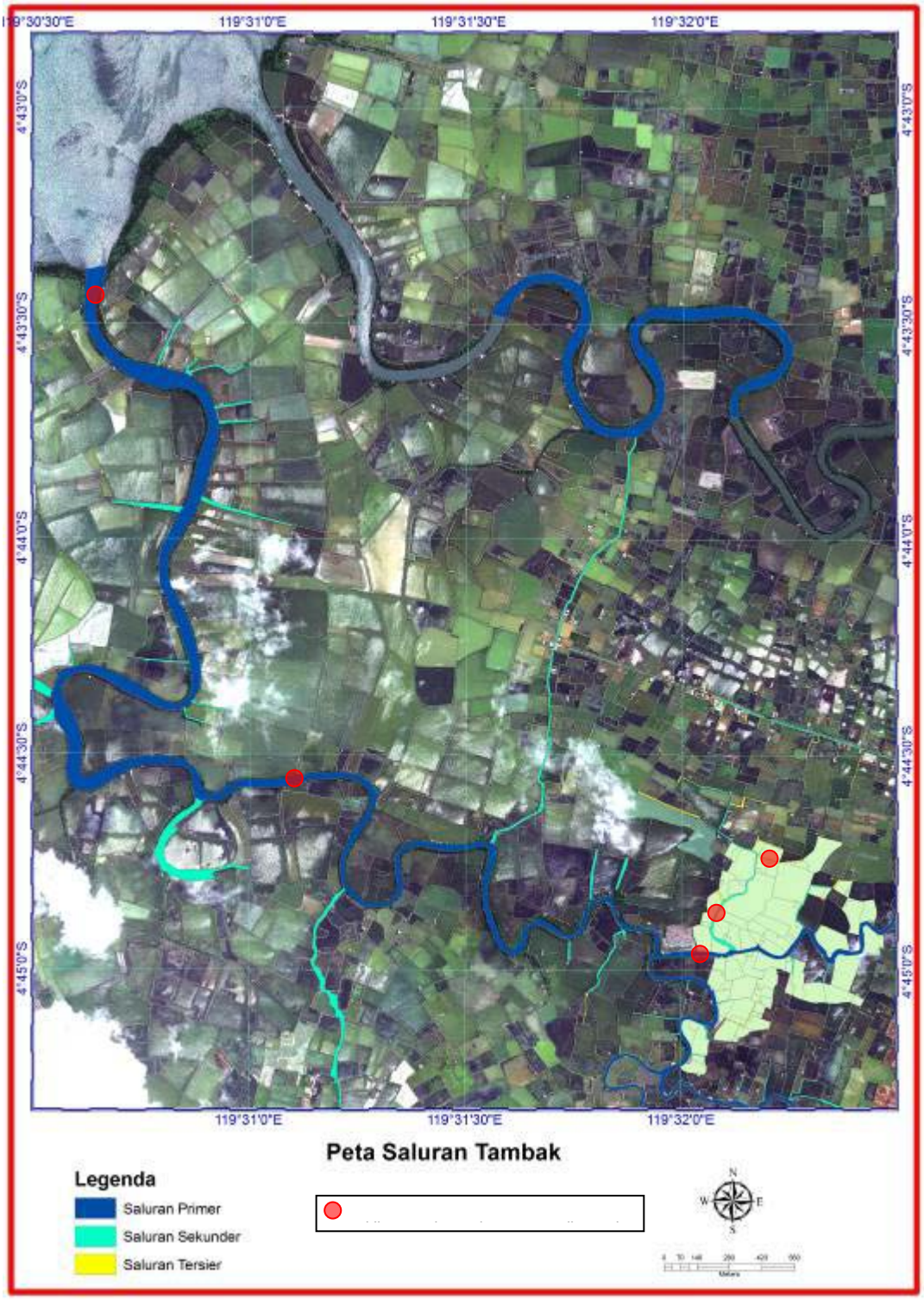
Kajian dilaksanakan di kawasan pertambakan yang terletak di Desa Gentung, Kecamatan Labakkang, Kabupaten Pangkep, Provinsi Sulawesi Selatan. Peta hasil klasifikasi tidak terbimbing dari Citra QuickBird akuisisi 2010 telah digunakan sebagai

petunjuk awal dalam penentuan lokasi pengamatan. Penentuan lokasi pengukuran dan pengambilan contoh tanah dilakukan secara *purposive* (bertujuan) yaitu dengan memilih petak tambak yang telah ditentukan sebagai tambak BMP (Best Management Practices), maupun tambak Kontrol serta tambak *Surveillance* (Pengamatan). Petak yang tergolong tambak BMP adalah tambak 45, 46, 47, 48, 49 dan 50 dimana dalam pengelolaan budidaya tambaknya diterapkan BMP. Petak yang tergolong tambak Kontrol adalah tambak 13, 33 dan 37 dimana dalam pelaksanaan budidayanya dilakukan pemantauan setiap hari. Sedangkan tambak yang tergolong tambak *Surveillance* adalah petak 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 34, 35, 36, 38, 39, 40, 41, 42, 43 dan 44 yang hanya dipantau pada saat terjadi serangan penyakit. Contoh tanah diambil pada semua petak tambak BMP dan tambak Kontrol, sedangkan tambak *Surveillance* hanya diambil contoh tanahnya pada petak tambak 16, 17 dan 18.

Contoh tanah diambil dengan menggunakan bor tanah pada permukaan tanah (0-0,25 m) dan kedalaman tanah 0,25-0,50 m. Contoh tanah diambil pada pelataran tambak dan saluran keliling tambak pada 23 titik pengambilan. Kualitas tanah yang diukur secara *in situ* adalah pH_F (pH tanah yang diukur langsung di lapangan) dengan pH-meter (Watling *et al*., 2004), pH_{FOX} (pH tanah yang diukur di lapangan setelah dioksidasi dengan hidrogen peroksida 30%) dengan pH-meter (Watling *et al*., 2004) dan potensial redoks diukur dengan redox-meter. Untuk analisis peubah kualitas tanah lainnya, maka diambil contoh tanah lalu dimasukkan kantong plastik dan selanjutnya ditempatkan dalam *cool box* yang berisi es sesuai petunjuk Ahern *et al*. (2004). Sebelumnya, sisa tumbuhan segar, kerikil dan kotoran lainnya dibuang dan bongkahan besar dikecilkan dengan tangan. Karena seluruh contoh tanah adalah tanah sulfat masam, maka contoh tanah diovenkan pada suhu 80-85^oC selama 48 jam (Ahern *et al*., 2004). Setelah kering, contoh tanah dihaluskan dengan cara ditumbuk pada lumpang porselin dan diayak dengan ayakan ukuran lubang 2 mm dan selanjutnya dianalisis di Laboratorium Tanah Balai Riset Perikanan Budidaya Air Payau di Maros. Kualitas tanah yang dianalisis di laboratorium meliputi pH_{KCl} (pH dari ekstrak KCl) (McElnea dan Ahern, 2004a), S_P (sulfur peroksida) (Melville, 1993; McElnea dan Ahern, 2004c), S_{KCl} (sulfur yang diekstrak dengan KCl) (Melville, 1993; McElnea dan Ahern, 2004d), S_{POS} (S_P-S_{KCl}) (Ahern dan McElnea, 2004), TPA (*Titratable Peroxide Acidity* atau sebelumnya dikenal dengan *Total Potential Acidity*) (McElnea dan Ahern, 2004b), TAA (*Titratable Actual Acidity* atau sebelumnya dikenal

dengan *Total Actual Acidity*) (McElnea dan Ahern, 2004a), TSA (*Titratable Sulfidic Acidity* atau sebelumnya dikenal dengan *Total Sulfidic Acidity*) (TPA-TAA) (McElnea dan Ahern, 2004b), pirit (Ahern *et al*., 1998a, 1998b), karbon organik dengan metode Walkley dan Black (Sulaeman *et al*., 2005), nitrogen-total dengan metode Kjedhal (Sulaeman *et al*., 2005), fosfat-tersedia dengan metode Bray 1 (Sulaeman *et al*., 2005), Fe dengan spektrofotometer (Menon, 1973), Al dengan spektrofotometer (Menon, 1973), dan tekstur dengan metode hidrometer (Agus *et al*., 2006).

Kecepatan dan arah aliran air ditentukan dengan menggunakan penanda dari zat pewarna serta meteran dan stopwatch yang dilakukan di saluran primer (sungai), saluran sekunder dan saluran tersier (Gambar 1). Pengukuran kecepatan dan arah aliran air dilakukan 2-3 jam sebelum surut terendah dan pasang tertinggi pada bulan Oktober 2010. Titik-titik pengukuran dan pengambilan contoh ditentukan posisinya dengan *Global Positioning System* (GPS). Data lain yang dikumpulkan adalah ada atau tidak adanya kematian udang windu serta penyebab kematian dari udang windu yang dipelihara di 50 petak tambak di lokasi kajian. Data pasang surut untuk bulan Oktober 2010 diambil dari stasiun terdekat dari lokasi kajian yaitu Biringkasi (4^{o}51’37,9” LS; 119^{o}23’00,6” BT) (Dinas Hidro-Oseanografi, 2010). Statistik deskriptif (minimum, maksimum, rata-rata, standar deviasi) digunakan untuk mendapatkan gambaran umum dari data yang ada. Karena pengambilan contoh tanah di lapangan dilakukan secara bertujuan, maka secara spasial data tersebar secara tidak teratur, maka dilakukan proses interpolasi terhadap titik-titik yang memiliki data. Sebanyak 14 contoh tanah (Mustafa *et al*., 2010) yang berada di luar lokasi kajian diperhitungkan pula dalam proses interpolasi data. Metode Kriging dalam Program Arc View 3.2 digunakan untuk proses interpolasi. Korelasi Pearson digunakan untuk mengetahui keeratan hubungan antarpeubah kualitas tanah pada kedalaman 0-0,25 m yang dianalisis dengan Program *Statistical Product and Service Solution* (SPSS) versi 15,0 (SPSS, 2006; Coakes *et al*., 2008).



Gambar 1. Kawasan pertambak dan lokasi pengukuran kecepatan aliran air di Desa Gentung, Kecamatan Labakkang, Kabupaten Pangkep

HASIL DAN BAHASAN

Karakteristik Lahan

Tanah

Berdasarkan pada Taksonomi Tanah (Soil Survey Staff, 2001), tanah tambak di Desa Gentung, Kecamatan Labakkang diklasifikasikan sebagai Sulfaquent dan Sulfihemits untuk kategori Kelompok Besar (*Great Groups*). Sulfaquent dicirikan dengan Aquents yang mempunyai bahan sulfidik atau pirit sampai 0,5 m dari permukaan tanah dan termasuk tanah sulfat masam potensial. Dalam kategori Kelompok Inti (*Ordo*), Sulfaquent dimasukkan dalam Entisol atau pada sistem Klasifikasi Tanah dari Pusat Penelitian Tanah dimasukkan dalam tanah Aluvial. Tanah sulfat masam tidak hanya didapatkan dalam tanah mineral, tetapi juga dalam tanah organik, termasuk tanah sulfat masam di Desa Gentung yang diklasifikasi sebagai Sulfihemits. Sulfihemits dicirikan dengan keberadaan bahan sulfidik sampai 1,0 m dari permukaan yang tidak teroksidasi dan tidak mempunyai horizon sulfurik pada kedalaman 0,5 dari permukaan tanah serta juga digolongkan sebagai tanah sulfat masam potensial. Dalam kategori Kelompok Inti, Sulfihemits adalah Histosol atau pada sistem Klasifikasi Tanah dari Pusat Penelitian Tanah dimasukkan dalam Organosol.

Hasil analisis peubah kualitas tanah yang merupakan peubah yang khas tanah sulfat masam dapat dilihat pada Tabel 1 dan 2 serta Lampiran Gambar 1 sampai dengan 22. Pada Tabel 1 dan 2 memperlihatkan distribusi vertikal kualitas tanah, sedangkan Lampiran Gambar 1 sampai dengan 22 memperlihatkan distribusi horisontal kualitas tanah di kawasan pertambakan Desa Gentung, Kecamatan Labakkang, Kabupaten Pangkep.

Potensial redoks tanah menunjukkan status tanah yang teroksidasi atau tereduksi. Potensial redoks adalah hasil pengukuran kuantitatif untuk menginformasikan suatu indeks diagnostik dari tingkat anaerobik atau anoksia tanah (Patrick dan Delaune, 1977). Potensial redoks adalah salah satu peubah penting dalam mengontrol persistensi berbagai senyawa organik dan anorganik tanah (Vorenhout *et al*., 2004; Zhang *et al*., 2009). Pengukuran kualitas tanah dilakukan dalam kondisi tanah yang tergenang, sebagai akibatnya potensial redoks tanah bernilai negatif baik pada kedalaman 0-0,25 m maupun

0,50-0,75 m. Potensial redoks tanah lebih rendah pada kedalaman 0-0,25 m daripada kedalaman 0,25-0,50 m. Pada kedalaman 0,25-0,50 m tanah lebih jenuh air, sehingga potensial redoksnya lebih rendah. Distribusi horisontal potensial redoks memiliki pola yang mirip dengan kandungan bahan organik atau karbon organik tanah (Lampiran Gambar 1, 13 dan 14). Dalam hal ini, potensial redoks tanah yang tinggi dijumpai pada tanah dengan kandungan karbon organik atau bahan organik yang rendah. Hal ini diperjelas dari nilai korelasi Pearson antara potensial redoks tanah dan kandungan bahan organik yang bernilai -0,428 (*P*=0,042) (Lampiran Tabel 1) yang menunjukkan bahwa semakin tinggi kandungan bahan organik semakin rendah potensial redoks tanah. Bahan organik adalah material yang dapat memegang air yang cukup besar, sebagai akibatnya lebih jenuh air sehingga potensial redoksnya lebih rendah. Secara umum, nilai potensial redoks yang negatif sebagai akibat pengukuran yang dilakukan pada tambak yang sedang dalam proses budidaya, sehingga tanah tambak tersebut jenuh air. Untuk budidaya tambak, disarankan nilai potensial redoks tambak sebaiknya bernilai positif. Walaupun pada persiapan tambak tambak di mana dilakukan pengeringan secara sempurna sehingga potensial redoks benilai positif, setelah proses budidaya berlangsung maka nilai potensial redoks tanah ini akan menurun seiring dengan bertambahnya masa pemeliharaan.

pH_F adalah pH tanah yang diukur langsung di lapangan dalam kondisi tanah basah atau segar. Pengukuran kemasaman tanah dengan pH-meter harus dilaksanakan dalam kondisi contoh basah untuk mencegah oksidasi pirit (senyawa yang umum dalam tanah sulfat masam) menjadi asam sulfat yang dapat menyebabkan penurunan nilai pH yang besar dibandingkan dengan yang normal jika diukur secara *in situ* (English *et al*., 1997). pH_F dapat digunakan untuk indikator secara cepat keberadaan dan kepelikan tanah sulfat masam aktual. pH_F tanah pada pada kedalaman 0-0,25 m lebih tinggi daripada kedalaman 0,25-0,50 m (Tabel 1 dan 2). Hal ini sebagai akibat proses remediasi alami yang berlangsung dalam waktu yang sangat lama yang menyebabkan terbilasnya senyawa atau unsur penyebab kemasaman tanah pada permukaan tanah tambak atau pada kedalaman 0-0,25 m.

Berbeda dengan pH_F adalah pH_{FOX} yaitu pH yang diukur di lapangan setelah tanah diberikan hidrogen peroksida (H_2O_2) 30%. Pemberian H_2O_2 30% dalam pengukuran pH_{FOX} dimaksudkan agar potensi kemasaman yang ada dalam tanah dapat teroksidasi semaksimum mungkin secara paksa. Sebagai akibatnya pH_{FOX} yang terukur

menjadi lebih rendah daripada hasil pengukuran pH_F pada semua kedalaman tanah. Salinitas dan alkalinitas yang tinggi pun tidak dapat menetralisir potensial kemasaman sehingga pH_{FOX} tetap menjadi rendah, apalagi tambak di Desa Gentung memiliki salinitas yang rendah. Seperti juga pada pH_F, maka pH_{FOX} tanah pada permukaan tanah dasar tambak lebih tinggi daripada pH_{FOX} pada kedalaman 0,25-0,50 m.

Tabel 1. Kualitas tanah kedalaman 0-0,25 m di tambak Desa Gentung, Kecamatan Labakkang, Kabupaten Pangkep, Provinsi Sulawesi Selatan (n=23)

Peubah	Minimum	Maksimum	Rata-rata	Standar Deviasi
Potensial redoks (mV)	-401	-170	-344	58
pH_F	6.78	7.96	7.10	0.246
pH_{FOX}	0.01	3.39	0.60	0.693
pH_F-pH_{FOX}	3.65	7.11	6.49	0.698
pH_{KCl}	3.93	6.75	5.64	0.772
S_{KCl} (%)	0.25	1.77	0.79	0.353
S_P (%)	2.20	4.48	3.76	0.591
S_{POS} (%)	1.78	3.76	2.97	0.515
TPA (mol H^+/ton)	501.00	1834.00	1108.72	368.905
TAA (mol H^+/ton)	0.00	17.00	1.67	4.010
TSA (mol H^+/ton)	501.00	1834.00	1107.04	367.856
Pirit (%)	2.24	8.19	4.94	1.642
C-Organik (%)	5.39	16.20	10.84	2.583
Bahan organik (%)	9.30	27.93	18.68	4.453
N-total (%)	0.12	1.49	0.93	0.293
Rasio C:N	7.66	96.64	15.09	17.922

PO_4 (ppm)	11.58	134.66	45.82	31.760
P_2O_5 (ppm)	8.65	100.64	34.25	23.737
Fe (ppm)	4317.00	5040.00	4730.17	169.852
Al (ppm)	138.50	593.50	307.57	120.755
Pasir (%)	58.00	88.00	70.87	8.286
Liat (%)	0.00	20.00	4.70	3.890
Debu (%)	8.00	42.00	24.43	9.184
Tekstur	Lempung berpasir (17 dari 23 contoh tanah) dan Pasir berlempung (6 dari 23 contoh tanah)			

Nilai pH_F-pH_{FOX} dapat digunakan sebagai indikator besarnya nilai potensi kemasaman pada tanah sulfat masam. Dari Tabel 1 dan 2 terlihat bahwa nilai pH_F-pH_{FOX} lebih tinggi pada kedalaman tanah 0,25-0,50 m daripada kedalaman tanah 0-0,25 m, yang menunjukkan bahwa potensi kemasaman tanah lebih tinggi pada kedalaman 0,25-0,50 m. Hal ini masih terkait dengan lebih seringnya tanah permukaan tanah terbilas secara alami dan kemungkinan mendapatkan bahan penetral kemasaman seperti kapur, sehingga potensi kemasamannya lebih rendah.

Seperti halnya dengan nilai pH_F dan pH_{FOX}, maka nilai pH_{KCl} (pH dengan pengekstrak 1 N KCl) menunjukkan nilai yang lebih tinggi pada kedalaman tanah 0-0,25 m (Tabel 1 dan 2). Pengukuran pH_{KCl} menunjukkan nilai pH tanah setelah H^+ dalam kompleks jerapan didesak keluar dan masuk ke dalam larutan tanah oleh kation lain sehingga disebut pula pH tanah potensial. pH_{KCl} biasanya diukur untuk membandingkan pH yang menggunakan pengekstrak H_2O (pH_{H2O}). Peubah kemasaman lainnya untuk tanah sulfat masam seperti TPA, TAA dan TSA juga menunjukkan bahwa baik kemasaman aktual maupun kemasaman potensial lebih rendah pada kedalaman tanah 0-0,25 m daripada 0,25-0,50 m. TSA juga mempunyai hubungan secara linear dengan kandungan pirit (Sutrisno, 1990 *dalam* Noor, 2004) pada tanah sulfat masam.

Tabel 2. Kualitas tanah kedalaman 0,25-0,50 m di tambak Desa Gentung, Kecamatan Labakkang, Kabupaten Pangkep, Provinsi Sulawesi Selatan (n=23)

Peubah	Minimum	Maksimum	Rata-rata	Standar Deviasi
Potensial redoks (mV)	-397	-276	-362	32
pH_F	6.69	7.98	7.01	0.271
pH_{FOX}	0.02	1.08	0.43	0.317
pH_F-pH_{FOX}	6.05	7.67	6.58	0.372
pH_{KCl}	1.70	6.52	4.41	1.202
S_{KCl} (%)	0,56	1.90	1.17	0.383
S_P (%)	2.64	5.17	4.19	0.566
S_{POS} (%)	1.94	4.14	3.03	0.535
TPA (mol H^+/ton)	950	2075	1503.17	333.220
TAA (mol H^+/ton)	0	96	18.22	25.795
TSA (mol H^+/ton)	940	2060	1484.96	327.013
Pirit (%)	4.20	9.20	6.63	1.460
C-Organik (%)	9.23	17.11	13.10	2.395
Bahan organik (%)	15.91	29.49	22.59	4.128
N-total (%)	0.48	1.23	0.83	0.174
Rasio C:N	7.93	24.20	16.36	4.331
PO_4 (ppm)	10.42	130.90	23.79	24.890
P_2O_5 (ppm)	7.79	97.83	17.78	18.602
Fe (ppm)	4018	4974	4567.39	247.520
Al (ppm)	130	556	255.41	112.311
Pasir (%)	56.00	86.00	70.09	7.874

Liat (%)	0.00	8.00	4.70	1.869
Debu (%)	10.00	42.00	25.22	8.129
Tekstur	Lempung berpasir (15 dari 23 contoh tanah) dan Pasir berlempung (8 dari 23 contoh tanah)			

Pada tanah sulfat masam yang dicirikan dengan kandungan pirit, maka salah satu sumber kemasamannya adalah sulfur. Pirit yang teroksidasi akan menghasilkan asam sulfat dan ferrosulfat yang apabila bereaksi dengan air melepaskan ferrisulfat yang selanjutnya apabila teroksidasi kembali akan menghasilkan asam sulfat Sebagai sumber kemasaman yang penting pada tanah sulfat masam, maka sulfur yang diukur dalam bentuk S_{POS} tanah telah digunakan oleh Ahern *et al*. (1998b) untuk menentukan kebutuhan kapur bagi tanah sulfat masam.

Pada tanah sulfat masam yang dicirikan dengan kandungan pirit, maka salah satu sumber kemasamannya adalah sulfur. Pirit yang teroksidasi akan menghasilkan asam sulfat dan ferrosulfat yang apabila bereaksi dengan air melepaskan ferrisulfat yang selanjutnya apabila teroksidasi kembali akan menghasilkan asam sulfat. Pirit adalah ciri utama dari tanah sulfat masam. Pirit sebagai salah salah satu sumber kemasaman di tanah sulfat masam juga menunjukkan konsentrasi yang rendah pada kedalaman 0-0,25 m daripada kedalaman tanah 0,25-0,50 m (4,94% *vs* 6,63%).

Kandungan bahan organik tanah tambak di Desa Gentung berkisar antara 9,30 dan 27,93 dengan rata-rata 18,68% pada kedalaman 0-0,25 m dan berkisar antara 15,91 dan 29,49 dengan rata-rata 22,59% pada kedalaman 0,25-0,50 m. Lebih rendahnya kandungan bahan organik pada kedalaman 0-0,25 m sebagai akibat telah terjadi proses mineralisasi yang lebih intensif pada kedalaman tanah tersebut. Mineralisasi bahan organik dapat menghasilkan unsur hara terutama karbon nitrogen. Bahan organik, selain sebagai sumber karbon, juga merupakan sumber nitrogen (Boyd, 2008). Hal ini juga dapat menjadi penyebab lebih tingginya kandungan N-total tanah pada kedalaman tanah 0-0,25 m (Tabel 1 dan Tabel 2). Lebih tingginya kandungan N-total tanah pada kedalaman tanah 0-0,25 m dapat juga berasal dari aplikasi pupuk yang mengandung

nitrogen yang diaplikasikan baik sebagai pupuk dasar pada saat persiapan tambak maupun pupuk susulan pada saat sedang berlangsung budidaya.

Telah disebutkan sebelumnya bahwa tanah tambak di Desa Gentung dikasifikasikan dalam Organosol. Organosol atau tanah organik atau tanah gambut adalah tanah yang mengandung karbon organik lebih besar 15% (Boyd *et al*., 2002). Tanah tambak dengan kandungan karbon organik antara 3,1 dan 15,0% tergolong tinggi dan kandungan karbon organik antara 1,1 dan 3,0% tergolong sangat baik untuk budidaya tambak (Boyd *et al*., 2002). Dari Tabel 1 dan Tabel 2 terlihat bahwa kandungan karbon organik taman tambak di Desa Gentung tergolong tinggi yaitu 5,39 dan 16,20% pada kedalaman 0-0,25 m dan antara 9,23 dan 17,11% pada kedalaman 0,25-0,50 m. Tanah dengan kandungan bahan organik tinggi tergolong tanah yang porous seperti tanah yang bertekstur pasir yang akan meningkatkan rembesan air yang juga membawa bahan organik ke dalam tanah tambak.

Kandungan bahan organik yang rendah dan dan kandungan nitrogen-total yang lebih tinggi menyebabkan rasio C:N tanah pada kedalaman 0-0,25 m lebih rendah daripada kedalaman 0,25-0,50 m. Secara umum, rasio C:N tanah tergolong tidak terlalu tinggi yaitu antara 7,66:1 dan 96,64:1 dengan rata-rata 15,09:1 pada kedalaman 0-0,25 m dan antara 7,93:1 dan 24,20:1 dengan rata-rata 16,36:1 pada kedalaman 0,25-0,50 m. Rasio C:N tanah yang ideal untuk lahan budidaya tambak seharusnya 8:1 sampai 12:1 (Boyd, 2008). Rasio C:N tanah di Desa Gentung ini masih lebih rendah daripada daripada rasio C:N tanah gambut yang biasanya lebih besar dari 31:1 (Mustafa, 1998; Barchia, 2006). Pada tanah dengan rasio C:N tinggi, maka terjadi immobilisasi nitrogen oleh mikrobiologi untuk memenuhi kebutuhan metabolismenya.

Kandungan fosfat tanah berbeda pada kedalaman tanah yang berbeda, dimana kandungan fosfat yang rendah dijumpai kedalaman 0,25-0,50 m. Kandungan fosfat yang rendah pada kedalaman tersebut sebagai akibat dari pH tanah yang lebih rendah. Pada tanah yang pHnya rendah, fosfat diikat secara kuat oleh Fe dan Al dalam bentuk $FePO_4$ atau $AlPO_4$ yang tidak larut (Kselik *et al*., 1992; Barchia, 2006; Mustafa dan Sammut, 2007). Faktor lain yang dapat menyebabkan lebih tingginya kandungan PO_4 tanah pada kedalaman 0-0,25 adalah pemberian pupuk yang mengandung PO_4 seperti TSP atau SP-36 yang diaplikasikan baik sebagai pupuk dasar maupun pupuk susulan. Distribusi PO_4 secara horizontal menunjukkan bahwa kandungan PO_4 yang rendah dijumpai pada

tambak yang tanahnya tergolong tinggi kandungan Fe nya (Lampiran Gambar 16 dan 18). Seperti telah dijelaskan sebelumnya bahwa fosfat dapat diikat oleh Fe, sehingga fosfat menjadi rendah kandungannya.

Fe adalah bagian dari pirit (FeS_2) pada tanah sulfat masam yang dapat berasal dari sedimen. Kandungan Al pada tanah sulfat masam meningkat pada pH yang lebih rendah, yaitu pH 4,0-4,5 (Dent, 1986). Selain itu, kandungan Al pada tanah sulfat masam berkaitan dengan oksidasi pirit. Suasana yang sangat masam mempercepat pelapukan mineral alumino-silikat akibat perusakan kisi dari mineral tipe 2:2 (seperti montmorillonit) menjadi mineral tipe 1:1 (kaolinit) dengan membebaskan dan melarutkan Al yang lebih banyak (Pons, 1973). Oleh karena itu, kandungan Fe dan Al tanah tambak di Desa Gentung tergolong tinggi. Kandungan Fe dan Al tanah tambak yang tergolong tinggi sangat umum dijumpai pada tambak tanah sulfat masam.

Satu-satunya sifat fisik tanah yang diamati dalamn kajian ini adalah tekstur. Tekstur tanah menggambarkan perbandingan antara fraksi liat, debu dan pasir dari tanah. Hanya ada 2 jenis tekstur tanah yang teridentifikasi di tambak Desa Gentung yaitu lempung berpasir dan pasir berlempung, dimana tekstur lempung berpasir lebih dominan daripada tekstur pasir berlempung. Pada kandungan pasir yang tinggi memudahkan pengelolaan kelebihan bahan organik dari sisa pakan atau kotoran udang. Namun demikian, kandungan pasir yang tinggi dan liat yang rendah dapat mengakibatkan tingginya porositas tambak. Fraksi pasir memiliki potensi untuk lebih kompak yang tergolong rendah, sedangkan liat tergolong tinggi (Schaetzl dan Anderson, 2005). Dikatakan oleh Boyd (1995) bahwa suatu material tanah yang merupakan campuran dari partikel yang berbeda ukuran dan mengandung minimum 30% liat adalah ideal untuk konstruksi tambak. Tekstur tanah yang baik untuk tambak adalah: liat, lempung berliat, lempung liat berdebu, lempung berdebu, lempung dan lempung liat berpasir (Ilyas *et al*., 1987). Dengan demikian, tekstur tanah tambak di Desa Gentung kurang mendukung untuk usaha budidaya tambak terutama dilakukan secara tradisional maupun tradisional plus, sebab tekstur tanahnya kurang baik untuk penumbuhan makanan alami seperti klekap.

Tambak dengan tekstur tanah yang tergolong kasar seperti tambak di Desa Gentung dimana fraksi pasirnya rata-rata 70,87 dan 70,09% masing-masing pada kedalaman 0-0,25 dan 0,25-0,50 m tergolong pada tambak yang porous sehingga

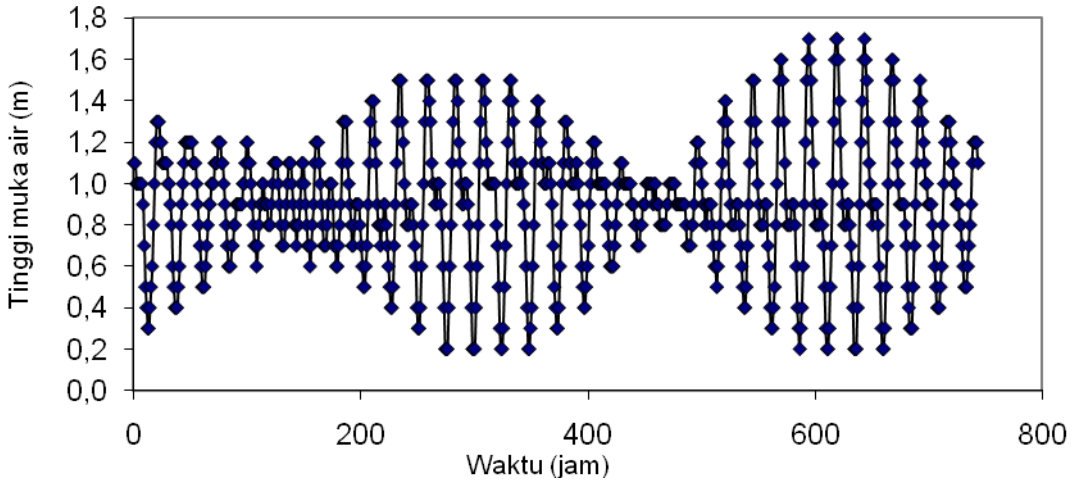
rembesan air juga cukup besar. Air yang merembes bisa saja membawa mikroorganisme penyebab penyakit sehingga dapat mempercepat proses penyebaran penyakit di tambak.

Hidrologi

Hasil analisis data pasang surut bulan Oktober 2010 dari stasiun Biringkassi (Kabupaten Pangkep) menunjukkan bahwa perbedaan pasang surut di kawasan pesisir Kabupaten Pangkep sebesar 1,5 m (Gambar 2). Dari Gambar 2 terlihat juga bahwa di kawasan pesisir Kabupaten Pangkep terjadi 2 kali pasang dalam sehari di mana salah satu pasang lebih tinggi dari pasang lainnya. Kisaran pasang surut antara 1 dan 3 m lebih baik dalam pengisian serta pengeingan dan pembuangan limbah dari dalam tambak (Chanratchakool *et al*., 1995). Dalam hal ini, kondisi pasang surut di kawasan pesisir Kabupaten Pangkep dapat mendukung usaha tambak di kawasan intertidal.

Sumber air laut untuk kawasan pertambakan di Desa Gentung berasal dari Selat Makassar melalui Sungai Gentung yang tergolong sungai pasang surut. Sungai tersebut memiliki banyak *meander* (kelokan) sehingga jarak tempuh air laut semakin panjang. Pendangkalan di muara sungai dapat juga memperlambat air pasang masuk pada saat pasang dan air surut ke luar pada saat surut. Di sepanjang sungai dijumpai lebih dari 30 unit alat perangkap ikan yaitu sero yang dapat juga menghambat laju air sebab perangkap ikan tersebut juga menjadi perangkap bagi sampah. Alat perangkap ikan tersebut ditempatkan pada kelokan luar sungai dari arah hulu, sehingga peluang sampah terperangkap semakin besar.

Saluran tambak di Desa Gentung pada termasuk tipe terbuka dengan penampang berbentuk trapesium terbalik dan airnya mengalir secara gravitasi. Lebar sungai yang menjadi sumber air bagi pertambakan di Desa Gentung bervariasi dari sekitar 8 m di sekitar pertambakan dan sekitar 30 m di muara sungai. Lebar saluran sekunder, saluran yang langsung dari sungai, yaitu dari 2 sampai 6 m. Saluran tersier, saluran dari saluran sekunder ke petak tambak, mempunyai lebar 0,6 sampai 2 m.



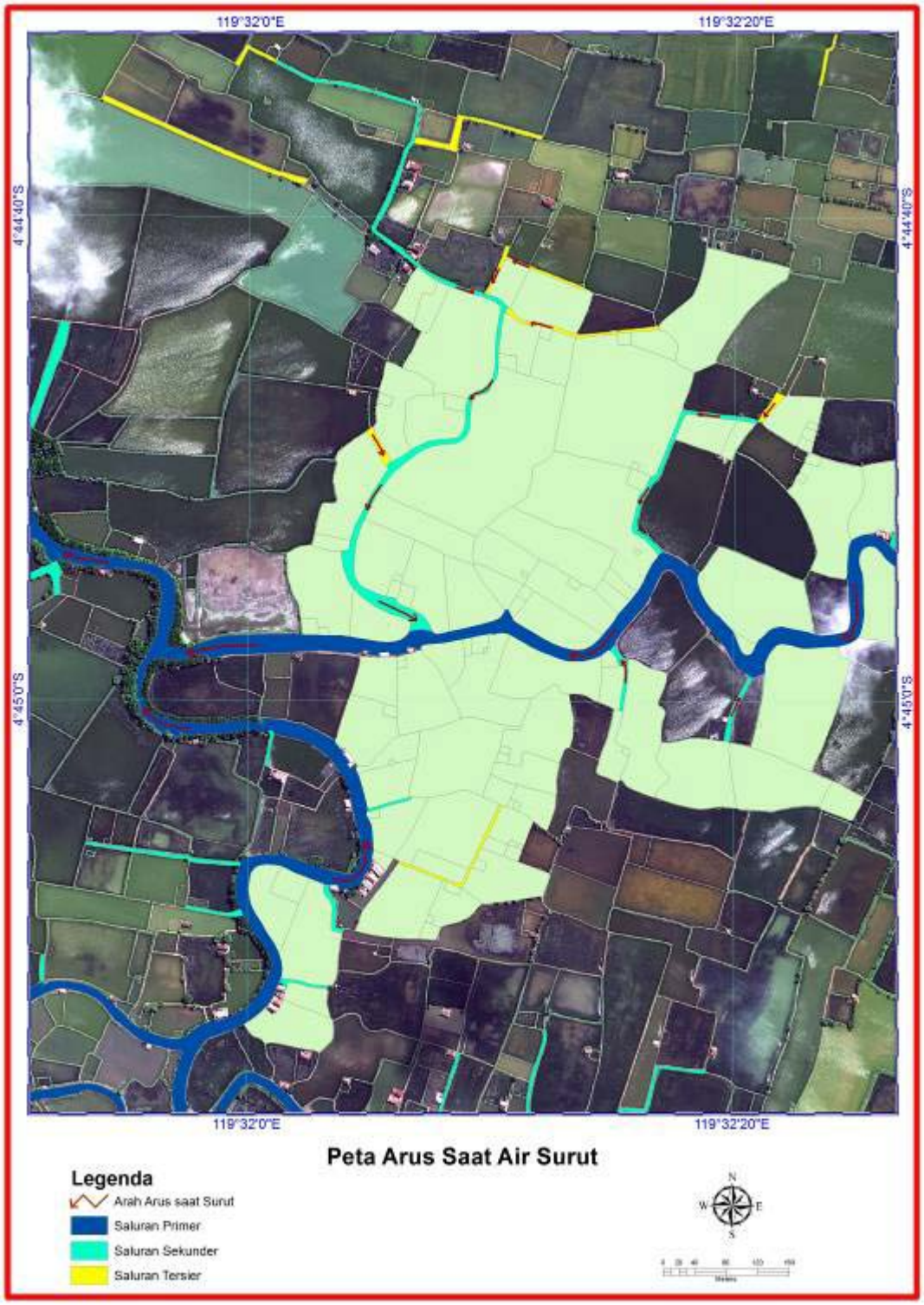
Gambar 2. Fluktuasi pasang surut di kawasan pesisir Kabupaten Pangkep (Data diolah dari Dinas Hidro-Oseanografi (2010))

Petak tambak ada yang mengambil langsung dari sungai (saluran primer) dan ada pula dari saluran sekunder dan tersier, tergantung pada posisi tambak terhadap saluran tersebut. Saluran yang ada merupakan saluran pemasukan yang juga merangkap sebagai saluran pengeluaran. Oleh karena itu, setiap petak tambak hanya memiliki satu pintu air yang juga merupakan pintu pemasukan dan pengeluaran air. Ada juga tambak yang memiliki pintu pengeluaran air berupa pipa pralon, tetapi juga dibuang pada saluran yang sama.

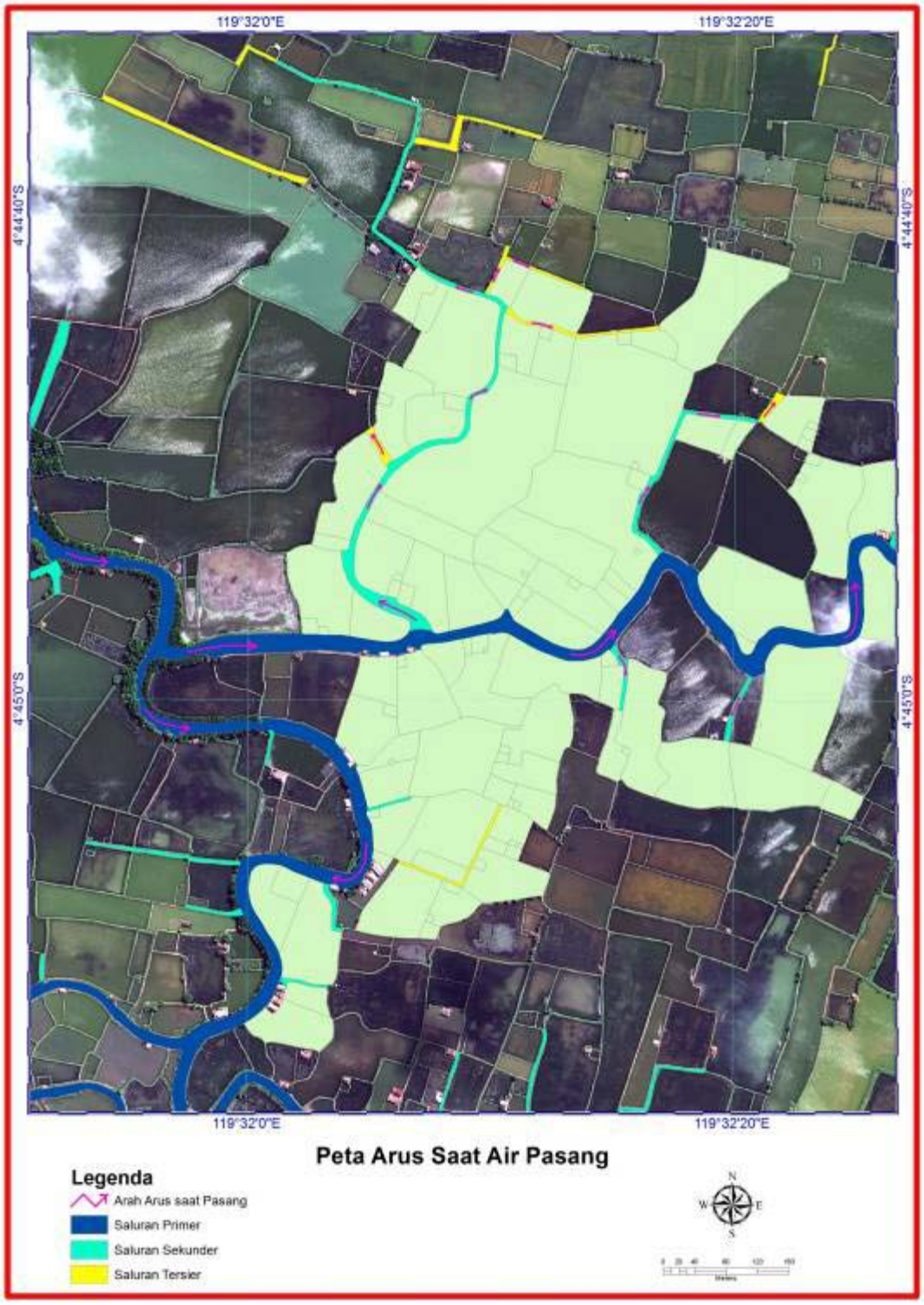
Kecepatan aliran air relatif bervariasi pada waktu pengukuran dan jenis saluran yang berbeda. Kecepatan aliran air yang tinggi dicatat pada saluran tersier pada saat 2 jam sebelum surut terendah yaitu rata-rata 0,17 m/detik. Pada waktu yang tidak terlalu berbeda kecepatan aliran air di saluran sekunder dan sungai masing-masing rata-rata 0,14 dan 0,09 m/dt. Lebih tingginya kecepatan aliran air di saluran tersier sebagai akibat adanya pembudidaya tambak yang melakukan pembuangan air untuk pergantian air. Penampang saluran sekunder dan sungai yang lebih besar juga merupakan penyebab lebih rendahnya kecepatan aliran alir di sungai dan saluran sekunder. Kecepatan aliran air yang rendah ini memungkinkan terjadinya pengendapan pada saluran, namun tidak menimbulkan erosi pematang. Agar tidak terjadi pengendapan pada saluran yang

permukaannya berupa tanah, maka kecepatan aliran air pada saluran sebaiknya lebih dari 0,3 m/dt (Wheaton, 1977). Kecepatan maksimum aliran air yang dapat ditolerir pada tanah endapan aluvial tanpa menimbulkan erosi adalah 1,52 m/dt. Biasanya kecepatan aliran air pada saluran yang permukaannya dari tanah adalah 0,5-0,7 m/dt (Ilyas *et al*., 1987).

Seperti telah dijelaskan sebelumnya bahwa tambak di Desa Gentung hanya memiliki saluran yang sama untuk pemasukan dan pengeluaran air. Dari hasil pengamatan menunjukkan bahwa arah aliran air yaitu dari tambak ke saluran tersier, saluran sekunder, sungai dan ke laut pada saat surut (Gambar 3) dan sebaliknya melalui saluran yang sama arah aliran air menuju ke tambak pada saat pasang (Gambar 4). Dalam hal ini, peluang penggunaan kembali air buangan dari tambak cukup besar yang juga membuka peluang tersebarnya penyakit di kawasan pertambakan. Seperti dikatakan oleh Boyd dan Musig (1992) bahwa tambak dapat menjadi sumber polusi bagi dirinya sendiri, apabila tambak tersebut menggunakan badan air yang sama untuk sumber air dan pembuangan limbah. Muara Sungai Gentung yang dangkal dan banyaknya alat perangkap ikan (sero) dapat menjadi faktor ketidaklancaran pengeluaran maupun pemasukan air di kawasan pertambakan Desa Gentung yang juga dapat memicu penyebaran penyakit.



Gambar 3. Arah aliran air pada saat surut di saluran pertambakan Desa Gentung, Kecamatan Labakkang, Kabupaten Pangkep

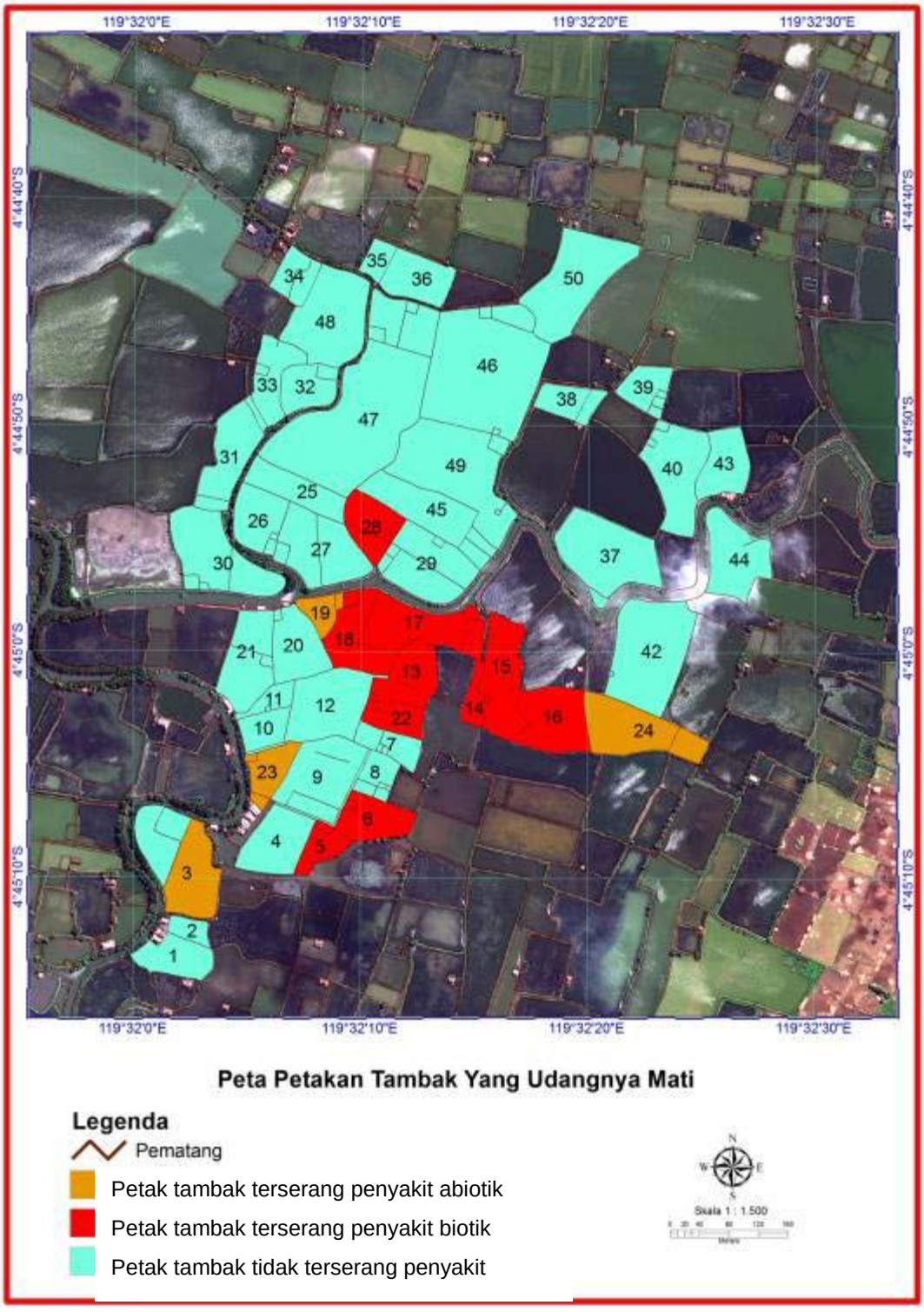


Gambar 4. Arah aliran air pada saat pasang di saluran pertambakan Desa Gentung, Kecamatan Labakkang, Kabupaten Pangkep

Serangan Penyakit

Penyakit pada budidaya udang dapat digambarkan sebagai kondisi atau faktor biotik dan abiotik yang berpengaruh kurang baik terhadap budidaya tambak (Lightner, 1996, Flegel *et al*., 2008). Penyakit biotik pada udang disebabkan oleh perantara organisme, sedangkan penyakit abiotik disebabkan oleh lingkungan atau kondisi ekstrem. Seperti halnya di Desa Gentung, dari 50 petak tambak yang dikaji, ternyata 10 petak tambak di antaranya terserang oleh penyakit yang menyebabkan kematian udang windu yang dipelihara dan 4 petak tambak juga mengalami kematian udang windu yang dipelihara sebagai akibat faktor lingkungan. Sepuluh petak tambak yang terserang penyakit biotik adalah petak 5, 6, 13, 14, 15, 16, 17, 18, 22 dan 28, sedangkan 4 petak yang terserang penyakit abiotik adalah petak 3, 19, 23 dan 24 (Gambar 5). Seperti telah dilaporkan sebelumnya bahwa faktor lingkungan adalah penyebab utama terjadinya serangan penyakit pada tambak udang di Sulawesi Selatan (Anonim, 2010a). Penyakit yang diidentifikasi yang menyebabkan kematian udang windu di Desa Gentung adalah bintik putih yang diakibatkan oleh WSSV (*White Spot Syndrome Virus*). Penyakit bintik putih sering dicirikan dengan kematian yang tinggi dan cepat serta terjadi sangat singkat sesudah muncul tanda-tanda klinik awal dan dapat menyerang semua kelompok umur dari udang (MPEDA/NACA. 2003). Juga telah dilaporkan sebelumnya bahwa Desa Kanaungang, Kecamatan Labakkang yang lokasinya relatif dekat dengan lokasi kajian telah terserang penyakit bintik putih sehingga panen udang windu pembudidaya tambak mengalami penurunan 40-50% (Anonim, 2010b).

Pada petak tambak 45 sampai 60 atau tambak BMP tidak dijumpai adanya kematian secara nyata dari udang windu yang dibudidayakan sebagai akibat dari penyakit. Hanya saja, pada petak tambak 48 dijumpai penyakit yang mengkerdilkan udang yang diidentifikasi sebagai IHHNV (*Infectious Hypodermal and Hematopoietic Necrosis Virus*). Hal ini dapat disebabkan karena keenam petak tambak tersebut menerapkan BMP. Pengelolaan tambak yang diaplikasikan pata tambak BMP tersebut adalah: mendapatkan air pasok yang bebas hama penular dan logam berat yang berbahaya; tambak dapat menampung air dan mempertahankan kedalaman sesuai yang diinginkan; mengeluarkan limbah dengan tingkat sedimen dan bahan organik terlarut yang rendah; menjaga keseimbangan proses mikrobiologis; menggunakan bahan yang aman bagi manusia dan lingkungan serta menebar benih yang sehat.



Gambar 5. Peta distribusi petakan tambak yang terserang penyakit abiotik, penyakit biotik dan tambak yang tidak terserang penyakit di Desa Desa Gentung, Kecamatan Labakkang, Kabupaten Pangkep

Hubungan Karakteristik Lahan dan Penyakit

Dari 19 peubah kualitas tanah yang dikaji dalam hubungannya dengan penyakit udang windu di tambak tanah sulfat masam di Desa Gentung, Kecamatan Labakkang ternyata ada enam peubah kualitas tanah yang memiliki kemiripan pola distribusi terhadap tambak yang terserang penyakit yaitu: pH_{KCl}, S_{POS}, TSA, TPA, pirit, bahan organik dan tekstur tanah. Namun demikian, kematian udang windu ini bukanlah dari akibat langsung dari kualitas tanah tersebut, seperti dikatakan oleh Rostamian (Tanpa tahun) bahwa tanah memiliki pengaruh tidak langsung terhadap pertumbuhan, sintasan dan serangan penyakit pada organisme akuatik termasuk udang windu. Kualitas lahan yang kurang sesuai atau perubahan kondisi lahan akibat kesalahan manajemen dapat mempercepat terjadinya serangan penyakit (Kautsky *et al*., 2000; Menavesta, 2002; Nimrat *et al*., 2008).

Udang windu yang terserang penyakit dijumpai pada tambak-tambak dengan pH_{KCl} tanahnya tergolong rendah. pH_{KCl} biasa juga disebut pH tanah potensial ternyata lebih baik dijadikan indikator terjadinya kematian udang windu dibandingkan dengan bentuk pH lain dari tanah sulfat masam seperti pH_F, pH_{FOX} dan pH_F-pH_{FOX}. Dari Lampiran Tabel 1 memang menunjukkan bahwa tidak ada hubungan yang kuat antara pH_{KCl} dengan pH_F, pH_{FOX} dan pH_F-pH_{FOX} padat tamah sulfat masam di tambak Desa Gentung. Pada pH tanah yang rendah dapat menyebabkan kelarutan unsur-unsur toksik seperti aluminium menjadi lebih tinggi yang diduga dapat memicu kematian udang windu. Hidrolisis aluminium dapat meningkatkan kemasaman dalam sistem (Minh *et al*., 2002) dan keberadaan aluminium yang dapat larut dapat megurangi produksi makanan alami, udang dan ikan (Sammut, 1999).

Peubah khas tanah sulfat masam yang memiliki kemiripan pola distribusi dengan tambak yang udangnya terserang penyakit adalah S_{POS}. Serangan penyakit pada udang windu di tambak tanah sulfat masam Desa Gentung juga dijumpai pada tambak yang memiliki nilai S_{POS} yang relatif tinggi. S_{POS} menggambarkan sulfur yang ada dalam tanah sulfat masam setelah diekstrak dengan KCl dan hidrogen peroksida. Sindrom kulit lunak, penyakit bintik merah dan udang biru terjadi karena terkait dengan masalah tanah sulfat masam (Baticados *et al.,* 1990; Brock, 1991).

Peubah kualitas tanah sulfat masam lainnya yang menggambarkan kemasaman tanah yang juga memiliki kemiripan pola distribusi dengan tambak yang udangnya terserang penyakit adalah TSA dan TPA. Dari Lampiran Tabel 1 memperlihatkan bahwa

TSA dan TPA memiliki hubungan yang erat dengan S_{POS} tanah. Telah dikatakan oleh McElnea *et al*. (2002a, 2002b) bahwa pada tanah sulfat masam yang rendah kandungan bahan organiknya, maka TSA berkorelasi baik dengan S_{POS}. TSA juga mempunyai hubungan secara linear dengan kandungan pirit (Sutrisno, 1990 *dalam* Noor, 2004) pada tanah sulfat masam. Oleh karena itu, pirit juga memiliki pengaruh tidak langsung terhadap kematian udang windu di tambak tanah sulfat masam di Desa Gentung. Tambak-tambak yang udangnya terserang penyakit didapatkan pada tambak yang memiliki kandungan TSA, TPA dan pirit yang tinggi.

Pirit yang teroksidasi akan menghasilkan asam sulfat dan ferrosulfat yang apabila bereaksi dengan air melepaskan ferrisulfat yang selanjutnya apabila teroksidasi kembali akan menghasilkan asam sulfat sehingga terjadi penurunan pH tanah. Penurunan pH tanah meningkatkan kelarutan unsur toksik seperti Fe, Al dan Mn yang dapat menyebabkan kematian pada udang windu. Secara umum logam seperti Fe dan Al dapat menyebabkan gangguan pada proses fisiologis organisme akuatik. Besi dapat menyebabkan penyumbatan pada insang ikan dan udang (Sammut, 1999). Penyumbatan pada insang ikan dan udang berarti mempersempit luas permukaan insang yang berdampak pada berkurangnya difusi oksigen ke permukaan organ pernafasan pada saat inspirasi dan difusi karbondioksida pada saat ekspirasi. White *et al*. (1996) telah melaporkan bawa keberadaan Fe terlarut dapat menyebabkan kematian ikan secara umum. Keberadaan Al berdampak negatif pada pakan alami, ikan dan udang (Sammut, 1999). Penurunan kualitas lingkungan sebagai akibat tanah sulfat masam, polutan dan organisme penyebab penyakit dapat menurunkan pertumbuhan dan sintasan udang serta mengintrodusir penyakit (Hambrey, 1996).

Tambak dengan kandungan bahan organik tanah yang tinggi kurang mendukung keberlanjutan budidaya tambak terutama sistem tertutup sebab dapat memproduksi amonia dan senyawa toksik lainnya yang dapat membahayakan kehidupan udang (Chanratchakool *et al*., 1995) seperti metan dan hidrogen sulfida (Rachmansyah dan Mustafa, 2011). Kandungan bahan organik yang tinggi juga menjadi media bagi berkembangbiaknya berbagai mikroorganisme termasuk mikroorganisme penyebab penyakit. Pendapat ini sesuai dengan temuan yang diperoleh dalam kajian ini. Udang windu yang terserang penyakit di tambak Desa Gentung secara umum dijumpai pada tambak yang memiliki kandungan bahan organik yang tinggi.

Udang windu yang terserang penyakit di tambak Desa Gentung juga dijumpai pada tanah tambak dengan fraksi liat yang rendah atau fraksi pasir yang tinggi. Kandungan pasir yang tinggi dan liat yang rendah dapat mengakibatkan tingginya porousitas pematang tambak. Fraksi pasir memiliki potensi untuk lebih kompak yang tergolong rendah, sedangkan liat tergolong tinggi (Schaetzl dan Anderson, 2005). Dikatakan oleh Boyd (1995) bahwa suatu material tanah yang merupakan campuran dari partikel yang berbeda ukuran dan mengandung minimum 30% liat adalah ideal untuk konstruksi pematang tambak. Tambak dengan tekstur tanah yang tergolong kasar merupakan tambak porous sehingga rembesan air juga cukup besar. Air yang merembes bisa saja membawa organisme penyebab penyakit sehingga dapat mempercepat proses penyebaran penyakit di tambak. Tambak dengan tekstur tanah yang tergolong kasar seperti di Desa Gentung kurang mendukung dalam hal penumbuhan makanan alami seperti klekap di tambak yang juga berarti kurang mendukung pertumbuhan maupun ketahanan penyakit dari udang yang dipelihara.

Pengelolaan Tanah

Oleh karena tanah tambak di Desa Gentung didominasi oleh tanah sulfat masam, dimana tanah sulfat masam tergolong tanah bermasalah untuk budidaya tambak. Dengan demikian upaya perbaikan melalui pengelolaan tanah yang tepat sesuai karakteristiknya, merupakan tindakan yang perlu dilakukan. Peningkatan pH tanah dan penurunan kandungan unsur-unsur beracun dapat dilakukan melalui remediasi. Remediasi adalah suatu aktivititas atau proses yang dilakukan untuk mengurangi unsur-unsur beracun dalam tanah. Remediasi yang dapat dilakukan berupa proses oksidasi dan pembilasan tanah serta pengapuran. Prinsip remediasi melalui oksidasi dan pembilasan tanah adalah pengeringan tanah untuk mengoksidasi pirit, perendaman untuk melarutkan dan menetralisir kemasaman atau menurunkan produksi kemasaman lanjut dan pembilasan untuk membuang hasil oksidasi dan meminimumkan cadangan unsur-unsur beracun dalam tanah.

Tanah dasar tambak sebaiknya dicangkul terlebih dahulu sedalam 0,2 m agar permukaan tanah bertambah luas sehingga proses oksidasi dapat lebih baik.

Pengeringan tanah dasar tambak dilakukan selama 2 minggu pada keadaan terik matahari atau tergantung pada keadaan iklim selama pengeringan. Tambak dibiarkan terendam selama 1 minggu dengan air bersalinitas tinggi (lebih besar 15 ppt) dan selanjutnya air rendaman dibuang. Air bersalinitas tinggi mengandung Ca, Mg dan Na yang tinggi pula, sehingga lebih banyak pula Fe dan Al yang dapat tergantikan unsur yang bersifat basa tersebut yang berdampak pada pengurangan Fe dan Al yang juga lebih banyak. Proses tersebut diulang 2 atau 3 kali sampai didapatkan kondisi tanah yang lebih baik. Disarankan proses tersebut dilakukan pada musim kemarau dimana curah hujan relatif rendah dan suhu udara relatif tinggi dan pada saat surut rendah agar pengeringan dapat lebih baik, salinitas air rendaman dapat lebih tinggi dan selanjutnya pembilasan air rendaman juga dapat lebih sempurna. Dalam pengeringan tambak, diharapkan juga proses dekomposisi bahan organik dapat lebih cepat sehingga kandungan bahan organik yang tinggi dapat menurun, di samping mengurangi senyawa-senyawa beracun. Bentuk lain remediasi berupa pengapuran dapat dilakukan untuk mengurangi unsur-unsur beracun yang masih tersisa dalam tanah. Bentuk kapur yang disarankan digunakan adalah kaptan dan dolomit.

Nilai S_{POS} telah digunakan oleh Ahern *et al*. (1998b) untuk menentukan kebutuhan kapur bagi tanah sulfat masam. Jika diasumsikan bahwa berat jenis tanah sebesar 0,75 g/cm^3 dengan S_{POS} permukaan tanah antara 1,78 dan 3,76 dengan rata-rata 2,97% dan kapur dapat bereaksi sampai kedalaman 0,05 m, maka tambak di Desa Gentung membutuhkan kapur pertanian antara 15,24 dan 31,19 dengan rata-rata 25,43 ton/ha (nilai netralisasi = 100, faktor keamanan = 1,5). Kebutuhan kapur ini masih lebih rendah dibandingkan dengan tambak tanah sulfat masam lainnya yang pernah dilaporkan. Kapur pertanian sebanyak 40,5 ton/ha dibutuhkan tambak tanah sulfat masam di Kecamatan Anggrek, Kabupaten Gorontalo Utara (Mustafa dan Pantjara, 2009). Tambak tanah sulfat masam di Kabupaten Mamuju membutuhkan kapur pertanian rata-rata 33,21 ton/ha (Mustafa *et al*., 2010).

Nitrogen-total > 500 ppm pada tanah tambak digolongkan sebagai *slight* atau baik dengan faktor pembatas yang mudah diatasi (Karthik *et al*., 2005). Oleh karena itu, pupuk yang mengandung nitrogen seperti urea tidak terlalu banyak dibutuhkan di tambak Desa Gentung, sebab kandungan nitrogen-totalnya sudah termasuk tinggi. Lain halnya dengan fosfat-tersedia yang masih dibutuhkan di tambak Desa Gentung. Rata-rata fosfat-tersedia di tanah tambak Desa Gentung adalah 45,82 ppm. Fosfat-tersedia lebih dari 60 ppm

dalam tanah tambak dapat digolongkan sebagai *slight* atau tergolong baik dengan faktor pembatas yang sangat mudah diatasi (Karthik *et al*., 2005). Fosfat adalah unsur esensial sebagai sumber energi pada banyak bentuk kehidupan. Pada sistem akuatik, fosforus juga merupakan unsur penting karena merupakan unsur esensial untuk produksi primer (Boyd, 1995). Pupuk yang mengandung fosfor sebaiknya diberikan dalam bentuk larutan untuk mengurangi kemungkinan kontak langsung pupuk dengan tanah yang dapat berdampak pada pengikatan fosfat oleh aluminium dan besi.

Selain perbaikan tanah, rekayasa tambak tepat dapat pula mengoptimumkan produksi di tambak tanah sulfat masam. Untuk mengurangi masuknya asam-asam organik dari pematang ke dalam tambak pada saat hujan (terutama setelah panas yang lama), maka pada tambak tanah sulfat masam sebaiknya pematang diberi berm dan ditanami rumput. Penanaman rumput pada pematang ini juga dapat mengurangi erosi pematang, namun jangan membiarkan rumput tumbuh dalam air tambak yang dapat mengganggu pengelolaan tambak. Secara umum, pematang tambak di Desa Gentung telah ditumbuhi rumput, sehingga erosi dan pencucian tanah dari pematang sudah dapat dikurangi. Mempertahankan tinggi air yang sama dalam petakan tambak yang berdekatan dan mengusahakan air dalam petakan tambak lebih tinggi dari yang ada dalam saluran untuk mengurangi masuknya unsur atau senyawa beracun ke dalam petakan tambak secara peresapan melalui pematang.

KESIMPULAN DAN SARAN

Tanah tambak di Desa Gentung, Kecamatan Labakkang, Kabupaten Pangkep didominasi oleh tanah sulfat masam yang dicirikan dengan kemasaman dan kandungan unsur toksik yang tinggi dengan tekstur tanah lempung berpasir dan pasir berlempung. Sumber air laut bagi pertambakan berasal dari Selat Makassar melalui Sungai Gentung dan saluran sekunder dan tersier yang berfungsi sebagai saluran pemasukan dan sekaligus sebagai saluran pengeluaran air tambak. Penyakit udang windu yang berhasil diidentifikasi adalah bintik putih yang diakibatkan oleh WSSV (*White Spot Syndrome Virus*) yang menyerang tambak Kontrol dan tambak *Surveillance* dan yang mengkerdilkan atau IHHNV (*Infectious Hypodermal and Hematopoietic Necrosis Virus*) yang menyerang satu petak tambak BMP (Best management Pracitices). Peubah kualitas tanah yang

memiliki kemiripan dengan pola distribusi tambak yang udangnya mengalami kematian adalah pH_{KCl}, S_{POS}, TSA, TPA, pirit, bahan organik dan tekstur tanah yang menunjukkan bahwa peubah kualitas tanah tersebut memberikan pengaruh tidak langsung terhadap kematian udang windu. Oleh karena tanah yang dominan adalah tanah sulfat masam yang tergolong tanah bermasalah, maka disarankan untuk dilakukan upaya perbaikan tanah terlebih dahulu berupa remediasi baik dalam bentuk pengoksidasian dan pembilasan tanah maupun dengan pengapuran.

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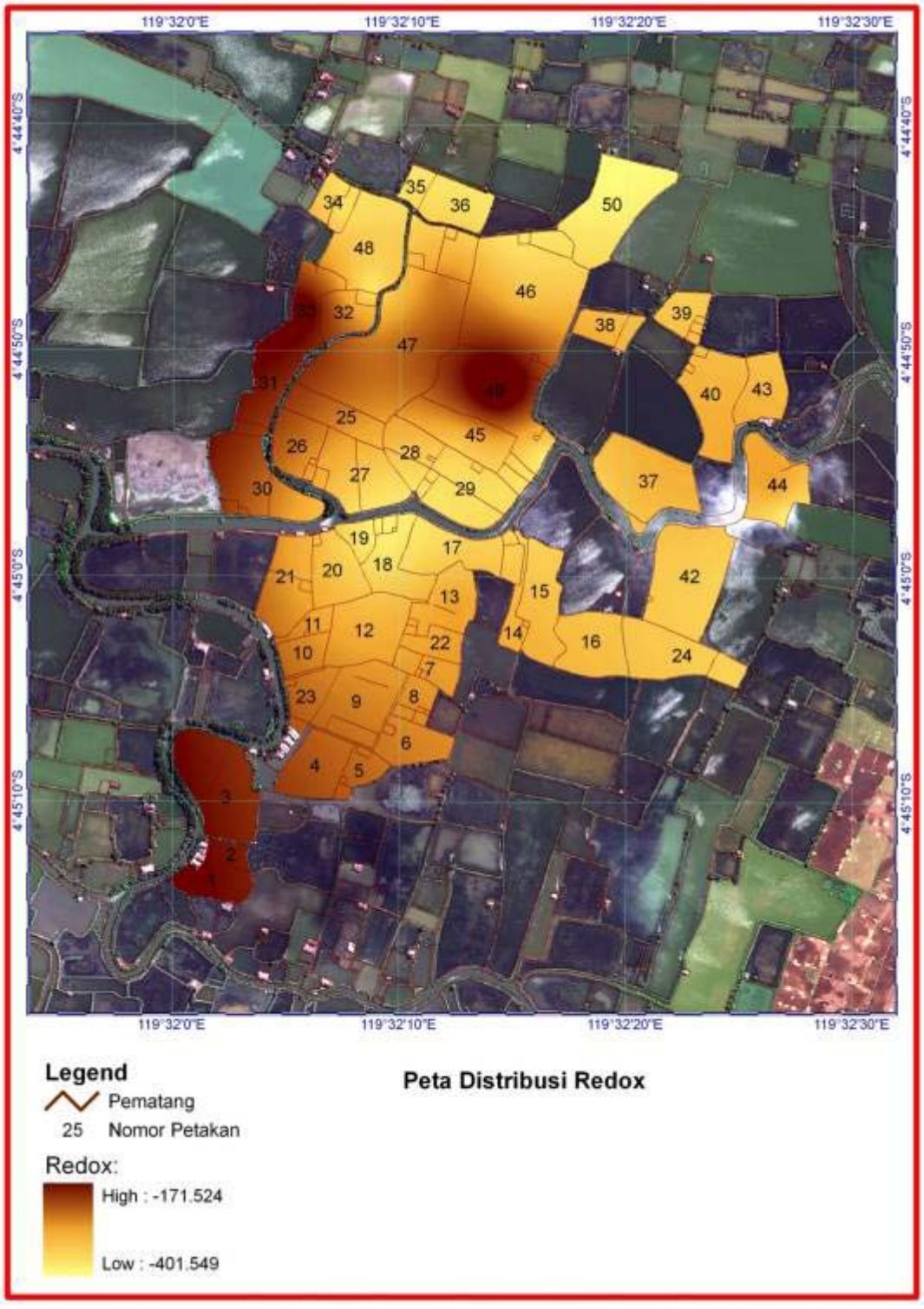
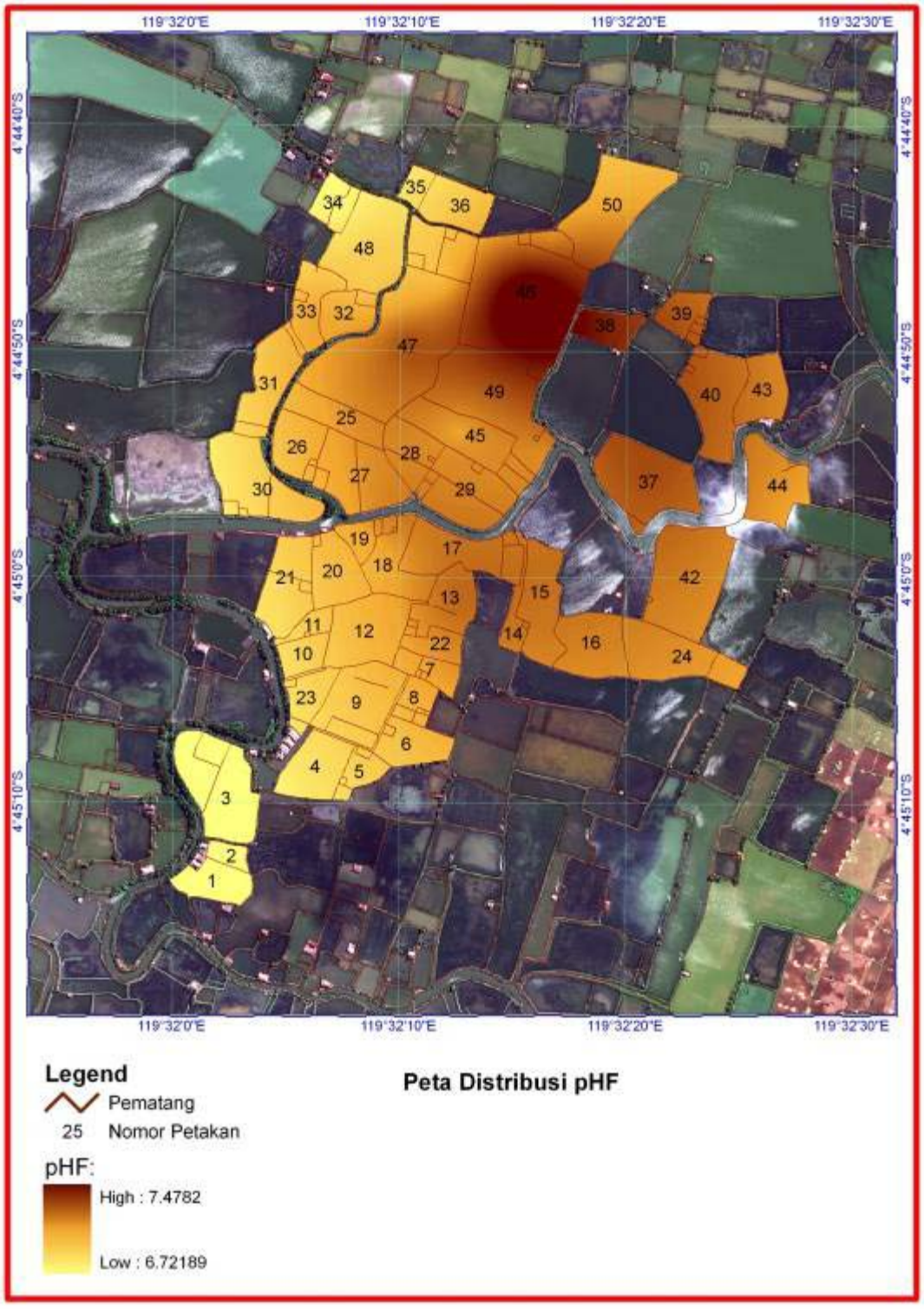
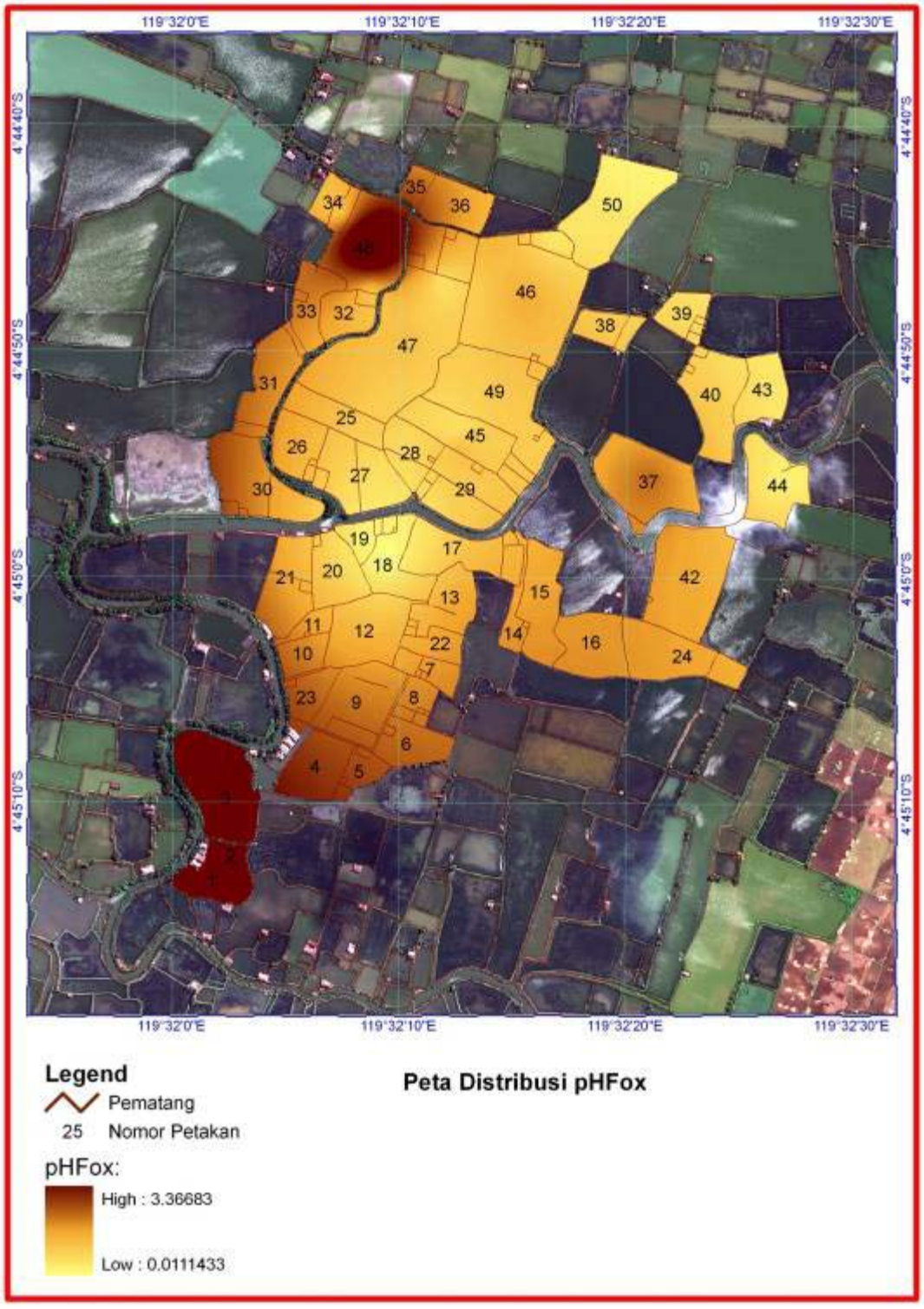


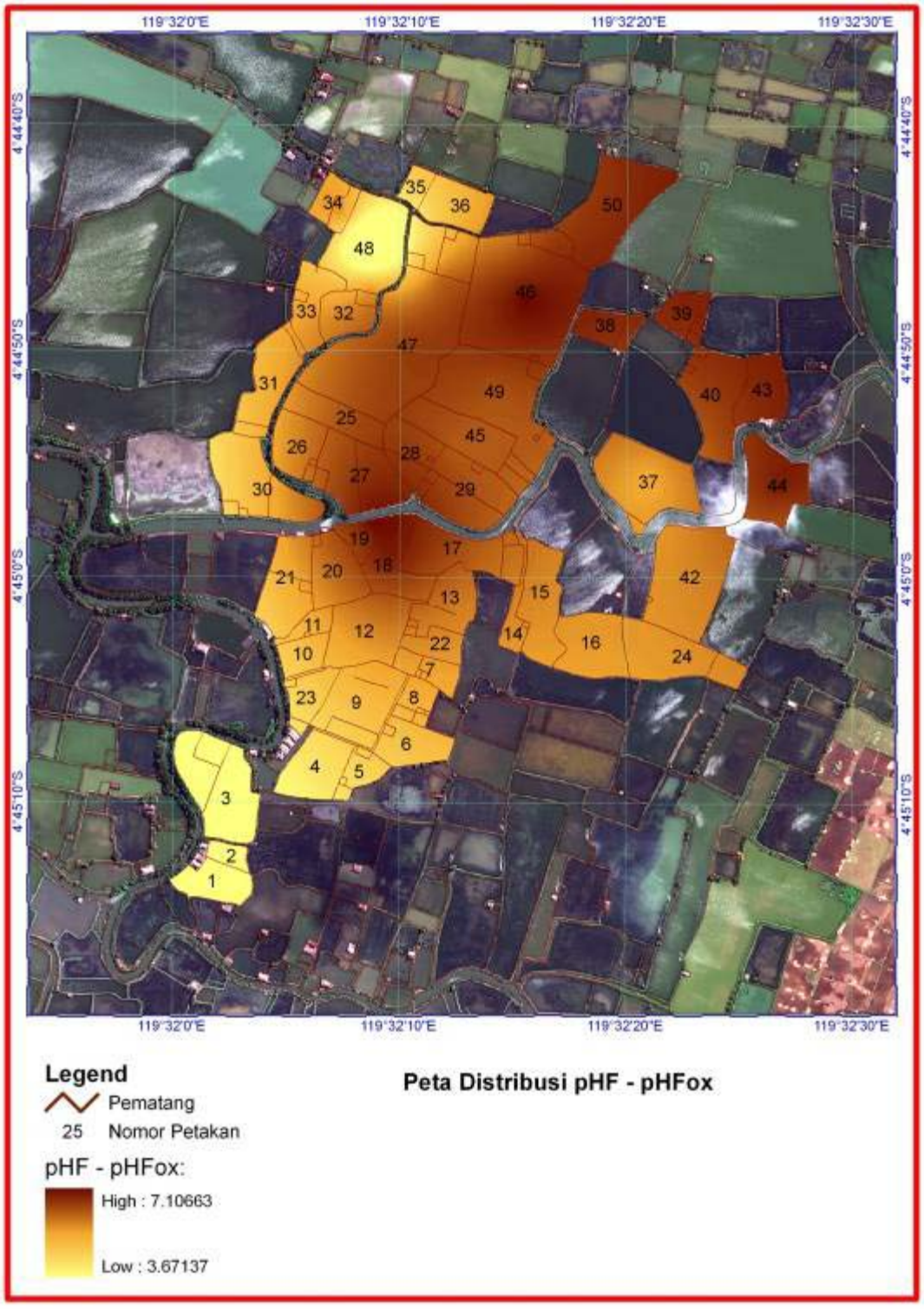
Lampiran Gambar 1. Peta distribusi potensial redoks (mV) tanah pada kedalaman 0-0,25 m di kawasan pertambakan Desa Gentung, Kecamatan Labakkang, Kabupaten Pangkep



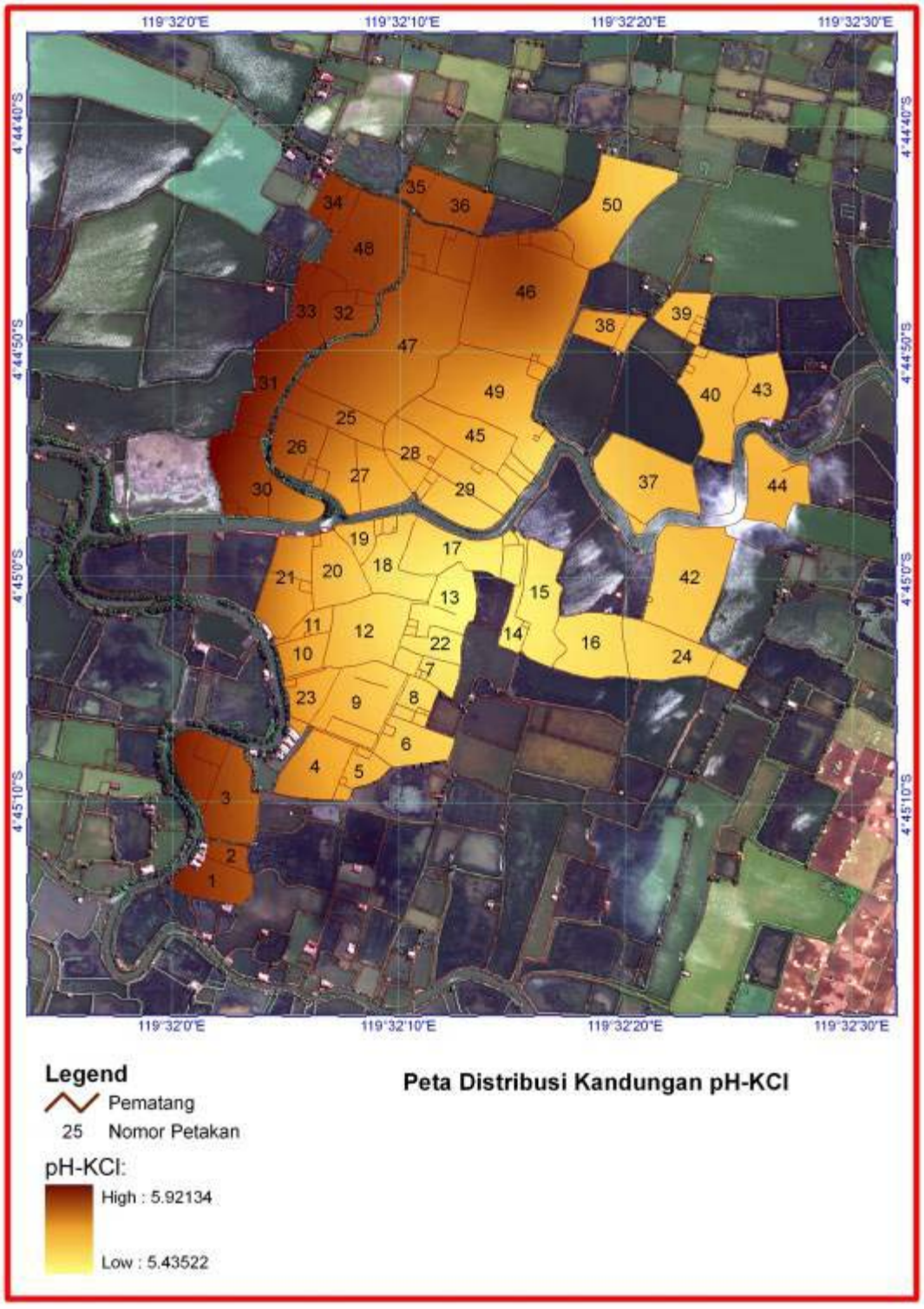
Lampiran Gambar 2. Peta distribusi pH_F tanah pada kedalaman 0-0,25 m di kawasan pertambakan Desa Gentung, Kecamatan Labakkang, Kabupaten Pangkep



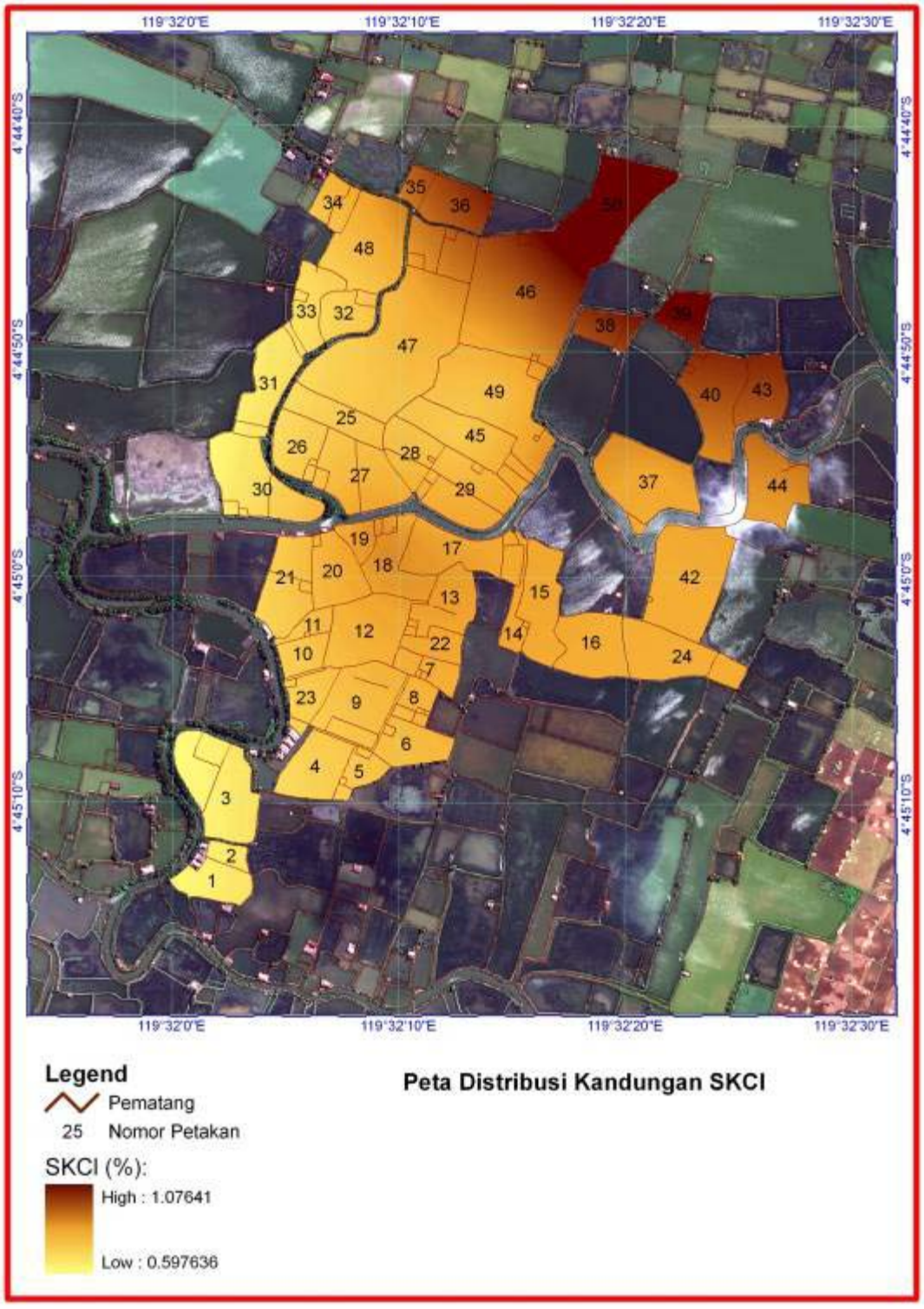
Lampiran Gambar 3. Peta distribusi pH_{FOX} tanah pada kedalaman 0-0,25 m di kawasan pertambakan Desa Gentung, Kecamatan Labakkang, Kabupaten Pangkep



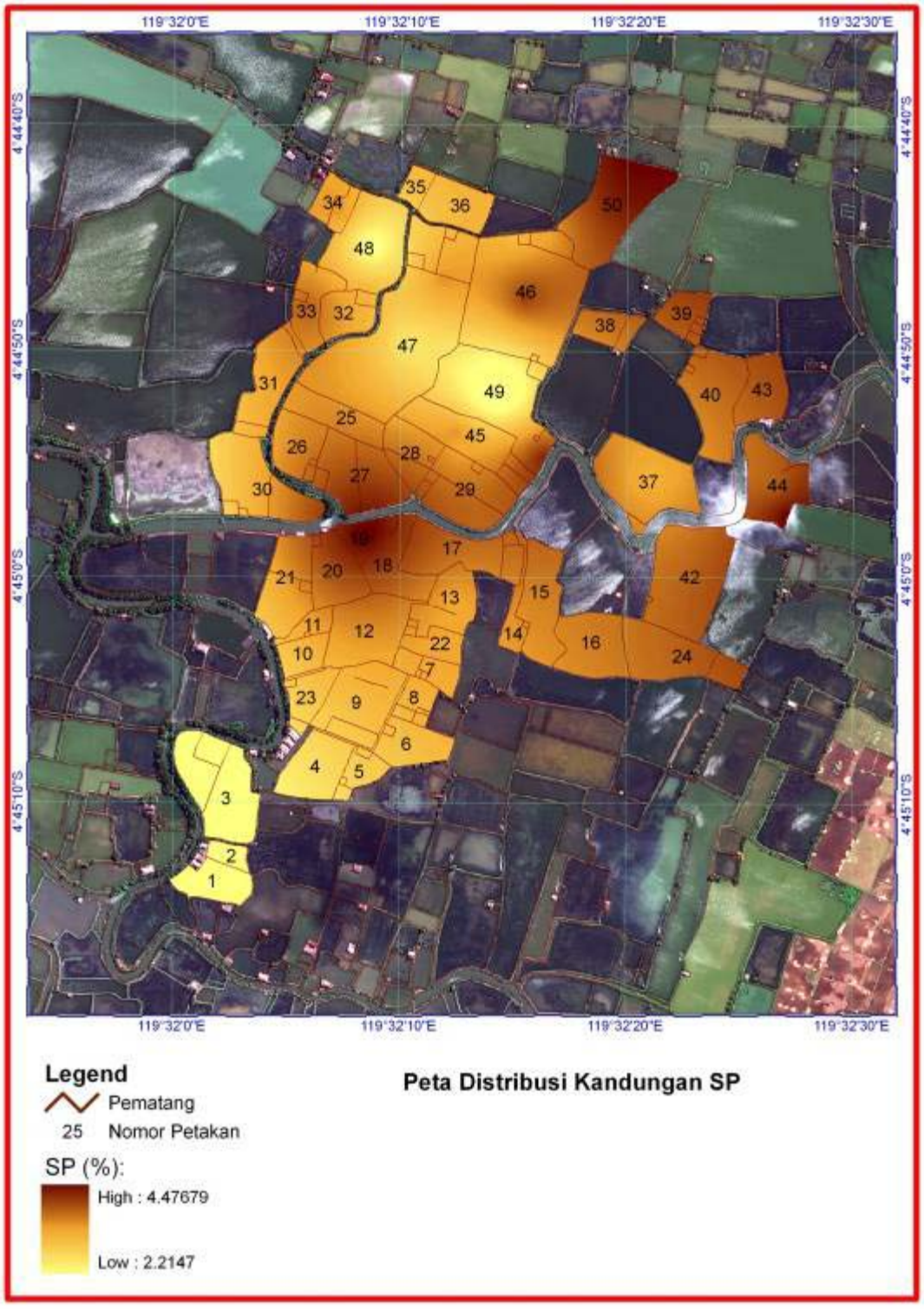
Lampiran Gambar 4. Peta distribusi pH_F-pH_{FOX} tanah pada kedalaman 0-0,25 m di kawasan pertambakan Desa Gentung, Kecamatan Labakkang, Kabupaten Pangkep



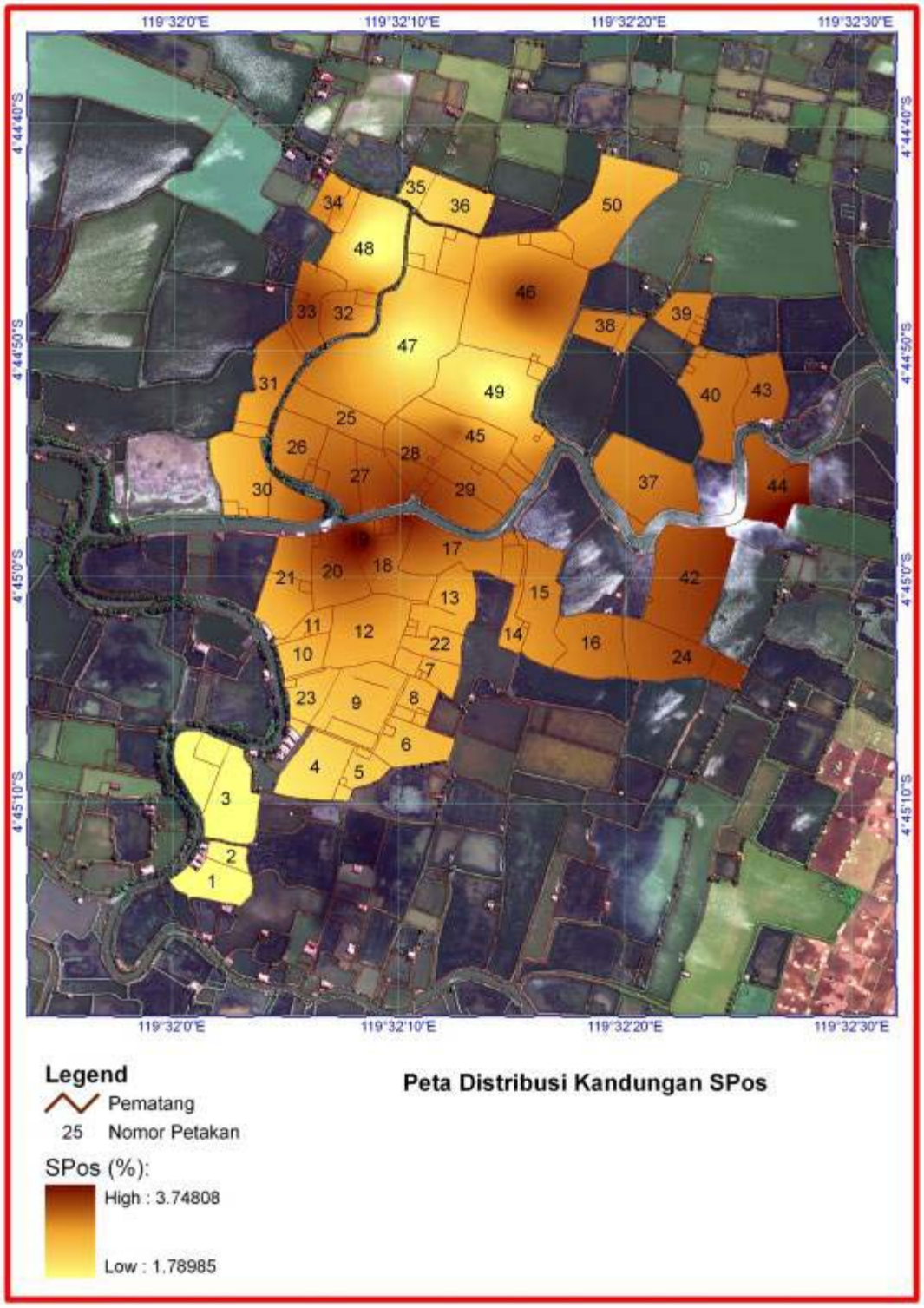
Lampiran Gambar 5. Peta distribusi pH_{KCl} tanah pada kedalaman 0-0,25 m di kawasan pertambakan Desa Gentung, Kecamatan Labakkang, Kabupaten Pangkep



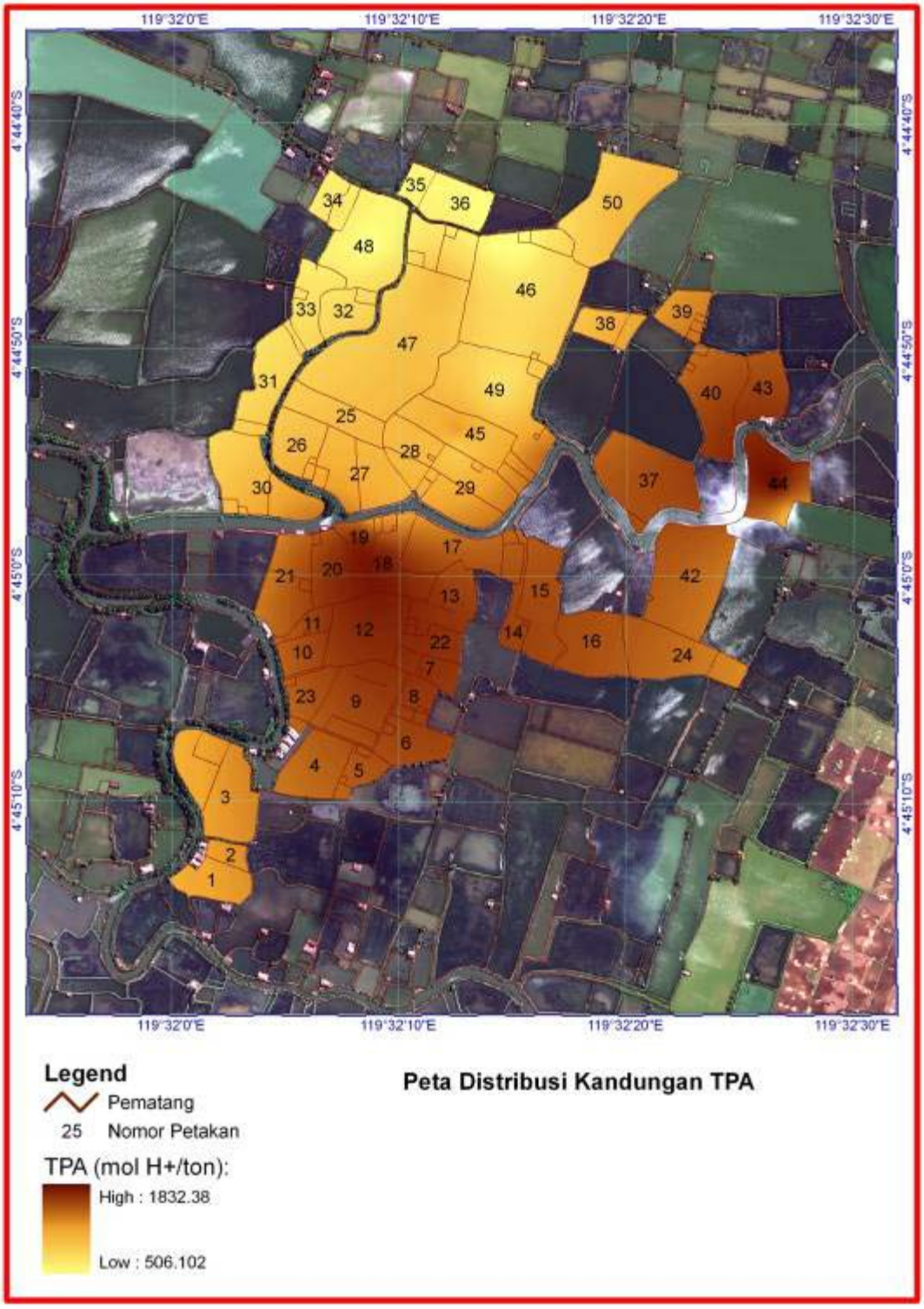
Lampiran Gambar 6. Peta distribusi S_{KCl} (%)tanah pada kedalaman 0-0,25 m di kawasan pertambakan Desa Gentung, Kecamatan Labakkang, Kabupaten Pangkep



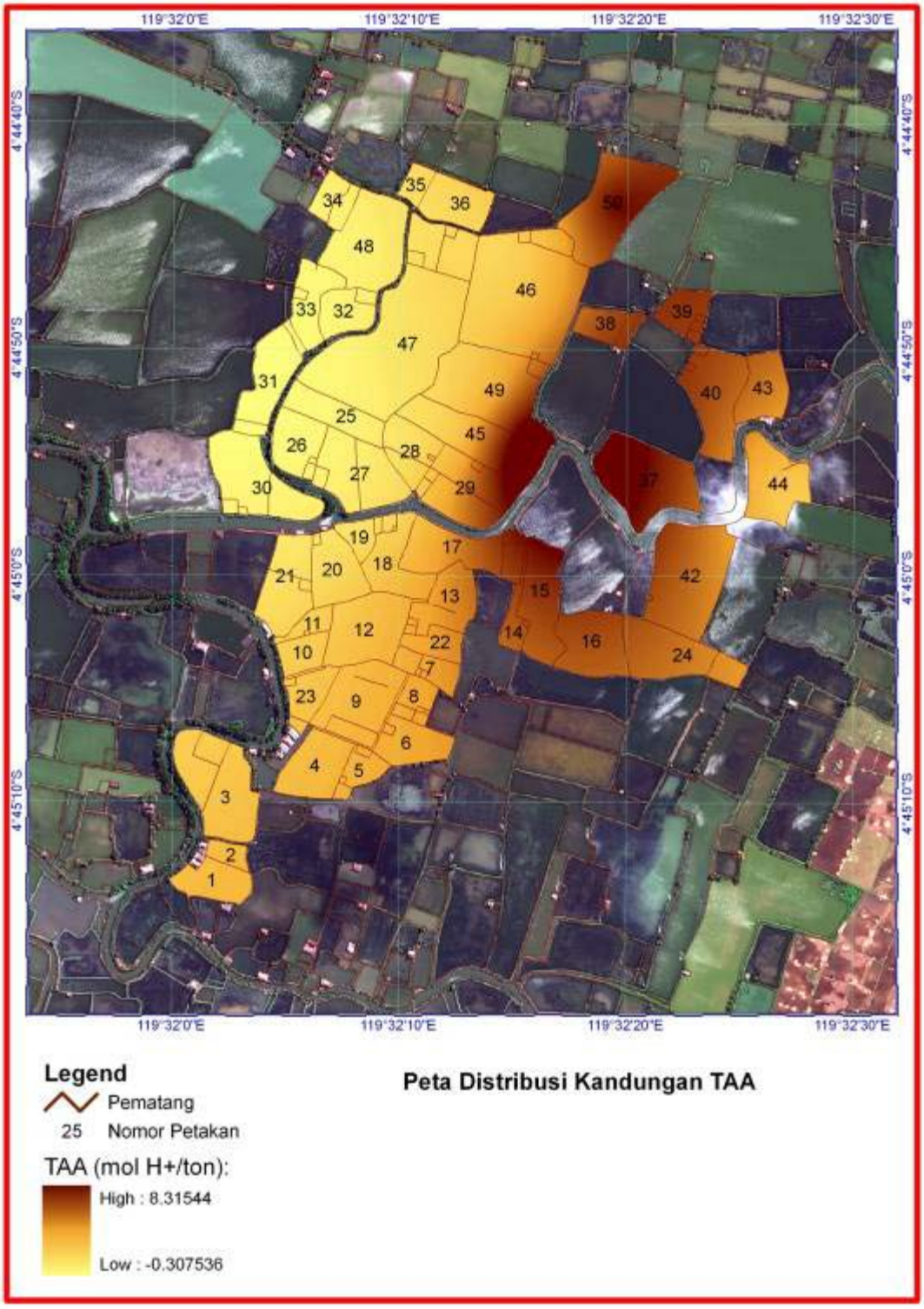
Lampiran Gambar 7. Peta distribusi S_P (%)tanah pada kedalaman 0-0,25 m di kawasan pertambakan Desa Gentung, Kecamatan Labakkang, Kabupaten Pangkep



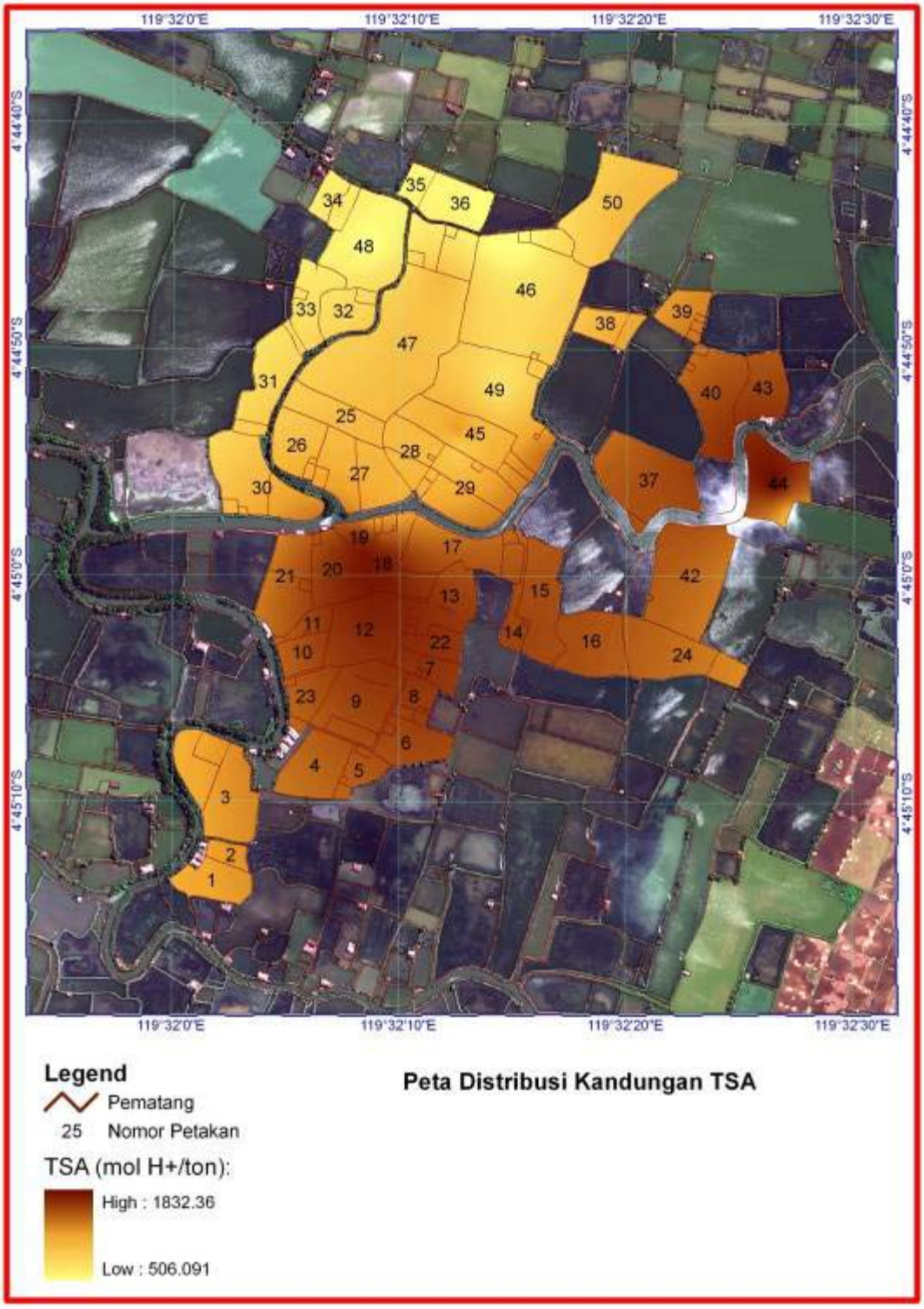
Lampiran Gambar 8. Peta distribusi S_{POS} (%)tanah pada kedalaman 0-0,25 m di kawasan pertambakan Desa Gentung, Kecamatan Labakkang, Kabupaten Pangkep



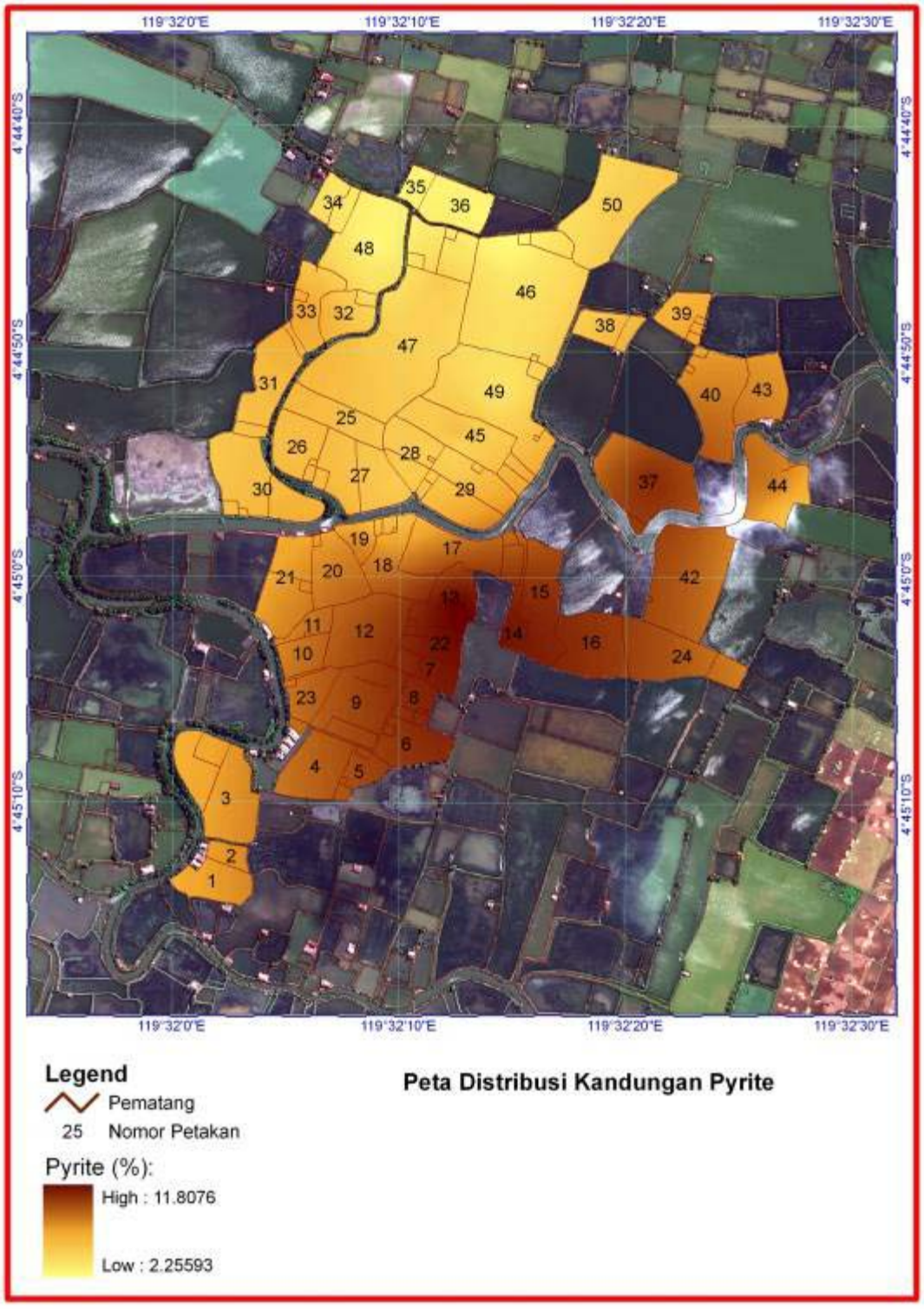
Lampiran Gambar 9. Peta distribusi TPA (mol H^+/ton) tanah pada kedalaman 0-0,25 m di kawasan pertambakan Desa Gentung, Kecamatan Labakkang, Kabupaten Pangkep



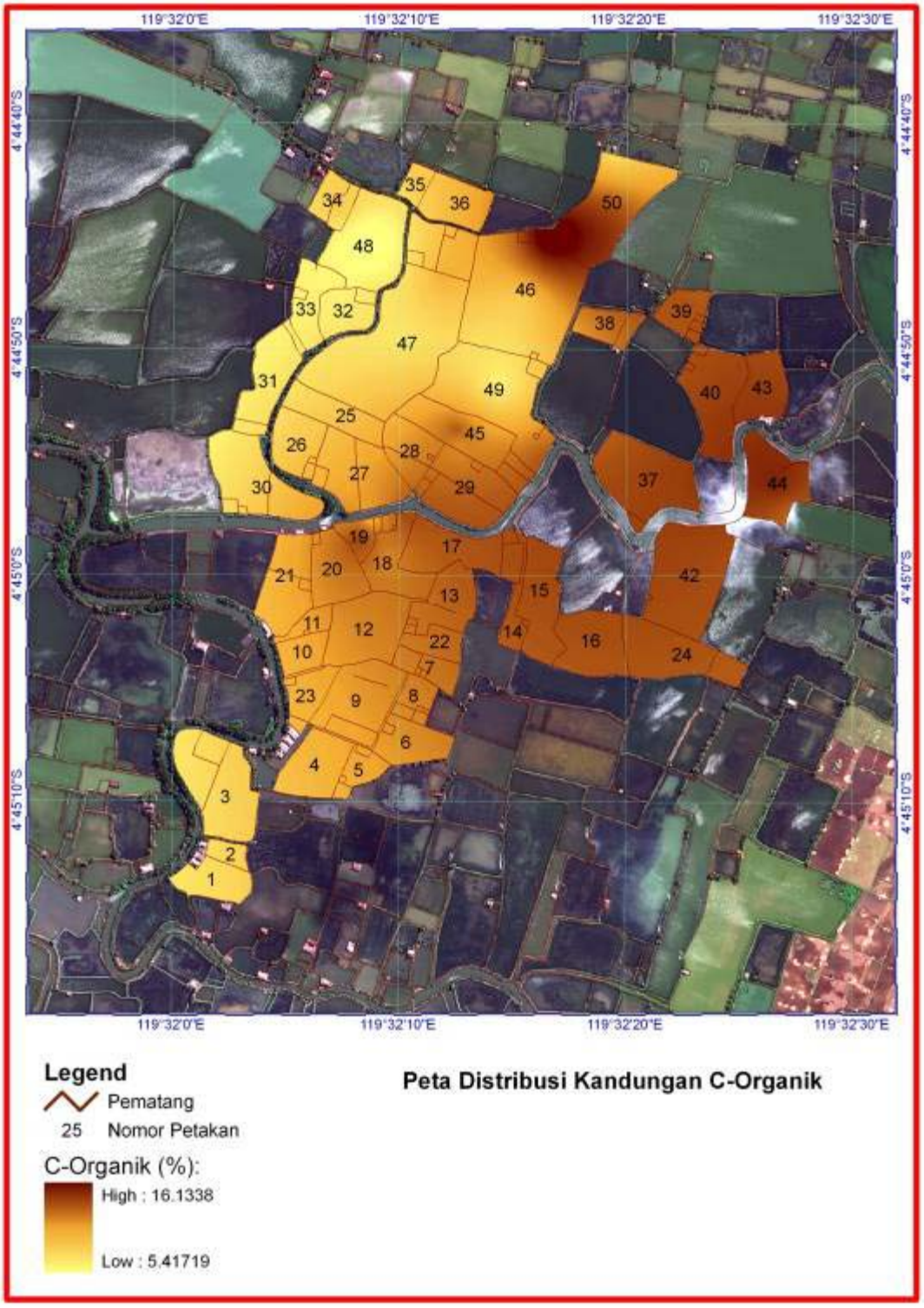
Lampiran Gambar 10. Peta distribusi TAA (mol H^+/ton) tanah pada kedalaman 0-0,25 m di kawasan pertambakan Desa Gentung, Kecamatan Labakkang, Kabupaten Pangkep



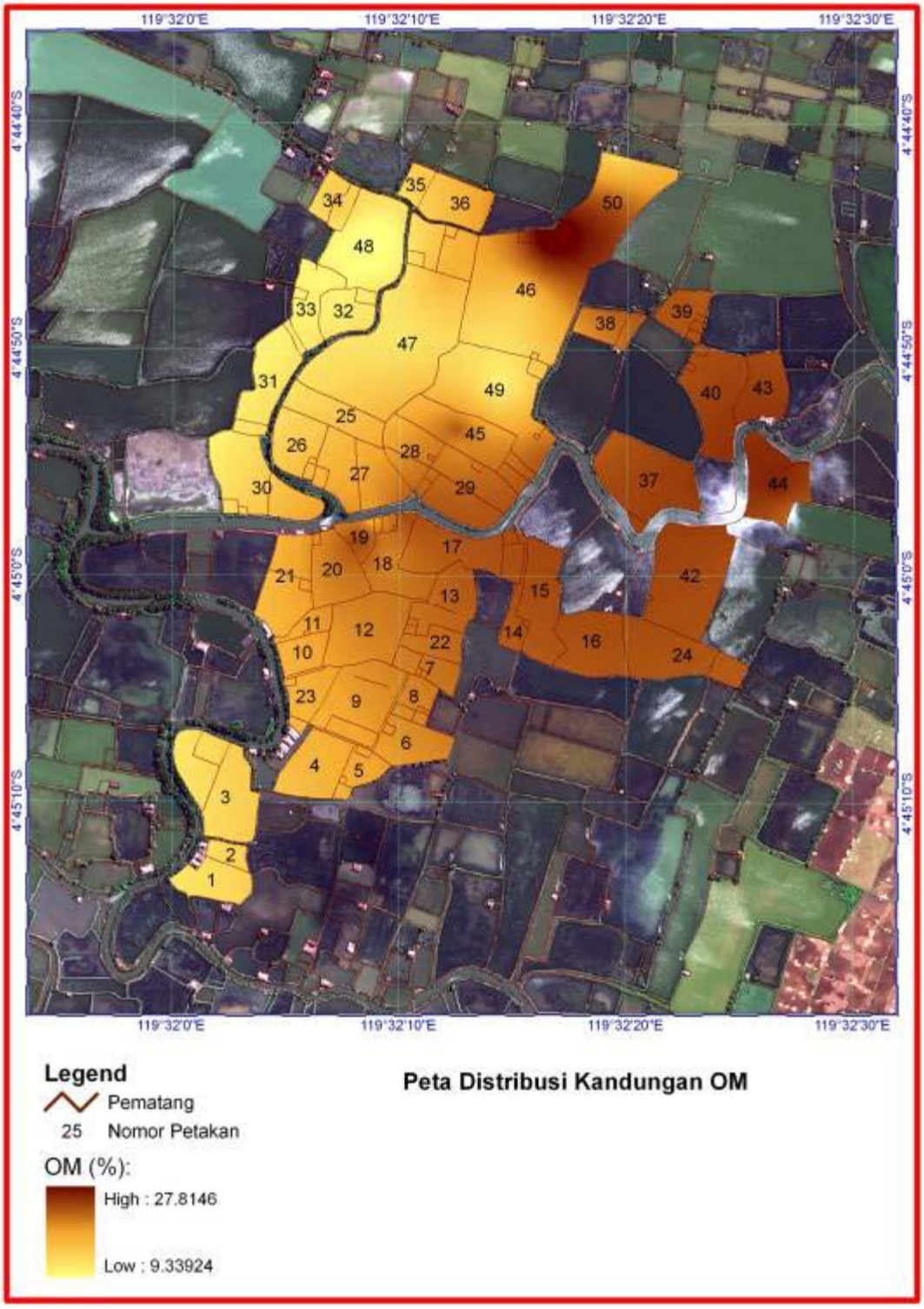
Lampiran Gambar 11. Peta distribusi TSA (mol H^+/ton) tanah pada kedalaman 0-0,25 m di kawasan pertambakan Desa Gentung, Kecamatan Labakkang, Kabupaten Pangkep



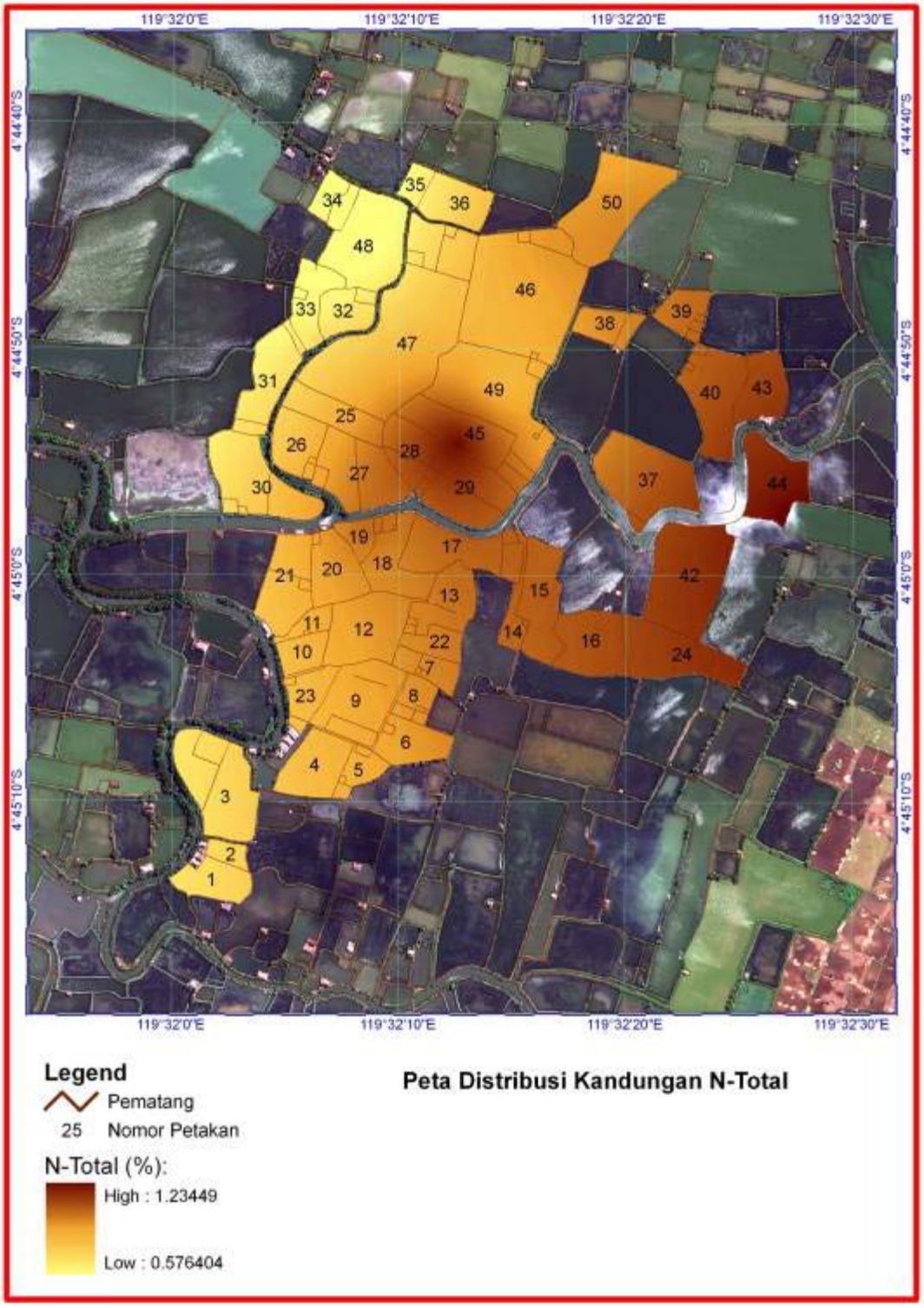
Lampiran Gambar 12. Peta distribusi pirit (%) tanah pada kedalaman 0-0,25 m di kawasan pertambakan Desa Gentung, Kecamatan Labakkang, Kabupaten Pangkep



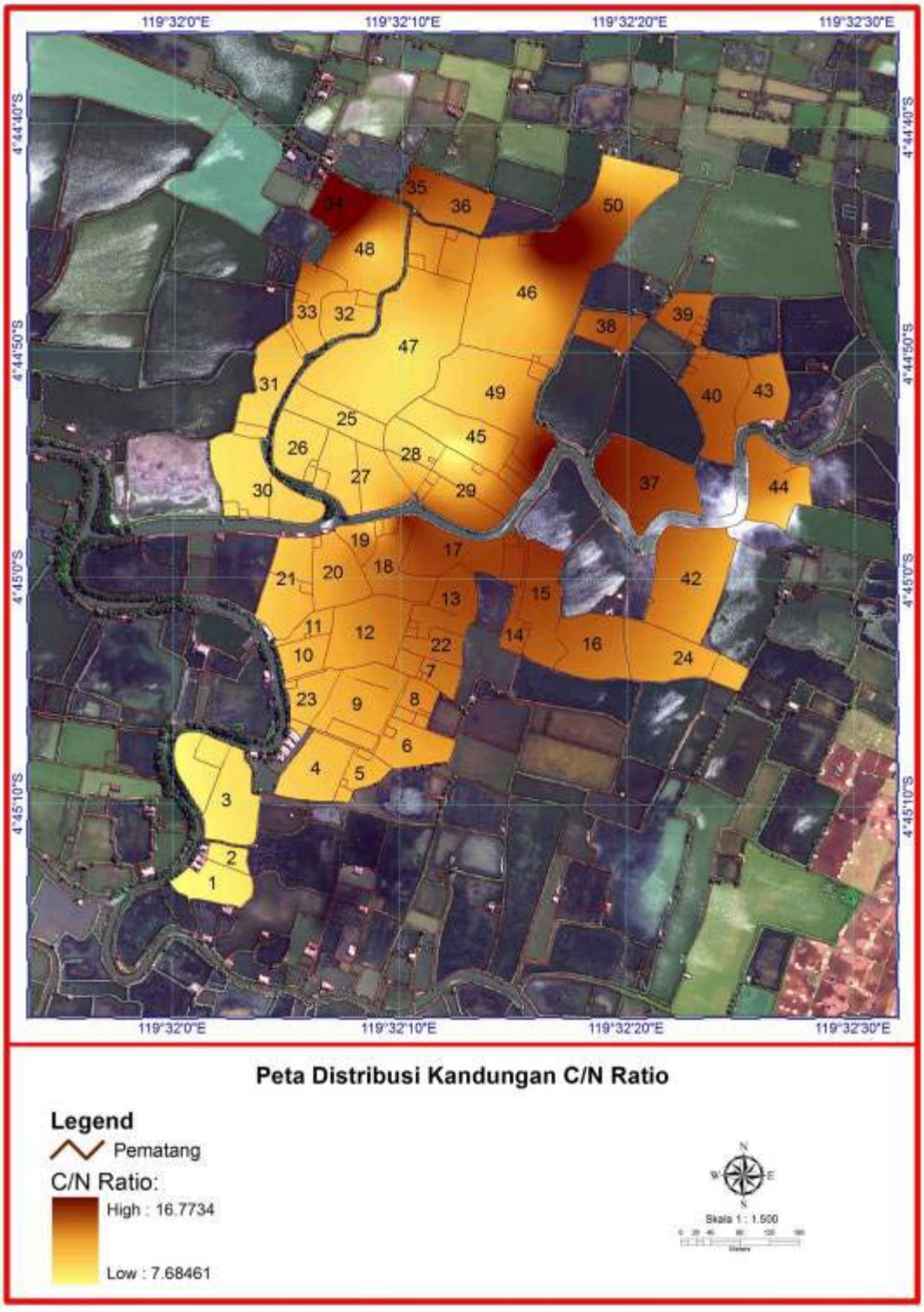
Lampiran Gambar 13. Peta distribusi karbon organik (%) tanah pada kedalaman 0-0,25 m di kawasan pertambakan Desa Gentung, Kecamatan Labakkang, Kabupaten Pangkep



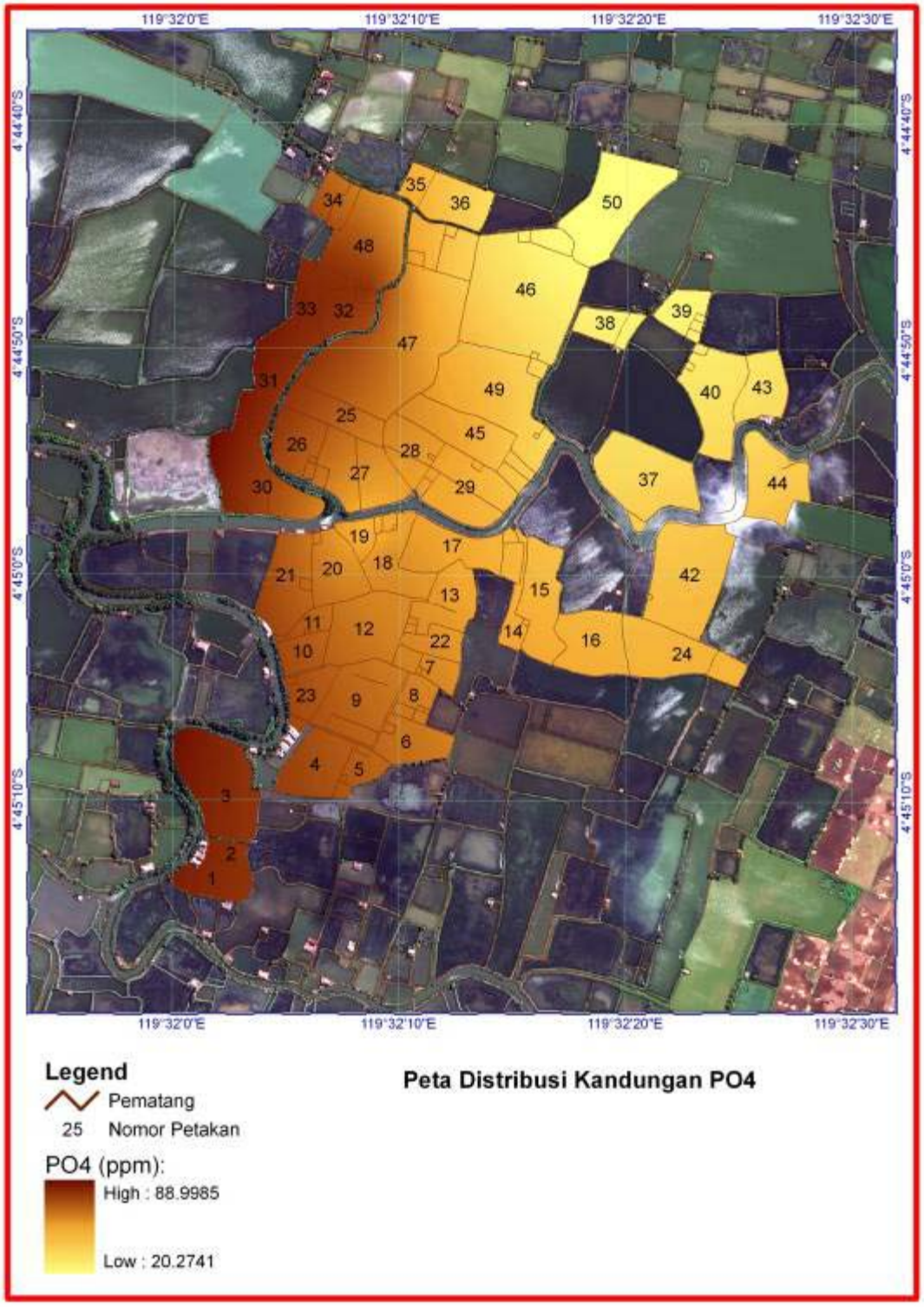
Lampiran Gambar 14. Peta distribusi bahan organik (%) tanah pada kedalaman 0-0,25 m di kawasan pertambakan Desa Gentung, Kecamatan Labakkang, Kabupaten Pangkep



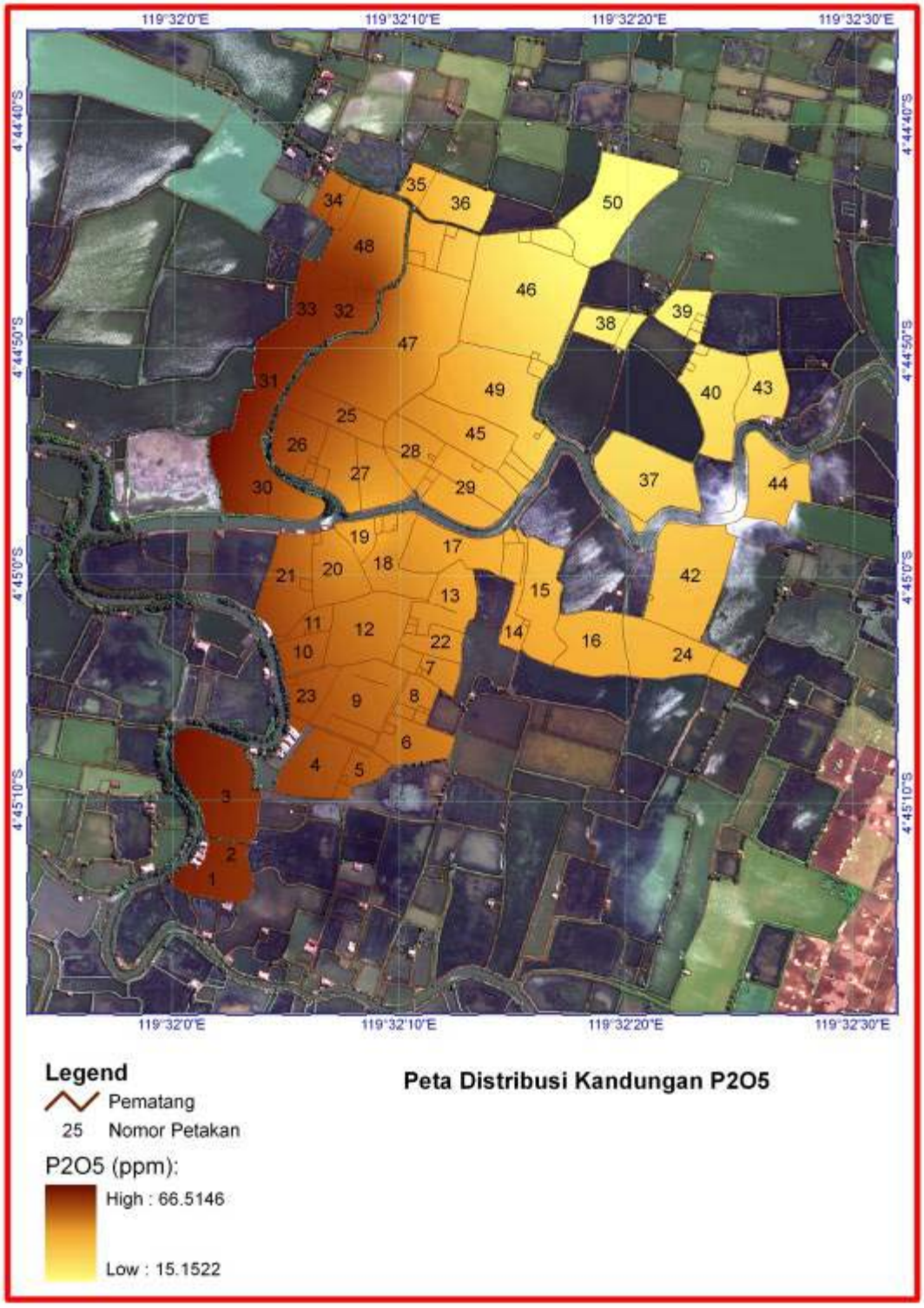
Lampiran Gambar 15. Peta distribusi nitrogen-total (%) tanah pada kedalaman 0-0,25 m di kawasan pertambakan Desa Gentung, Kecamatan Labakkang, Kabupaten Pangkep



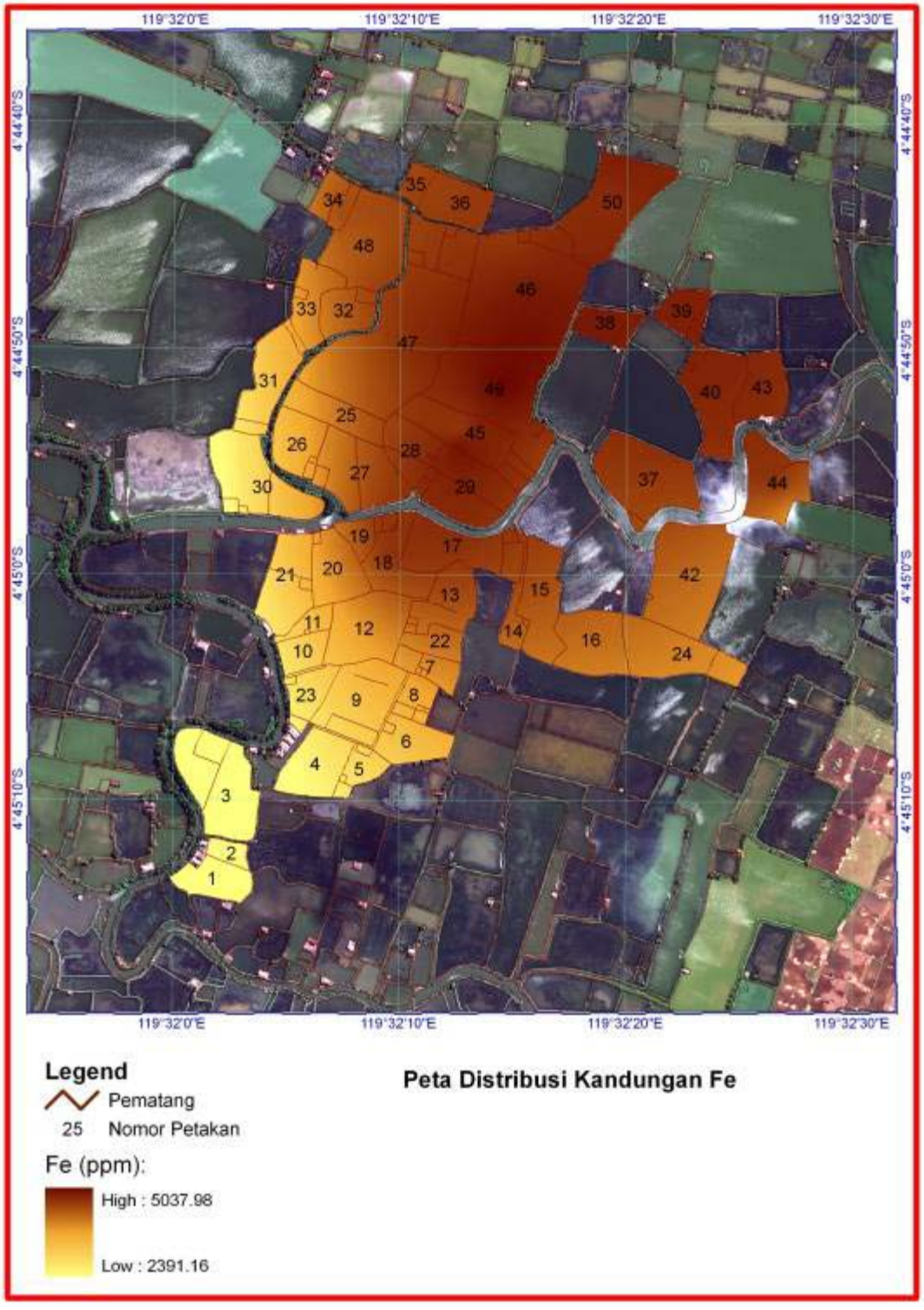
Lampiran Gambar 16. Peta distribusi rasio C:N tanah pada kedalaman 0-0,25 m di kawasan pertambakan Desa Gentung, Kecamatan Labakkang, Kabupaten Pangkep



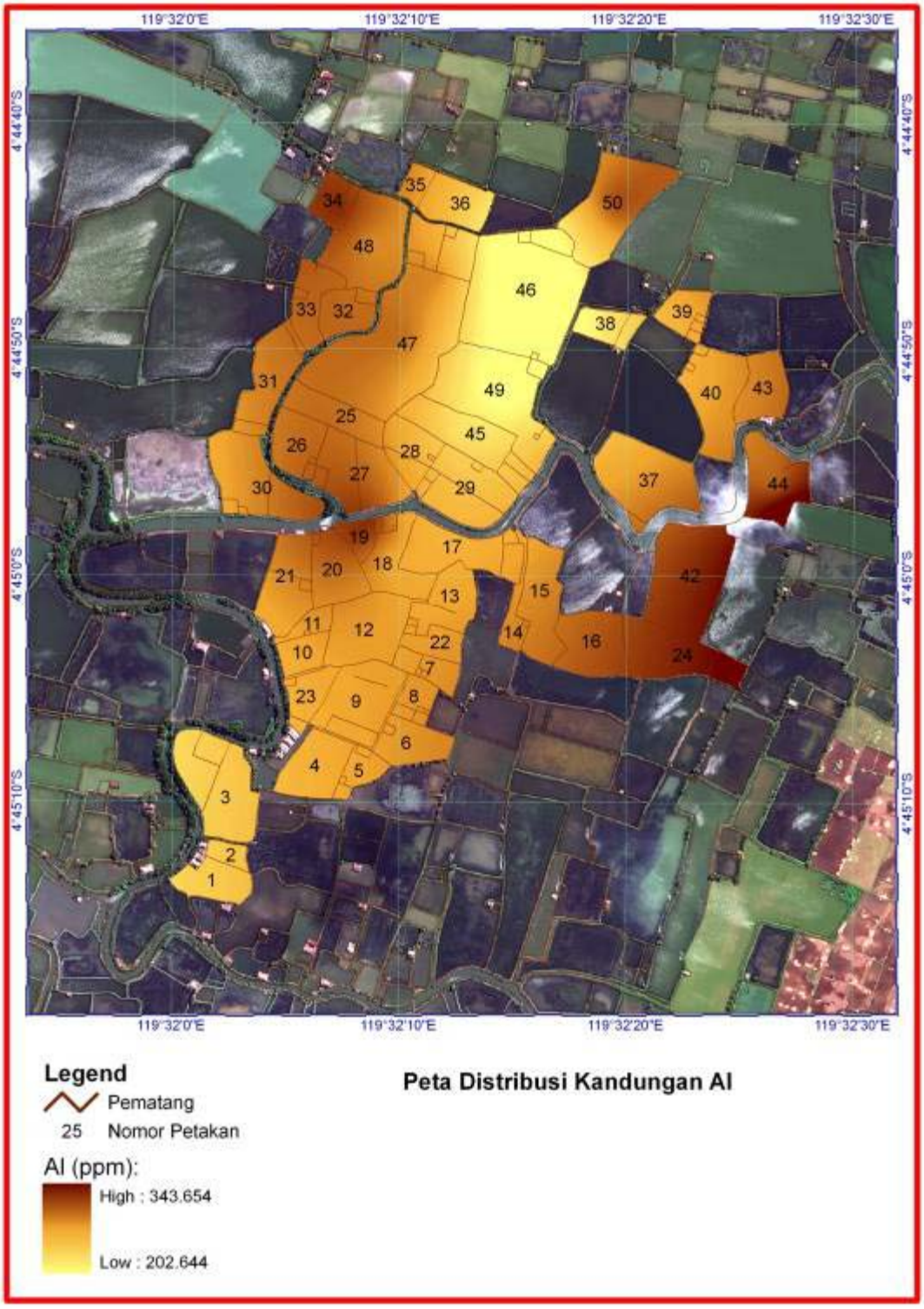
Lampiran Gambar 17. Peta distribusi PO_4 (ppm) tanah pada kedalaman 0-0,25 m di kawasan pertambakan Desa Gentung, Kecamatan Labakkang, Kabupaten Pangkep



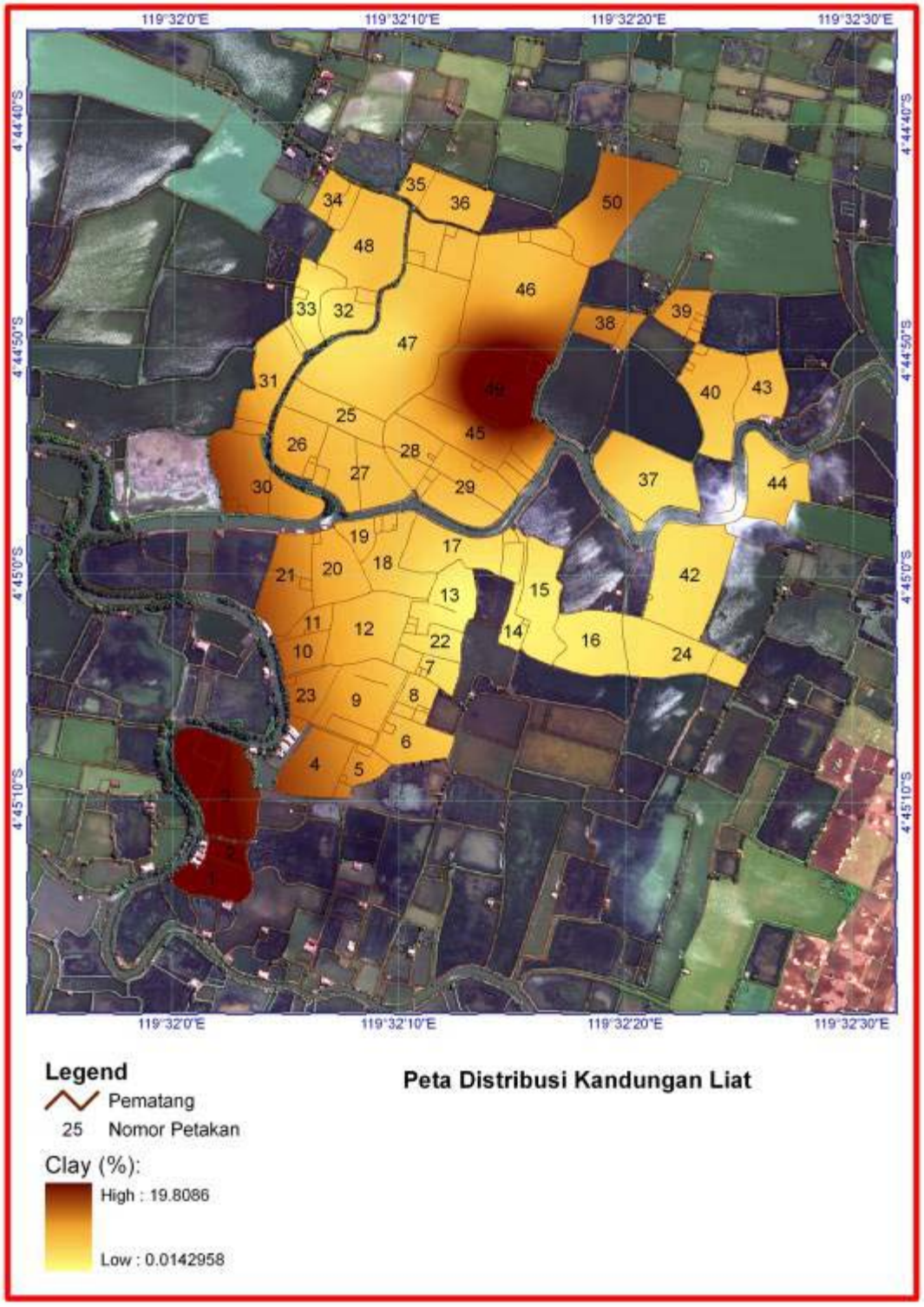
Lampiran Gambar 18. Peta distribusi P_2O_5 (ppm) tanah pada kedalaman 0-0,25 m di kawasan pertambakan Desa Gentung, Kecamatan Labakkang, Kabupaten Pangkep



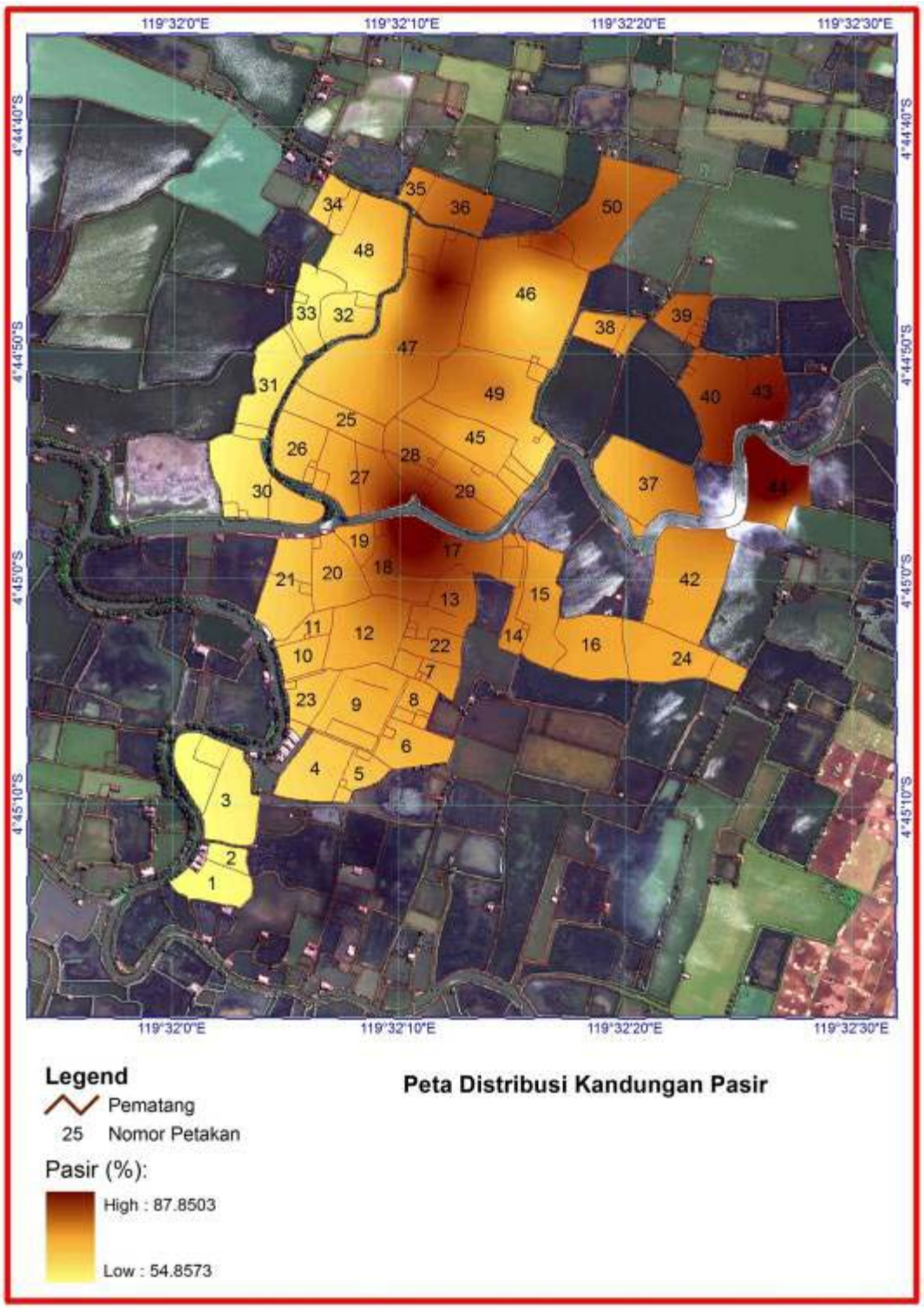
Lampiran Gambar 19. Peta distribusi besi atau Fe (ppm) tanah pada kedalaman 0-0,25 m di kawasan pertambakan Desa Gentung, Kecamatan Labakkang, Kabupaten Pangkep



Lampiran Gambar 20. Peta distribusi aluminium atau Al (ppm) tanah pada kedalaman 0-0,25 m di kawasan pertambakan Desa Gentung, Kecamatan Labakkang, Kabupaten Pangkep



Lampiran Gambar 21. Peta distribusi fraksi liat (%) tanah pada kedalaman 0-0,25 m di kawasan pertambakan Desa Gentung, Kecamatan Labakkang, Kabupaten Pangkep



Lampiran Gambar 22. Peta distribusi fraksi pasir (%) tanah pada kedalaman 0-0,25 m di kawasan pertambakan Desa Gentung, Kecamatan Labakkang, Kabupaten Pangkep

Lampiran Table 1. Matriks korelasi Pearson antarpeubah kualitas tanah tambak kedalaman 0-0,25 m di Desa Gentung, Kecamatan Labakkang, Kabupaten Pangkep

Peubah		Potensial redoks	pH_F	pH_{FOX}	pH_F - pH_{FOX}	pH_{KCl}	TPA	TAA	TSA	S_{KCl}	S_P	S_{POS}	Bahan organik	N-total	Rasio C:N	Pirit	Fe	Al	PO_4	Pasir	Liat	Debu
Potensial redoks	KP	1,000																				
	Sig.	0,000																				
pH_F	KP	0,211	1,000																			
	Sig.	0,355	0,000																			
pH_{FOX}	KP	0,190	0,156	1,000																		
	Sig.	0,386	0,478	0,000																		
pH_F - pH_{FOX}	KP	-0,114	0,198	-0,937**	1,000																	
	Sig.	0,605	0,365	0,000	0,000																	
pH_{KCl}	KP	-0,326	-0,213	-0,265	0,188	1,000																
	Sig.	0,129	0,330	0,221	0,389	0,000																
TPA	KP	-0,129	-0,096	-0,501*	0,463*	-0,045	1,000															
	Sig.	0,557	0,665	0,015	0,026	0,838	0,000															
TAA	KP	-0,213	-0,109	-0,127	0,088	0,016	0,267	1,000														
	Sig.	0.328	0,621	0,564	0,691	0,943	0,219	0,000														
TSA	KP	-0,127	-0,095	-0,501*	0,464*	-0,045	1,000**	0,257	1,000													
	Sig.	0,563	0,668	0,015	0,026	0,837	0,000	0,237	0,000													
S_{KCl}	KP	-0,480*	-0,083	-0,220	0,189	0,206	0,207	0,519*	0,202	1,000												
	Sig.	0,020	0,707	0,312	0,387	0,344	0,344	0,011	0,356	0,000												
S_P	KP	-0,606**	-0,020	-0,574**	0,562**	0,082	0,528**	0,276	0,526**	0,500*	1,000											
	Sig.	0,002	0,926	0,004	0,005	0,709	0,010	0,202	0,010	0,015	0,000											
S_{POS}	KP	-0,365	0,036	-0,506*	0,515*	-0,044	0,462*	-0,037	0,464*	-0,112	0,804**	1,000										
	Sig.	0,087	0,872	0,014	0,012	0,843	0,026	0,865	0,026	0,610	0,000	0,000										

Bahan organik	KP	-0,428*	-0,150	-0,607**	0,550**	0,099	0,497*	0,213	0,496*	0,082	0,507*	0,526*	1,000								
	Sig.	0042	0,495	0,002	0,007	0,654	0,016	0,330	0,016	0,711	0,014	0,010	0,000								
N-total	KP	-0,306	0,000	-0,400	0,397	0,384	0,118	-0,133	0,119	-0,016	0,355	0,422*	0,464*	1,000							
	Sig.	0,156	1,000	0,058	0,061	0,070	0,593	0,544	0,587	0,944	0,096	0,045	0,026	0,000							
Rasio C:N	KP	0,085	0,078	0,057	-0,029	-0,576**	0,304	0,166	0,303	0,008	-0,043	-0,059	0,136	-0,634**	1,000						
	Sig.	0,701	0,723	0,797	0,897	0,004	0,159	0,449	0,160	0,972	0,846	0,789	0,535	0,001	0,000						
Pirit	KP	-0,127	-0,095	-0,501*	0,464*	-0,046	1,000**	0,256	1,000**	0,202	0,526**	0,464*	0,496*	0,119	0,303	1,000					
	Sig.	0,564	0,668	0,015	0,026	0,836	0,000	0,238	0,000	0,356	0,010	0,026	0,016	0,587	0,160	0,000					
Fe	KP	0,155	0,213	-0,283	0,355	0,505*	-0,164	0,032	-0,165	0,119	-0,197	-0,306	-0,025	0,215	-0,353	-0,165	1,000				
	Sig.	0,481	0,330	0,191	0,096	0,014	0,455	0,884	0,453	0,589	0,367	0,156	0,910	0,324	0,098	0,452	0,000				
Al	KP	0,086	-0,078	0,067	-0,094	-0,708**	0,182	0,153	0,181	0,095	0,216	0,179	-0,079	-0,299	0,499*	0,181	-0,724**	1,000			
	Sig.	0,696	0,723	0,760	0,668	0,000	0,405	0,486	0,408	0,666	0,322	0,413	0,720	0,166	0,015	0,408	0,000	0,000			
PO_4	KP	0,057	-0,200	0,205	-0,274	0,178	-0,110	-0,353	-0,106	-0,473*	-0,320	-0,362	-0,362	-0,083	-0,146	-0,106	-0,079	-0,181	1,000		
	Sig.	0,795	0,360	0,349	0,206	0,417	0,618	0,099	0,630	0,023	0,136	0,090	0,090	0,706	0,506	0,629	0,720	0,408	0,000		
Pasir	KP	-0,037	-0,104	-0,452*	0,411	0,115	0,394	-0,136	0,397	0,145	0,096	-0,362	0,532**	0,192	0,107	0,397	0,219	-0,159	-0,233	1,000	
	Sig.	0,868	0,636	0,030	0,051	0,601	0,063	0,536	0,061	0,509	0,968	0,090	0,009	0,380	0,626	0,061	0,315	0,469	0,285	0,000	
Liat	KP	0,292	-0,088	-0,141	0,109	0,393	-0,225	0,108	-0,227	-0,036	-0,361	-0,386	-0,157	0,077	-0,267	-0,227	0,587**	-0,413*	0,014	0,009	1,000
	Sig.	0,177	0,691	0,522	0,622	0,064	0,301	0,622	0,297	0,869	0,091	0,069	0,474	0,727	0,219	0,297	0,003	0,050	0,950	0,969	0,000
Debu	KP	-0,090	0,131	0,467*	-0,417*	-0,270	-0,260	0,077	-0,262	-0,115	0,066	0,156	-0,414*	-0,206	0,016	-0,262	-0,446*	0,318	0,204	-0,906**	-0,431*
	Sig.	0,682	0,550	0,025	0,048	0,212	0,231	0,727	0,228	0,600	0,765	0,479	0,050	0,346	0,942	0,228	0,033	0,139	0,350	0,000	0,040

Keterangan:

KP = Korelasi Pearson

Sig. = Signifikansi

* = Korelasi nyata pada taraf 0,05

** = Korelasi nyata pada taraf 0,01

9.1 Appendix 2: Pond variables collected

Summary of pond variables collected:

Variable	Description	Code/units	Type of variable
OUTCOME VARIABLE			
WSD outbreak	Whether the pond was determined to have had a mortality event caused by WSD	0 = no, 1 = yes	Categorical: ordinal
EXPLANATORY VARIABLES			
50 PONDS			
Pond characteristics			
BMP status BMP binary	Which BMP protocol applied to the study pond	1 = Full BMP, 2 = Basic BMP, 3 = no BMP 3 = no BMP, 4 = BMP (full+ basic)	Categorical: nominal
Pond area	Pond area	hectares	Measurement: continuous
DOP	Exit date minus Stocking date = days of production	days	Measurement: discrete
Soil variables			
Redox	Reducing/oxidising potential	1 = low, 2 = medium, 3 = high [6]	Categorical: ordinal
PhF	Field pH	1 = low, 2 = medium, 3 = high	Categorical: ordinal
Pyrite		1 = low, 2 = medium, 3 = high	Categorical: ordinal
C organic	Organic carbon	1 = low, 2 = medium, 3 = high	Categorical: ordinal
Organic matter		1 = low, 2 = medium, 3 = high	Categorical: ordinal
N total	Total nitrogen	1 = low, 2 = medium, 3 = high	Categorical: ordinal
CN ratio	Carbon : nitrogen ratio	1 = low, 2 = medium, 3 = high	Categorical: ordinal
PO4	Phosphate	1 = low, 2 = medium, 3 = high	Categorical: ordinal
Fe	Iron	1 = low, 2 = medium, 3 = high	Categorical: ordinal
Clay		1 = low, 2 =	Categorical: ordinal

[6] *Note: low – high is only relative to the range of that variable within the ponds studied NOT relative to normal functional range for producing shrimp*

		medium, 3 = high	
Sand		1 = low, 2 = medium, 3 = high	Categorical: ordinal
Shares bank	Shares an embankment with a pond that has been affected with WSD within the last 7 days	0 = no, 1 = yes	Categorical: ordinal
Shares high bank	Shares a high sand embankment with a pond that has been affected with WSD within the last 7 days	0 = no, 1 = yes	Categorical: ordinal
Water flow variables			
Primary canal	Water intake from same primary canal system (including dependent secondary or tertiary canals) as previously affected WSD pond(s)	0 = no, 1 = yes	Categorical: ordinal
Secondary canal	Water intake from same secondary canal system (including dependent tertiary canal) as previously affected WSD pond(s)	0 = no, 1 = yes	Categorical: ordinal
Tertiary canal	Water intake from same tertiary canal system as previously affected WSD pond(s)	0 = no, 1 = yes	Categorical: ordinal
Distance to outbreak	Distance to closest WSD outbreak pond via canal system while exposed pond still holding shrimp for at least 7 days post outbreak (m)	metres	Measurement: continuous
9 PONDS			
Pond preparation			
Trench prep ox	Post pond preparation oxidised surface layer >1cm deep at representative sites in trench	0 = no, 1 = yes	Categorical: ordinal
Plat prep ox	Post pond preparation oxidised surface layer >1cm deep at representative sites in plateau	0 = no, 1 = yes	Categorical: ordinal
MP removed	Macrophtyes removed	0 = no, 1 = yes	Categorical: ordinal
Trench prep pH	Post preparation soil pH in trench	pH	Measurement: continuous
Plat prep pH	Post preparation soil pH in plateau	pH	Measurement: continuous
Trench prep org	Post preparation organic matter in trench	%	Measurement: continuous
Plat prep org	Post preparation organic matter in plateau	%	Measurement: continuous
Water quality			
Trench water depth	Depth of water in the trench	centimetres	Measurement: continuous
Transparency	Transparency of pond water	centimetres	Measurement:

			continuous
Salinity	Salinity of pond water	parts per thousand (ppt)	Measurement: continuous
DO sunrise	Pond water dissolved oxygen at sunrise	parts per million (ppm)	Measurement: continuous
DO 1700	Pond water dissolved oxygen at 1700 hrs	parts per million (ppm)	Measurement: continuous
pH sunrise	Pond water pH at sunrise	pH	Measurement: continuous
pH 1700	Pond water pH at 1700 hrs	pH	Measurement: continuous
Temp sunrise	Pond water temperature at sunrise	degrees Celsius	Measurement: continuous
Temp 1700	Pond water temperature at 1700 hrs	degrees Celsius	Measurement: continuous
Alkalinity	Pond water alkalinity	parts per million (ppm)	Measurement: continuous
Trench ox	Depth of oxidised surface layer at representative site in trench	millimetres (mm)	Measurement: continuous
Plat ox	Depth of oxidised surface layer at representative site in plateau	millimetres (mm)	Measurement: continuous
MP live	Live macrophyte abundance	0 = none, 1 = low, 2 = moderate, 3 = high	Categorical: ordinal
MP dead	Dead macrophyte abundance in plateau	0 = none, 1 = low, 2 = moderate, 3 = high	Categorical: ordinal
NON-EXPLANATORY VARIABLES			
50 PONDS			
Pond ID	Pond ID	Consecutive whole numbers	Categorical: nominal
Stocking day	Day pond stocked with PLs (4 May 2010 = 0)	days	Measurement: discrete
Exit day	Last day of shrimp harvest from pond (4 May 2010 = 0)	days	Measurement: discrete
Type of exit	Reason for ending cropping period	nh = normal harvest, eh = emergency harvest, cf = crop failure, nheh = normal harvest then emergency harvest, nhcf = normal harvest then crop failure.	Categorical: nominal
Shrimple test (disease outbreak investigation ponds only)	Shrimple test result for ponds with mortality events	0 = negative, 1 = positive	Categorical: ordinal

PCR at exit	PCR test result at final shrimp harvest	0 = negative, 1 = positive	Categorical: ordinal
Nursery used	Nursery used to acclimatise shrimp before grow-out pond	0 = no, 1 = yes	Categorical: ordinal
Water release	A WSD affected pond releases untreated water into the canal system within 7 days of a WSD outbreak	0 = no, 1 = yes	Categorical: ordinal
Productivity			
Total harvest	Total harvest of shrimp from all harvest events during the crop	Kg	Measurement: continuous
Productivity	Total harvest/Pond area	Kg/ha	Measurement: continuous
Survival	Number of shrimp stocked/number of shrimp harvested x 100	%	Measurement: continuous
9 PONDS			
Profitability			
Total prod cost	Total production costs excluding family labour	IDR = Indonesian rupiah	Measurement: continuous
Family cost	Family labour costs	IDR = Indonesian rupiah	Measurement: continuous
Total revenue	Revenue shrimp + revenue milkfish + revenue tilapia	IDR = Indonesian rupiah	Measurement: continuous
Revenue shrimp		IDR = Indonesian rupiah	Measurement: continuous
Revenue milkfish		IDR = Indonesian rupiah	Measurement: continuous
Revenue tilapia		IDR = Indonesian rupiah	Measurement: continuous
Profit		IDR = Indonesian rupiah	Measurement: continuous
Profit per ha	Profit per hectare	IDR = Indonesian rupiah	Measurement: continuous

9.1 Appendix 3: Soil data summary

Soil study data summary

Soil quality at 0-0.25m depth in ponds in Gentung Village, District Labakkang, Pangkep regency, South Sulawesi Province:

Variable	Minimum	Maximum	Mean	Standard deviation
Redox (mV)	-401	-170	-344	58
pH_F	6.78	7.96	7.10	0.246
pH_{FOX}	0.01	3.39	0.60	0.693
pH_F-pH_{FOX}	3.65	7.11	6.49	0.698
Pyrite (%)	2.24	8.19	4.94	1.642
C-Organic (%)	5.39	16.20	10.84	2.583
Organic matter (%)	9.30	27.93	18.68	4.453
N-total (%)	0.12	1.49	0.93	0.293
C:N ratio	7.66	96.64	15.09	17.922
PO_4 (ppm)	11.58	134.66	45.82	31.760
Fe (ppm)	4317.00	5040.00	4730.17	169.852
Al (ppm)	138.50	593.50	307.57	120.755
Sand (%)	58.00	88.00	70.87	8.286
Clay (%)	0.00	20.00	4.70	3.890
Texture	Sandy loam (17/23 samples) and argillaceous sand (6/23 samples)			

9.1 Appendix 4: RVC abstract

Student Research Team Expedition Report 'Better Management Practices in Indonesian Shrimp Farming' (2011) Royal Veterinary College, London

Abstract

The Royal Veterinary College Student Research Team (RVC SRT) is a student-led group interested in researching the effect of animal disease in developing countries by working with farmers to support sustainable farming.

The SRT 2011 team focused on sustainability in shrimp farming in Asia, an industry that has boomed over the last decade, increasing worldwide concerns regarding responsible aquaculture. To target these issues, key organisations developed 'Better Management Practices' (BMPs), a series of practical measures that can be adopted by shrimp farmers to improve productivity and farm responsibly.

The team visited a cluster of 50 extensive shrimp farms during a month-long stay in South Sulawesi, Indonesia, interviewed over 30 farmers and assessed the pond water quality. The goal of the project was to establish why there had been a low level adoption of BMPs recently, to determine whether BMP compliance was associated with reduced disease occurrence and to establish whether extensive shrimp farming had an effect on local water quality.

The preliminary findings do not support the impact of social background or variable costs in shrimp production confounding the low level of BMP status farms, but highlight concerns that, without a structured means of promoting BMPs, incorrect procedures may be adopted. Our results also show that a number of factors are associated with fewer disease occurrences, including overall BMP compliance, certain farming practices and inversely to years of farming experience. Compliance with certain components of BMPs were found to be important in the farmer population investigated. Environmental findings conclude that the waterways and ponds had a sub-optimal water quality, with evidence of eutrophication. Quality of water in BMP farms was shown to be higher, which, correlating well with water monitoring practices used on these farms results in earlier detection of problems.

The findings will help to ascertain the support still required by the small-scale shrimp impact and the effectiveness of health management strategies.