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Mekong River Commission

Biomonitoring of the lower Mekong River and selected tributaries, 2004–2007

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Meeting the Needs, Keeping the Balance

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Editors: B.C. Chessman, V.H. Resh and T.J. Burnhill
Graphic design: T.J. Burnhill

184 Fa Ngoum Road, Unit 18, Ban Sithane Neua, Sikhottabong District,
Vientiane 01000, Lao PDR
Telephone: (856-21) 263 263 Facsimile: (856-21) 263 264
E-mail: mrcs@mrcmekong.org
Website: www.mrcmekong.org

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Abbreviations and acronyms

ATSPI	Average Tolerance Score Per Individual
ATSPT	Average Tolerance Score Per Taxon
BDP	Basin Development Programme of the MRC
DO	Dissolved Oxygen
EC	Electrical Conductivity
MRC	Mekong River Commission
MRCS	Mekong River Commission Secretariat
NTU	Nephelometric Turbidity Units
SDS	Site Disturbance Score

Glossary of biomonitoring terms

Abundance: This is a measurement of the number of individual plants or animals belonging to a particular biological indicator group counted in a sample. Low species abundance is sometimes a sign that the ecosystem has been harmed.

Benthic macroinvertebrates: In this report, the use of this term refers to animals that live in the deeper parts of the riverbed and its sediments, well away from the shoreline. Because many of these species are not mobile, benthic macroinvertebrates respond to local conditions and, because some species are long living, they may be indicative of environmental conditions that are long standing.

Biological indicator group: These are groups of animals or plants that can be used to indicate changes to aquatic environments. Members of the group may or may not be related in an evolutionary sense. So while diatoms are a taxon that is related through evolution, macroinvertebrates are a disparate group of unrelated taxa that share the character of not having a vertebral column, or backbone. Different biological indicator groups are suitable for different environments. Diatoms, zooplankton, littoral and benthic macroinvertebrates, and fish are the most commonly used biological indicator groups used in aquatic freshwater environments. In addition, although not strictly a biological group, planktonic primary productivity can also be used as an indicator. However, for a number of logistical reasons fish and planktonic primary production are not suitable for use in the Mekong.

Diatom: Single celled microscopic algae (plants) with a cell wall made of silica. They drift or float in the river water (planktic/planktonic) or are attached to substrate such as rocks on the riverbed and aquatic plants growing in the river (benthic/benthonic). They are important primary producers in the aquatic food chain and are an important source of food for many invertebrate animals. Diatoms are a diverse group that respond in many ways to physical and chemical changes to the riverine environment. Because, they have a short generation time diatom populations respond rapidly to changes in the environment.

Environmental variables: These are chemical and physical parameters that were recorded at each sampling site at the same time as samples for biological indicator groups were collected. The parameters include, altitude, water transparency and turbidity, water temperature, concentration of dissolved oxygen (DO), electrical conductivity (EC), acidity (pH), and concentrations of chlorophyll-a, as well as the physical dimensions of the river at the site.

Littoral macroinvertebrates: In this report, the use of this term refers to animals that live on, or close to, the shoreline of rivers and lakes. They are the group of animals that are most widely used in biomonitoring exercises worldwide. They are often abundant and diverse and are found in a variety of environmental conditions. For these reasons littoral macroinvertebrates are good biological indicators of environmental changes.

Littoral organisms: Those organisms that live near the shores of rivers, lakes, and the sea.

Macroinvertebrate: An informal name applied to animals that do not have a vertebral column, including snails, insects, spiders, and worms, which are large enough to be visible to the naked eye. Biomonitoring programmes often use both benthic and littoral macroinvertebrates as biological indicators of the ecological health of water bodies.

Primary producer: Organisms at the bottom of the food chain, such as most plants and some bacteria and blue-green algae, which can make organic material from inorganic matter.

Primary production: The organic material made by primary producers. Therefore, planktonic primary production is the primary production generated by plants (including diatoms), bacteria and blue-green algae that live close to the surface of rivers lakes and the sea.

Primary productivity: The total organic material made by primary producers over a given period of time.

Reference sites: These are sampling sites that are in almost a natural state with little disturbance from human activity. To be selected as a reference site in the MRC biomonitoring programme, a site must meet a number of requirements including pH (between 6.5 and 8.5), electrical conductivity (less than 70 mS/m), dissolved oxygen concentration (greater than 5 mg/L) and average SDS (between 1 and 1.67). Reference sites provide a baseline from which to measure environmental changes.

Richness: This is a measurement of the number of taxa (types) of plants or animals belonging to a particular biological indicator group counted in a sample. Low species richness is often a sign that the ecosystem has been harmed.

Sampling sites: Sites chosen for single or repeated biological and environmental sampling. Although locations of the sites are geo-referenced, individual samples may be taken from the different habitats at the site that are suitable for particular biological indicator groups. Sites

were chosen to provide broad geographical coverage of the basin and to sample a wide range of river settings along the mainstream of the Mekong and its tributaries. There are 51 sampling sites from which 14 reference sites were selected.

Site Disturbance Score (SDS): This is a comparative measure of the degree to which the site being monitored has been disturbed by human activities, such as urban development, water resource developments, mining, and agriculture. In the MRC biomonitoring programme, the SDS is determined by a group of ecologists who attribute a score of 1 (little or no disturbance) to 3 (substantial disturbance) to each of the sampling sites in the programme after discussion of possible impacts in and near the river.

Richness: This is a measurement of the number of taxa (types) of plants or animals belonging to a particular biological indicator group counted in a sample. Low species richness is often a sign that the ecosystem has been harmed.

Taxon/taxa (plural): This is a group or groups of animals or plants that are related through evolution. Examples include species, genera, or families.

Tolerance, or Average Tolerance Score per Taxon (ATSPT): Each taxon of a biological indicator group is assigned a score that relates to its tolerance to pollution. ATSPT is a measure of the average tolerance score of the taxa recorded in a sample. A high ATSPT may indicate harm to the ecosystem, as only tolerant taxa survive under these disturbed conditions.

Zooplankton: Small or microscopic animals that drift or float near the surface of rivers, lakes, and the sea. They can be single celled or multi-cellular. They are often secondary producers that live off phytoplankton (including diatoms) or other zooplankton. Zooplankton can be useful biological indicators of the ecological health of water bodies because they are a diverse group that have a variety of responses to environmental changes. Because they have a short generation time, zooplankton populations tend to respond more rapidly to changes in the environment.

Summary

A biological monitoring programme was established for the lower Mekong River and its major tributaries by the MRC and its member nations in response to article 7 of the 1995 Agreement that established the Commission. The biomonitoring programme complements the previously established monitoring programmes on physical-chemical water quality, and helps to determine whether harmful effects on aquatic ecosystems are resulting from the development and use of the water resources of the Lower Mekong Basin.

The groups of organisms to be monitored in the programme were nominated in 2003 for their relevance to the interests of the general public, practicality of measurement in a broad-scale, routine monitoring programme, and likely sensitivity to water resources development and waste discharge, as indicated by international experience in biomonitoring over the past century. A pilot study in 2003 tested and refined the groups to be measured. As a result, diatoms, zooplankton, littoral macroinvertebrates and benthic macroinvertebrates were retained in the programme. Unfortunately, fish could not be retained for reasons of cost and logistics, but this could be re-considered in the future. Selected environmental measurements were also included in the programme to assist in interpretation of the biological data and testing of biological indicators.

Full-scale data collection with standardized methods began in 2004, when 20 sites were sampled. In 2005, 16 sites were sampled, in 2006, 21 sites, and in 2007, 20 sites. In total, 51 sites were sampled, with some sites being sampled in two or more years. All sampling was done in the dry season (March) because high water levels and rapid currents made sampling in the wet season impossible or dangerous.

Specific indicators of ecological harm were calculated for each sample of diatoms, zooplankton, littoral macroinvertebrates and benthic macroinvertebrates collected during the programme. These were richness (number of types of organisms in the sample), abundance (number of individual organisms in the sample) and average tolerance (a measure of how resistant the species in the sample are to stresses caused by humans). Because biological indicators can vary naturally as well because of human activities, data from reference sites were used to define thresholds of harm. Reference sites with low levels of development were selected from the total set of sites sampled after consideration of chemical water quality data, human activity at the site, and human activity upstream. Data from 14 reference sites were used to generate 12 interim biological guidelines, similar to the physical and chemical guidelines proposed for the MRC water quality assessment programme. Data from all sites were then compared with guideline values.

Potentially harmful effects at a sampling site were inferred if the average richness or abundance of a group of organisms was below the applicable guideline, because reduced richness or abundance can be construed as harm. For tolerance, potential harm was inferred

if the average value calculated for a site was above the applicable guideline, because a more tolerant fauna indicates a loss of sensitive species. In order to produce an overall assessment , each site was classified for each sampling occasion according to the number of guidelines met:

Class A (excellent): 10 – 12 guidelines met

Class B (good): 7 – 9 guidelines met

Class C (moderate): 4 – 6 guidelines met

Class D (poor): 0 – 3 guidelines met.

Of the 77 sampling events conducted over four years, 28 were in Class A, 32 in Class B, and 17 in Class C. None was in Class D. This rating suggests that the principal rivers of the Lower Mekong Basin have not yet suffered severe harm from the development of water resources or waste disposal. However, some rivers are showing signs of stress.

The data collected in this programme provide a basis for actions to avoid, minimise and mitigate harm to the river's ecosystems, as required by the 1995 Agreement. They also provide a sound baseline from which to monitor future change.

1. Introduction

1.1 The need for river monitoring

The people of the Lower Mekong Basin and their governments are naturally concerned about the ecological well being of the river, its major tributaries, and their associated floodplains, lakes and wetland habitats. This is because the river system supports plant and animal life on which the livelihoods and food supply of the great majority of the population of 60 million people have traditionally depended. These concerns are embedded in the 1995 Agreement that established the MRC. In particular, Article 7 of the agreement states that 'harmful effects on aquatic ecosystems resulting from the development and use of the water resources of the lower Mekong Basin, or the discharge of wastes and return flows, are to be avoided, minimised or mitigated.'

However, the governments of the four riparian countries (Cambodia, Lao PDR, Thailand, and Viet Nam) also want to alleviate poverty in their countries and to raise the standard of living of their people using the revenue gained from developing other uses of the river, such as hydropower generation, irrigated agriculture, improved navigation, and tourism. Although these new developments will inevitably change the natural state of the river system, predictions about how these modifications will affect people's livelihoods is made difficult by the complex ecological relationships among the river system, its plant and animal life, and the people who make a living from the river's resources. Therefore, governments and their line agencies need monitoring systems that will give them early warning of changes in the ecology of the river, so that they can take remedial action if it is necessary.

The MRC, acting on behalf of its member states, already has routine monitoring systems in place for hydrology and climate (water level, flow, and rainfall) and water quality (the chemical and physical properties of the river water, including natural and man-made pollutants). These systems are designed for regional-scale monitoring reflecting the MRC's remit to address issues that cross the national borders of its member states. However, there was no routine biological monitoring of the Mekong River system prior to the programme described in this paper.

1.2 The value of biological monitoring

Biological monitoring, or biomonitoring, of fresh waters began in Germany at the start of the 20th century (Rosenberg and Resh, 1993). Routine, broad-scale biomonitoring has been well established in Australia, Europe, Japan and North America for 20–30 years (Bonada *et al*., 2006; Carter *et al*., 2006 a, b; Ziglio *et al*., 2006). More recently, biomonitoring has expanded into developing countries, where it has been advocated because its relatively low cost and the ability of biomonitoring to involve local populations in decision making (Resh, 1995, 2007).

Biomonitoring provides a third type of monitoring that complements physical and chemical monitoring (Campbell, 2007). Biomonitoring provides important additional information because plants and animals are sensitive to a wide range of environmental factors, including many that are not practical to measure routinely in physical and chemical monitoring programmes. Biomonitoring can therefore provide an indication of environmental problems that are not detected by physical and chemical monitoring.

In addition, plants and animals are affected by episodic or intermittent pollution that may not be present at the times when physical and chemical sampling takes place. Populations of animals and plants that are sensitive to pollution take time to recover after pollutants have dispersed, and so are indicative of water quality in the recent past as well as quality at the time of sampling. For this reason, biomonitoring has been likened to a 'video replay' of conditions that existed in the recent past, rather than a 'snapshot' of conditions at a single moment in time (Carter *et al*., 2006a).

Equally importantly, biomonitoring records the condition of living things that are very important to people's way of life, and to which they can relate. For example, people will notice declines in fish populations, changes in vegetation, and the disappearance of certain types of animals. These sorts of changes cannot be predicted accurately from physical and chemical monitoring because of the complexity of ecological relationships and the huge variety of physical and chemical variables that can affect animals and plants.

1.3 The types of organisms included in biological monitoring

Early biomonitoring of fresh waters in Germany focused on bacteria because of concerns about public health (Hynes, 1960). However, as other management issues emerged, additional organisms, and eventually entire aquatic communities, were included (Cairns and Pratt, 1993; Bonada *et al*., 2006; De Pauw *et al*., 2006). When Hellawell (1986) reviewed the scientific literature to determine which biological groups were most popular for monitoring, he found that benthic macroinvertebrates were recommended in 27% of studies, and followed by algae (25%), protozoa (17%), bacteria (10%), and fish (6%). Other biotic groups such as macrophytes, fungi, yeasts, and viruses were seldom recommended.

More recently, most attention has been paid to three groups: benthic macroinvertebrates, algae (especially diatoms), and fish (De Pauw *et al*., 2006). In the USA, all states monitor benthic macroinvertebrates except Hawaii, where a programme is under development; two-thirds of the states monitor fish and one-third monitor algae (Carter *et al*., 2006b). Resh (2007) examined 50 recent biomonitoring studies conducted in developing countries and found that 34 of these used benthic macroinvertebrates, 9 involved fish, 3 algae, and 2 aquatic macrophytes. Gallacher (2001) reported that benthic macroinvertebrates are the most widely used organisms in biomonitoring in Asia (in 10 of 12 countries examined), followed by bacteria (8), algae and fish (7), and protozoans.

Resh (2008) reviewed 65 journal articles, websites, and books that listed attributes as advantages and disadvantages of different groups of organisms for biomonitoring. His results are summarized in Tables 1.1 and 1.2. The number of sources listing advantages and disadvantages of the different groups follows the pattern of frequency of use in biomonitoring programmes.

Table 1.1 *Percentage of sources describing an attribute as an advantage of a group of organisms for biomonitoring (after Resh, 2008).*

Attribute	Benthic macroinvertebrates (42 sources)	Algae (periphyton) (22 sources)	Fish (15 sources)	Zooplankton (9 sources)
Widespread: Group is abundant, common, ubiquitous, etc.	60%	36%	17%	33%
Diverse: Group has many species, varying in responses to environmental change	81%	45%	26%	67%
Important to ecosystem: Group has important trophic positions or ecological roles	29%	23%	63%	56%
Limited mobility: Group is sedentary and therefore useful for inferring local conditions	69%	14%	0%	0%
Longer generation time: Group is useful for tracking over time, long-term integrators, bioaccumulate toxins	55%	5%	63%	0%
Shorter generation time: Groups has rapid responses to change, quick recovery	14%	45%	0%	33%
Economic: Group is inexpensive to conduct research with, has good benefit-cost ratio	21%	9%	11%	0%
Easy taxonomy: Group has easily identified specimens, good taxonomic keys are available	36%	23%	58%	0%
Easy sampling: Group requires low field effort	60%	50%	22%	22%
Pre-existing information: Group with good background information, existing expertise	19%	18%	53%	0%
Easy transport/storage: Group is easily taken back from the field, moved, stored for future use	2%	14%	0%	0%
Field examination: Group could be at least partly processed/identified while in the field	2%	0%	21%	0%
Low impact of sampling: Group for which sampling has a low impact on its own population and of other fauna	7%	14%	5%	0%
Stable/persistent populations: Group with populations that are predictable, and remain in the environment over time and through various conditions	0%	5%	16%	0%
Use by agencies/volunteers: Group has been used for biomonitoring by an agency/volunteer group	7%/7%	0%/0%	11%/0%	0%/0%

Table 1.2 *Percentage of sources describing an attribute as a disadvantage of a group of organisms for biomonitoring (after Resh, 2008).*

Attribute	Benthic macroinvertebrates (19 sources)	Algae (periphyton) (9 sources)	Fish (14 sources)	Zooplankton (6 sources)
Sampling difficulties: Group requires high effort, or has seasonal/daily fluctuations, patchy spatial distributions, equipment needs, variable populations	68%	33%	36%	67%
Identification: Group requires expertise for identification, fewer taxonomic keys available	58%	67%	7%	17%
Undesirable response levels: Group has low sensitivity, with tolerances	42%	11%	4%	0%
Lack of social recognition by public: Public does not consider group important	5%	11%	0%	0%
Affected by natural conditions: Group affected by predators, changes in physical conditions	21%	22%	7%	50%
Mobile: Group swims, drifts, not useful as a local indicator, affected elsewhere (e.g. spawning grounds)	21%	0%	64%	0%
Problems with methods/use: Group has poor metrics/indices available, poor documentation, laboratory difficulties, requires expertise	21%	78%	21%	67%
Not found/abundant in certain habitats: Group does not regularly inhabit area	11%	0%	14%	33%
Short generation time: Poor integrators, do not show bioaccumulation	0%	33%	0%	33%
Signs of stress hard to trace to source: Changes in population/community structure of group does not necessarily point to cause of change	21%	11%	7%	0%

1.4 Biological monitoring in Asia

Table 1.3 provides examples of freshwater biomonitoring in Asian countries. Some countries not included in the table, such as India and Indonesia, also have biomonitoring in place (e.g. Sivaramakrishnan *et al*., 1996; Sudaryanti *et al*., 2001). Asian countries have made varying levels of progress in the establishment of biomonitoring, with Japan being most advanced and Thailand having made excellent progress, particularly within the Ping River system. Several studies (e.g. Mustow, 2002) have applied methods developed outside of Asia to examine their applicability to Asian water bodies (e.g. Thailand). This is a common approach in water quality monitoring in developing countries.

Table 1.3 *Examples of freshwater biomonitoring in Asia (based on information in Resh, 1995; Gallacher, 2001; Resh, 2007; Morse* et al.*, 2007)*

Country	Previous studies	Current practices	Future needs and issues	References
Asian Russia	Hydrobiologists at Institute of Biology and Soil Sciences began using macroinvertebrates for water quality monitoring in 2001.	Russian Clean Water Project (RCWP) developing policies to protect freshwater resources. RCWP and Clean Water Center (CWC) aim to develop rapid bioassessment technology using macroinvertebrates. Network of public ecological agencies provides extensive monitoring. Bioassessment data and conclusions passed through CWC to federal and regional nature protection departments, who then investigate sources of pollution. Rapid bioassessment protocols adapted from those used in the USA. CWC organizes regular freshwater clean-ups.	Taxonomic and applied research needed. Development of university courses and mentors. Investment in modern, ecological and taxonomical literature. Environmental monitoring by government agencies based on obsolete methods, with very little use of macroinvertebrates. General public uninformed and uninterested in ecology and nature conservation. Little or no ecological monitoring carried out by private consultants.	Vshivkova and Nikulina, 1998, Vshivkova *et al.*, 2000, Vshivkova *et al.*, 2003, Vshivkova *et al.*, 2005
China	National survey of hydrobiological measures and environmental variables for major aquatic resources began in late 1950s. Point-source, pollution studies began in 1963. Biotic indices and species diversity indices used to evaluate Yangtze, Yellow, Zhujiang and other rivers in late 1970s. Modified Shannon-Wiener Diversity Index used by government agencies in 1982. 'Manual for Water Quality Biomonitoring' issued in 1993. 'Aquatic Insects of China Useful for Monitoring Water Quality' published in 1994. Workshops held at several universities and volunteer monitoring groups established. Tolerance values in east China developed in 2004. Benthic index of biotic integrity developed in 2005.	Ecological monitoring by remote sensing implemented. Conservation programs for Chinese alligator and Chinese sturgeon implemented. Legislation on chemical effluents implemented. 40 NGOs active in China, but biological monitoring by them is rare.	Biological monitoring is lagging behind chemical monitoring. Requirements exist for faunal inventories, establishment of tolerance values, University training programs, training programs for government agencies and specific protocols.	Hwang *et al.*, 1982; Yang *et al.*,; 1992, Morse *et al.*, 1994; Wang, 2002; Wang and Yang, 2004; Wang *et al.*, 2005.

Country	Previous studies	Current practices	Future needs and issues	References
Japan	Biomonitoring with macroinvertebrates adapted from German practices in late 1950s. Comprehensive species lists compiled in 1962. Introduction of saprobic system and biotic index in 1962. Testing of indices to measure organic pollution since 1980s. Identification guides produced in 1985 and 2005.	National biomonitoring programme for organic pollution. Nationwide survey of aquatic organisms (macroinvertebrates) has 800,000 participants. 30 species of macroinvertebrates used as indicators. National census of river environments (109 rivers) describes macroinvertebrates, fish and riparian plants. Huge volunteer programs, with participation by 23% of NGOs and 74% of public schools.	National and public institutions rarely involved in surveys. No standardization of sampling or analysis methods. Some taxonomic problems with databases.	Tsuda, 1962; Tsuda, 1964; Kawai, 1985; Kawai and Tanida, 2005.
Malaysia	One of the first studies of the macroinvertebrate fauna of a tropical river in 1973. Guide to macroinvertebrates published in 2004. Impact of a variety of disturbances on macroinvertebrate distribution studied by university research groups, but largely unpublished. Comparative study of macroinvertebrate fauna in urban and pristine streams in 2005. Several macroinvertebrate species identified as potential bioindicators in 2005 study.	Biomonitoring uncommon; most studies focus on biodiversity. National monitoring network (902 stations, 462 rivers) measures various abiotic water quality parameters and determines pollution status. The only biological data collected are for microbial analysis. 72% of rivers considered polluted or slightly polluted.	Macroinvertebrates poorly known and relatively few species have been described. More intensive monitoring of rivers using macroinvertebrates needed. Increased protection and rehabilitation of aquatic ecosystems required. Training programs for taxonomists and aquatic biologists needed. Educational programs required for the general public and government officials.	Bishop, 1973; Yule and Yong, 2004; Chin, 2003; Azrina *et al*., 2005; Che-Salmah and Abu-Hassan, 2005; Yap, 2005.
Mongolia	Hydrobiological studies carried out by Russian and Mongolian scientists since late 1800s. Interest in aquatic insects as bioindicators began in late 1990s with the introduction of university courses.	Biomonitoring carried out through National Institute of Meteorology and Hydrology to investigate biodiversity and evaluate water quality and ecology. Aquatic insect research carried out at National Institute of Meteorology and Hydrology and Mongolian Academy of Sciences (supported by World Bank and US National Science Foundation). Western Lakes Survey Project focuses on diatoms, ostracods and Chironomidae. Selenge River Basin insect survey project provides inventory of entomofauna. Selenge River Basin insect survey project provides inventory of entomofauna. The two projects above aim to establish baseline data on biota for use in biomonitoring programmes and to develop indigenous expertise and infrastructure.	Laws and regulations must keep pace with accelerating degradation of water resources. Water pollution management requires prioritisation. Biomonitoring data need to form a resource for management decisions. Data on species responses to defined toxicant levels need to be made available to monitoring agencies. Adequate training and equipment for biomonitoring staff required.	

Country	Previous studies	Current practices	Future needs and issues	References
South Korea	Community indices introduced in 1970s. Nature conservation and restoration promoted in 1990s. Korean biotic index introduced and modified in 1995. Neural network methods introduced in 1996. Dominant species index created in 2005. Physiological measures and molecular biomarkers introduced in 2002.	Ministry of Environment of Korea (MEK) requires macroinvertebrate studies in environmental impact assessments. Long-term 'Eco-technopia 21 project' to develop technology. Protocols using macroinvertebrates, fish and algae are being investigated in order to establish regular biomonitoring at check points throughout the country. MEK supports long-term biomonitoring in major freshwater systems. Biomonitoring popular in schools. Governmental and NGO public education programs include biomonitoring subjects.	Insufficient taxonomic knowledge. Educational programs and materials for public participation required.	Bae and Lee, 2001; Bae *et al*., 2005; Bae, 2005a; Bae, 2005b; Yoon, 2005.
Thailand	Green World Foundation started river and stream investigation project for youth in 1997 (58 schools participated). Report on water quality in 48 major rivers published by Pollution Control Department in 2005 (51% moderately polluted). Studies on adult stages of aquatic insects carried out in northern Thailand to detect environment disturbance. Pollution surveillance system using macroinvertebrates initiated along Ping River after 1996.	Water quality of inland surface waters is monitored with physical and chemical analysis. Total coliforms and fecal coliforms are the only biological parameters included. Preliminary rapid bioassessment studies using USEPA protocols are in progress in northern and north-eastern Thailand.	Research is needed on the taxonomy and biology of Thai macroinvertebrates. Calibration of bioassessment procedures is required.	Sangpradub *et al*.; 1996 Thorne and Williams, 1997; Kanjanavanit and Moonchinda, 1999; Luadee *et al*., 2002; Thorne and Williams, 2002; Inmuong *et al*., 2003, Boonsoong *et al*., 2005; PCD, 2005.

Various short-term or issue-specific studies of freshwater organisms have been done in the Mekong River basin. Fish have been the best studied organisms but this has mainly been from the perspective of fish taxonomy and fishery productivity. Lists of invertebrates and algae have also been prepared but vary greatly in their completeness and accuracy. Perhaps the best studied organism that occurs in the river is the snail *Neotricula aperta*, which is the intermediate host of *Schistosoma mekongi*, the vector of schistosomiasis in the Mekong region.

Grimås (1988) examined 28 sites for benthic macroinvertebrates in Lao PDR, Thailand and Viet Nam, specifically to consider water quality issues. Concurrently, the Ministry of Fisheries of Viet Nam conducted a series of studies on the Cambodian section of the Mekong and included zooplankton, phytoplankton, and benthic invertebrates in their analysis. However, neither study was detailed, and the results are best considered as preliminary to the programme described here.

1.5 Development of the MRC biomonitoring programme

In 2003, the MRC undertook a pilot survey in the four riparian countries to test the potential of five biological groups, and one ecological process, for routine monitoring of the Mekong River and its major tributaries. These groups and process, selected in consideration of prior international experience in freshwater biomonitoring, were as follows:

1. Planktonic primary production (a process critical to the well being of the Mekong's fisheries);

2. Benthic algae, including microscopic diatoms and macro-algae such as the 'river weed' that is processed and sold or eaten by local people;

3. Zooplankton, which are microscopic animals floating and drifting in open water;

4. Littoral macroinvertebrates (invertebrate animals visible to the naked eye), living in the shallow water at the river's edge;

5. Benthic macroinvertebrates, living in or on the sediments at the bottom of the river;

6. Fish.

The pilot study confirmed that diatoms, zooplankton, littoral macroinvertebrates and benthic macroinvertebrates were practical and cost-effective for routine sampling and identification with standard protocols. However, the pilot study showed that planktonic primary production, macro-algae, and fish were not practical for immediate adoption in the Mekong River system. The measurement of planktonic primary production required mooring a boat on site for several hours through the middle part of the day, and transporting a large amount of equipment, including chemicals, from site to site. These logistical requirements meant that measuring

primary production was a costly exercise relative to other components. Macro-algae were not present in sufficient quantities to allow representative sampling at most sites. And pilot sampling of fish showed that not enough specimens for reliable assessment could be collected with nets, even when most of the day was spent in sampling one site.

A routine biomonitoring programme began in 2004, based on the four groups of organisms and associated sampling protocols that proved most successful in the pilot, and continued annually through to 2007. The overall objectives of this programme were to:

1. Survey the priority biological groups at a set of sites of interest for management purposes, across all of the sub-areas of the Lower Mekong Basin;

2. Choose a set of reference sites to create a biological benchmark against which data from any site in the Lower Mekong Basin can be compared;

3. Specify characteristics of the biological groups that indicate harm to the aquatic ecosystem (biological indicators);

4. Use values of the biological indicators measured at the reference sites to develop a set of guidelines to rate and classify the sites;

5. Prepare a 'report card' that provides non-specialists and the general public with information on the purpose and methods of biomonitoring, and indicates the current condition of the river's ecosystems.

The programme was undertaken by biologists and ecologists from the member states, supported by the MRC secretariat and international experts in the field of biomonitoring. All sampling was confined to the dry season (March) because sampling in the wet season would be too logistically difficult and dangerous. However, because of the long life span of many of the organisms collected, the data reflect prior conditions as well as conditions during the time of sampling.

This paper summarises and interprets the results of the four years of monitoring. It describes the sampling locations and dates, the sampling protocols, the environmental variables measured at each site, and the types and numbers of plants and animals recorded at each site. It analyses the statistical significance of relationships among these factors and describes the rating and classification of all the sites sampled.

2. Sampling sites

2.1 Rationale for site selection

Biomonitoring sites were chosen to provide broad geographical coverage of the basin, to include each of the sub-basins defined by the MRC's Basin Development Plan (BDP), and to sample the mainstream of the Mekong River and each of its major tributaries. Sites were selected each year by the MRC secretariat in consultation with the National Mekong Committees.

The four years of sampling covered 51 sites spread across the Lower Mekong Basin (Table 2.1, Figure 2.1). Some sites were visited more than once, and so the study included 77 sampling occasions. The sites covered a wide range of river settings, including rocky channels in northern Lao PDR and northeast Thailand, the alluvial channels and floodplains of southern Lao PDR and Cambodia, and the distributary system of the Mekong Delta in Cambodia and Viet Nam. The sites also had a range of disturbances from human activity. Some were located in or close by villages or cities, some were next to fields where crops are grown and livestock graze, some were upstream or downstream of dams and weirs, and at some there was heavy river traffic.

Table 2.1 *List of sites sampled in 2004 – 2007.*

Site code	River	Location	Year sampled	Coordinates (UTM)	
CKL	Bassac	Koh Khel	2006	48P E0503327	N1246641
CKM	Se Kong	River mouth	2005	48P E0615596	N1500691
			2006	48P E0615508	N1500632
			2007	48P E0615573	N1500696
CKT	Mekong	Kampi pool	2004	48P E0610951	N1393569
			2006	48P E0609207	N1393544
CMR	Mekong	Stung Treng Ramsar site	2005	48P E0607964	N1537129
			2006	48P E0604976	N1539456
			2007	48P E0605696	N1539736
CNL	Mekong	Nak Loeung	2006	48P E0528321	N1250852
CPP	Tonle Sap	Phnom Penh Port	2004	48P E0492492	N1279903
			2006	48P E0491666	N1280205
CPS	Pursat	4 km upstream of Prek Thot	2004	48P E0381258	N1382944
CPT	Prek Te		2006	48P E0613899	N1374811
CSJ	Se San	Downstream of confluence with Sre Pok	2005	48P E0621005	N1499145
			2006	48P E0620973	N1499412
			2007	48P E0615573	N1500688

Site code	River	Location	Year sampled	Coordinates (UTM)	
CSK	Stoeng Sangke	Battambang	2006	48P E0348375	N1465699
CSN	Stoeng Sen	Kapongthom	2006	48P E0490998	N1401845
CSP	Sre Pok	Kampong Saila, Lumpat	2004	48P E0716971	N1490691
			2005	48P E0716971	N1490691
			2006	48P E0717424	N1490804
			2007	48P E0717104	N1490800
CSS	Se San	Veunsai District, Rattanakiri Province	2004	48P E0696445	N1545480
			2005	48P E0695488	N1546145
CSU	Se San	Pum Pi village, Rattakiri Province	2005	48P E0764687	N1526041
			2006	48P E0764506	N1526065
			2007	48P E0764707	N1526063
CTU	Tonle Sap	Prek Kdam ferry	2004	48P E0477884	N1309367
			2006	48P E0478364	N1307071
LBF	Se Bang Fai		2007	48Q E0498437	N1888075
LBH	Se Bang Hieng		2007	48Q E0540315	N1779816
LDN	Mekong	Done Ngieu island	2007	48P E0596621	N1650516
LKD	Nam Ka Ding	Haad Sai Kam	2004	48Q E0398871	N2023713
			2007	48Q E0398583	N2023903
LKL	Se Kong	Ban Xou Touat, Attapeu Province	2005	48P E0673642	N1622904
			2007	48P E0670721	N1623450
LKU	Se Kong	Ban Xakhe, Attapeu Province	2005	48P E0701679	N1653515
			2007	48P E0702400	N1653117
LMH	Mekong	Near Houa Khong water quality station	2005	47Q E0723733	N2383320
LMX	Mekong	Near Ban Xieng Kok, Muang Luang	2005	47Q E0670860	N2311778
LNG	Nam Ngum	Upstream of confluence with Nam Lik	2004	48Q E0240744	N2050118
			2007	48Q E0237411	N2049992
LNK	Nam Khan	Between Hat Hian and Ban Houay Ung	2005	48Q E0203428	N2200953
LNM	Nam Mo	Upstream of bridge near mine	2007	48Q E0280667	N2088210
LNO	Nam Ou	About 5 km from river mouth	2004	48Q E0212495	N2222855
LNT	Nam Ton	50 km from Vientiane	2007	48Q E0208083	N2016581
LOU	Nam Ou	Between Ban Pak Ou and Ban Hat Mat	2005	48Q E0219345	N2229380
LPB	Mekong	Above Luang Prabang, upstream of Pak Nam Karn	2004	48Q E0201739	N2203028
			2005	48Q E0206113	N2206957
LPS	Mekong	Pakse, upstream of Se Done mouth	2004	48P E0587623	N1671756
LSD	Se Done	Ban He, upstream of Pakse	2007	48P E0586345	N1673985

Site code	River	Location	Year sampled	Coordinates (UTM)	
LVT	Mekong	Upstream of Vientiane	2004	48Q E0239871	N1988731
			2007	48Q E0229378	N1990015
TCH	Nam Chi	Wat Srithararm, Yasothon	2004	48P E0407724	N1745362
TKO	Nam Mae Kok	About 15 km upstream of Chieng Rai Weir	2004	47Q E0576165	N2205993
			2005	47Q E0576410	N2205793
TMC	Mekong	Wiangkhain, between Sop Ing Tai and Ban Huai Ian, near Cham Pong	2005	47Q E0655974	N2231281
TMI	Nam Mae Ing	Near Ban Ten	2005	47Q E0640355	N2213637
TMM	Nam Mun – Chi	Mekong (Mun - Kong Chiam)	2007	48P E0552854	N1692378
TMU	Nam Mun	Ban Tha Phae, Ubon Ratchathani	2004	48P E0553283	N1692193
TNK	Nam Kham	Na Kae	2007	48Q E0450473	N1874626
TSK	Nam Songkhram	About 8 km from river mouth	2004	48Q E0438501	N1946480
			2007	48Q E0440989	N1948666
TSM	Nam Songkhram	Mekong	2007	48Q E0444135	N1951422
VCD	Bassac	Chau Doc	2004	48P E0515263	N1187502
			2006	48P E0510969	N1188413
VCL	Cao Lanh		2006	48P E0563807	N1153868
VCT	Bassac	Can Tho	2006	48P E0588365	N1110673
VLX	Long Xuyen		2006	48P E0551878	N1143546
VSP	Sre Pok	Ban Don hydrographic station	2004	48P E0802270	N1426825
VSR	Sre Pok	Upper Sre Pok	2006	48P E0817329	N1396950
VSS	Se San	Kon Tum hydrographic station	2004	49P E0180575	N1587838
			2006	48P E0180527	N1588158
VTC	Mekong	Tan Chau	2004	48P E0528931	N1194535
			2006	48P E0524259	N1195808
VTR	Vinh Long	Vinh Long	2006	48P E0603976	N1135759

2004 survey

The sites surveyed in 2004 were chosen to provide a broad geographic coverage across the Lower Mekong Basin. They included localities on the Mekong and its major tributaries, in each of the BDP sub-areas and MRC member states.

2005 survey

The geographic coverage was more focused for the 2005 survey. The sites fell into two groups: (i) northern Lao PDR and the northern provinces of Thailand (mainly Chiang Rai), which lie in BDP sub-areas 1 (Northern Lao PDR) and 2 (Chiang Rai), and (ii) southern Lao PDR and eastern Cambodia, which lie largely in sub-area 7 (Se San/Sre Pok/Se Kong).

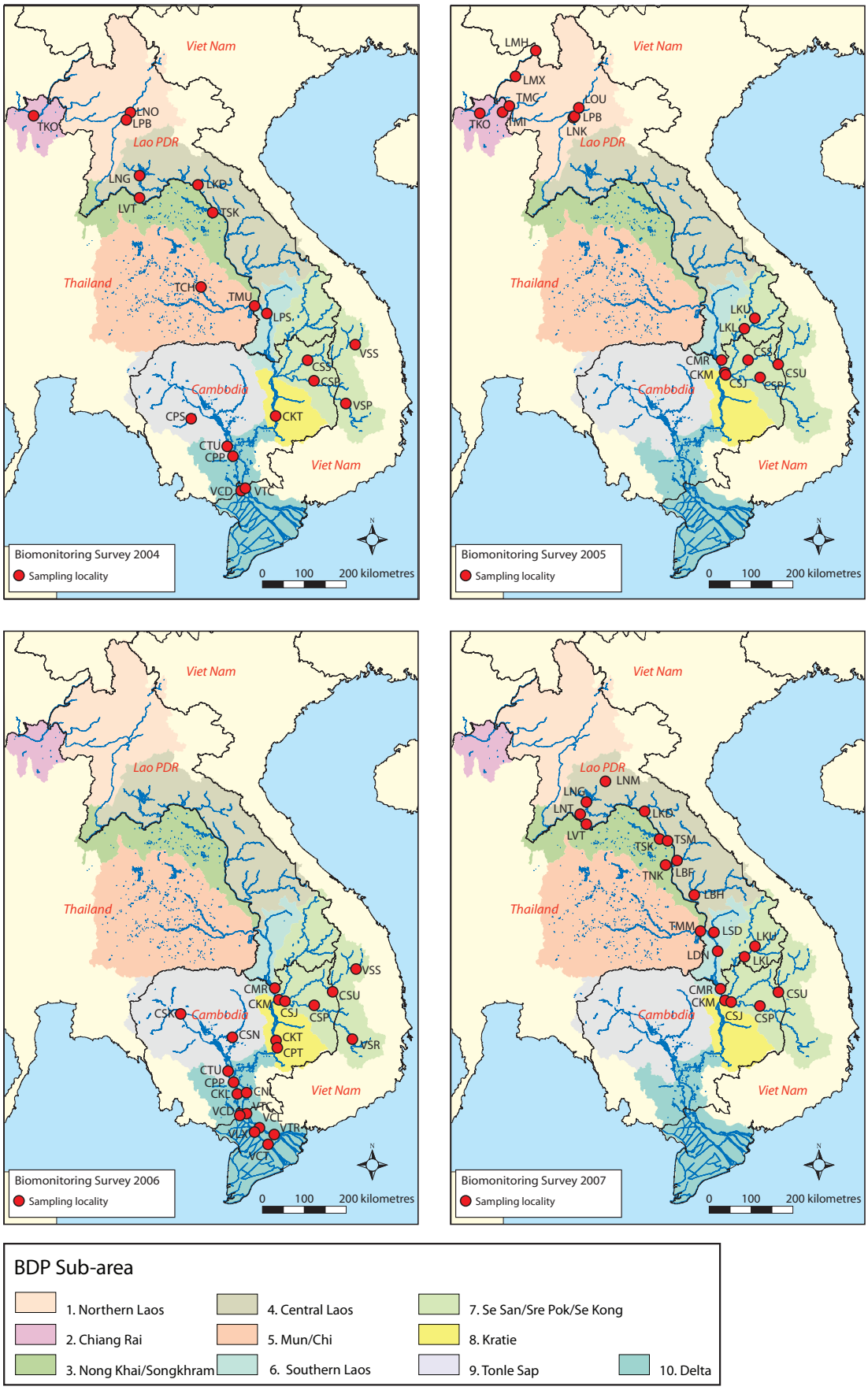

Figure 2.1 Maps of sites surveyed in 2004, 2005, 2006, and 2007.

2006 survey

The 2006 survey focused on the mainstream and its major tributaries downstream of the Ramsar site at Stung Treng in northern Cambodia. The survey included localities in sub-areas 6 (Southern Lao), 7 (Se San/Sre Pok/Se Kong), 8 (Kratie), 9 (Tonle Sap), and 10 (Delta).

2007 survey

The 2007 survey covered a large area of the lower Mekong Basin in central Lao PDR, and along the border of Lao PDR and Thailand. Sites from previous years were re-sampled in the Se Kong river in Lao PDR and Cambodia, and the Se San and Sre Pok rivers in Cambodia. The sites included fell in sub-areas 3 (Nong Khai/Songkhram), 4 (central Lao PDR), 5 (Mun – Chi), 6 (southern Lao PDR), and 7 (Se San/Se Kong/Sre Pok).

2.2 Designation of reference sites

Reference sites are used in both physical-chemical monitoring (e.g. to set water-quality criteria) and biological monitoring programmes worldwide. In biomonitoring, the sites chosen to be reference sites are usually selected on the basis of water quality and the degree of disturbance caused by human activities. They are commonly those sites that are in a most natural, or pristine, state. Reference sites for the Mekong provide benchmark data against which all sites in the system can be compared. They are located where anthropogenic impacts, such as from water resource development or waste disposal, are minimal.

Accordingly, reference sites were selected from those sampled in the biomonitoring programme by the application of six criteria related to water quality, human disturbance in the vicinity of the site, and human disturbance upstream. The water quality criteria were based on those proposed for the MRC's Environment Programme Water Quality Index (MRC 2008). Site disturbance was scored by the national and international experts present on each sampling occasion, having regard to site-scale activities such as the following (Figure 2.2):

1. Sand and gravel extraction;
2. Dredging and mining;
3. Removal of natural riparian vegetation for agriculture or housing;
4. Agricultural cultivation;
5. In-stream aquaculture;
6. Fishing intensity;

7. Road building;

8. Unnatural bank erosion;

9. Cattle and buffalo grazing;

10. Boat traffic;

11. Waste disposal from villages, farms, towns etc.;

12. Village activities such as bathing and washing of clothes;

13. Unnatural fluctuations in water level.

A Site Disturbance Score (SDS) ranging from 1 (little or none of any of these types of disturbance) to 3 (substantial disturbance of one or more types) was assigned independently by each of the participants following group discussion about potential anthropogenic impacts (on average there were eight participants, with a range of between five and nine). The individual scores were then averaged to determine a measure of human disturbance at a site. Visual assessment was used because it was not possible to make quantitative measurements of all of these types of disturbance. Visual scoring systems are widely used in stream assessments for features that are not amenable to quantitative measurement. Averaging of the scores of several observers evens out the influence of individual differences, in the same way that scores are averaged among judges of sporting and artistic competitions.

To be selected as a reference site, a site had to meet all of the following requirements:

1. The pH of the site at the time of biological sampling was between 6.5 and than 8.5.

2. The electrical conductivity at the time of biological sampling was less than 70 mS/m.

3. The dissolved oxygen concentration at the time of biological sampling was greater than 5 mg/L.

4. The average SDS was between 1 and 1.67 on a scale of 1 to 3, that is, in the lowest one-third of possible scores. A typical site with a score between 1 and 1.67 might have low-level rural development, such as low-density village activities, but not major urbanization, intensive agriculture or waste disposal.

5. There was no major dam or city within 20 km upstream of the site, and flow at the site was not affected by inter-basin water transfers. Downstream development was also considered where a site has upstream flow because of tidal influence.

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Figure 2.2 Clockwise from top left (i) reference site; examples of disturbance caused by human activity (ii) bank erosion, (iii) over-fishing, (iv) mining, (v) waste disposal, (vi) agricultural discharge, (vii) urban development, (viii) aquaculture, and (ix) agricultural cultivation.

Fourteen of the 51 sites sampled in the programme met all criteria and were selected as reference sites (Table 2.2).

Table 2.2 *Evaluation of all sites against reference site criteria.*

Site	Number of sampling occasions	pH (range if applicable)	Maximum EC (mS/m)	Minimum DO (mg/L)	Site disturbance score	Upstream and downstream disturbance	Reference site (yes or no)
CKL	1	7.17	12.32	7.56	2.19	Phnom Penh City	N
CKM	3	5.16–7.77	7.30	6.32	1.33		N
CKT	2	7.69–8.40	19.62	6.89	1.19		Y
CMR	3	7.74–8.41	23.02	8.15	1.59		Y
CNL	1	7.54	19.35	7.02	1.97		N
CPP	2	7.18–7.94	10.47	3.94	2.88	Phnom Penh City	N
CPS	1	7.30	8.40	5.07	2.22		N
CPT	1	7.13	11.03	4.56	2.33		N
CSJ	3	7.22–7.48	4.93	6.00	1.34	Dam 200 km upstream	Y
CSK	1	6.99	18.18	3.76	2.00	Battambang City and agriculture	N
CSN	1	7.22	8.10	7.13	2.00		N
CSP	4	7.32–7.63	6.85	5.91	1.22		Y
CSS	2	7.24–7.52	4.23	6.19	1.75		N
CSU	3	7.05–7.32	4.30	6.98	1.95		N
CTU	2	7.00–7.01	9.08	3.79	2.08		N
LBF	1	8.05	32.88	7.54	1.72		N
LBH	1	7.86	15.25	7.70	1.63	Interbasin transfer	N
LDN	1	8.27	22.87	8.51	1.53		Y
LKD	2	7.71–7.97	10.70	7.67	1.50	Dam 100 km upstream with interbasin transfer	N
LKL	2	7.18–7.24	7.07	5.56	1.59	Dam next year	Y
LKU	2	6.98–7.18	5.14	5.99	1.33	Dam next year	Y
LMH	1	8.19	34.80	9.34	1.94		N
LMX	1	8.10	33.00	8.25	1.94		N
LNG	2	6.87–7.45	7.51	6.93	1.67	Dam 3 km upstream	N
LNK	1	8.27	25.10	7.47	1.38		Y
LNM	1	7.95	9.65	8.87	2.31	Gold mine	N
LNO	1	8.46	24.72	8.59	1.00		Y
LNT	1	7.43	14.67	8.69	1.69	Town	N
LOU	1	8.15	21.27	8.16	1.00		Y
LPB	2	8.17–8.47	27.40	7.87	1.48		Y
LPS	1	8.38	22.86	7.17	1.57		Y
LSD	1	7.80	11.90	7.42	1.97	Rubber plantation	N

Site	Number of sampling occasions	pH (range if applicable)	Maximum EC (mS/m)	Minimum DO (mg/L)	Site disturbance score	Upstream and downstream disturbance	Reference site (yes or no)
LVT	2	7.79–8.63	28.80	8.61	1.78		N
TCH	1	7.83	18.38	7.71	1.86		N
TKO	2	6.62–7.95	11.75	6.22	1.87		N
TMC	1	6.80	22.68	7.60	1.64		Y
TMI	1	6.80	10.18	6.40	2.25		N
TMM	1	7.52	20.94	7.25	2.17	Dam 10 km upstream	N
TMU	1	7.30	9.59	7.44	1.71	Ubon City	N
TNK	1	7.15	16.92	7.11	2.44	Series of weirs	N
TSK	2	7.47–8.01	76.66	7.15	2.05		N
TSM	1	8.12	24.95	8.65	1.86		N
VCD	2	7.10–7.68	18.05	3.91	2.50	Town downstream and tidal movement; agriculture; shipping	N
VCL	1	7.58	18.87	8.01	1.91	Town upstream; agriculture; shipping	N
VCT	1	7.18	18.60	5.20	2.64	City upstream and downstream; agriculture; shipping	N
VLX	1	7.13	18.57	6.59	2.69	City upstream; agriculture; shipping	N
VSP	1	7.77	6.26	5.87	1.29		Y
VSR	1	7.14	5.15	7.31	2.00	Dam 7 km upstream	N
VSS	2	6.62–7.66	3.97	7.28	2.14		N
VTC	2	7.64–8.33	18.28	5.70	2.39	Town downstream and tidal movement; agriculture; shipping	N
VTR	1	7.33	18.11	6.70	2.44	Town downstream and tidal movement; agriculture; shipping	N

3. Environmental variables

3.1 Introduction

In the past, physical and chemical information was often the sole basis for monitoring the environmental quality of rivers and lakes. Today, with the widespread implementation of biological monitoring programmes, physical and chemical information is complemented by biological data. Physical and chemical data can assist in the interpretation of information obtained from biological monitoring programmes by revealing potential causes of biological changes. For this reason, physical and chemical measurements were included in the biomonitoring programme.

This chapter describes the physical and chemical environment of the sites sampled in the biomonitoring programme from 2004 to 2007. Information is provided on site locations and dimensions, water transparency and turbidity, water temperature, the concentration of dissolved oxygen (DO), electrical conductivity (EC), pH, and concentrations of chlorophyll-a. Later chapters relate these physical and chemical measurements to biological indicators.

3.2 Methods

The map coordinates and altitudes of the sampling sites were determined with a Garmin GPS 12xL device, and river width was measured with a Newcon Optik LRB 7x50 laser rangefinder. All water quality measurements were taken in three sections of the river at each site, near the left bank, near the right bank, and in the centre of the river, and averaged. Temperature, DO, EC, and pH were measured with Enviroquip TPS meters and later with a YSI 556MP5 meter, calibrated according to the manufacturer's instructions. Readings were taken at the surface and at a depth of 3.5 m, or the maximum of the river, whichever was less. A Secchi disc was used to determine water transparency. The disc was slowly lowered into the water, and the depth at which it could no longer be seen was recorded. The disc was then lowered another metre and slowly pulled up until it reappeared. If it reappeared at a depth more than 0.05 m different from the depth at which it disappeared, the procedure was repeated. Water turbidity and the concentration of chlorophyll-a were measured at the water surface in 2006 and 2007 only, with a Hach 2100P turbidity meter and Aquaflour handheld fluorimeter respectively.

3.3 Results

Overall variability and relationships among variables

Site averages of the environmental variables had a broad range across the 51 study sites over 77 visits during the four years (Appendix 1). Altitude varied from 3 to 565 m above sea level, with

most of the lowland sites being in Cambodia and Viet Nam and the high-altitude sites in Lao PDR, Thailand, and Viet Nam. Water width in the rivers varied from 11 to 2660 m, and tended to be greater as the altitude decreased (Figure 3.1).

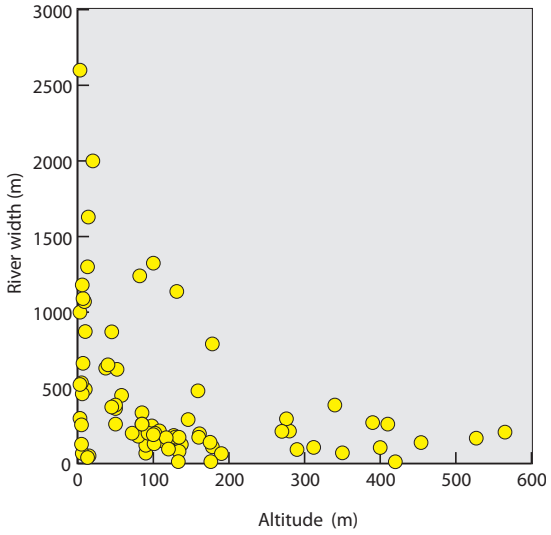

Figure 3.1 Relationship between river width and altitude.

Water temperature ranged from 16.7 ºC in a small, high-altitude river in Lao PDR to 31.4 ºC at a site in Cambodia, with an overall average of 27.7 ºC. As would be expected, temperature tended to be lower at the higher altitudes, although there was considerable variation (Figure 3.2).

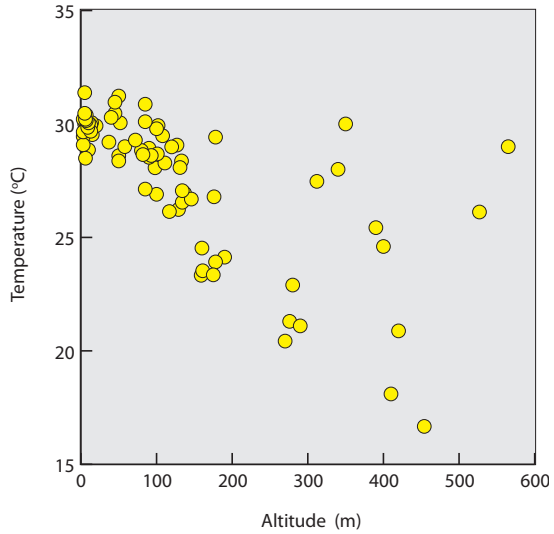

Figure 3.2 Relationship between average water temperature and altitude.

The concentration of dissolved oxygen was generally high, ranging from 2.7 to 10.5 mg/L with an average of 7.1 mg/L. DO was generally lower where temperature was higher, usually in low-elevation sites, which was expected because the solubility of oxygen is lower in warmer water (Figure 3.3).

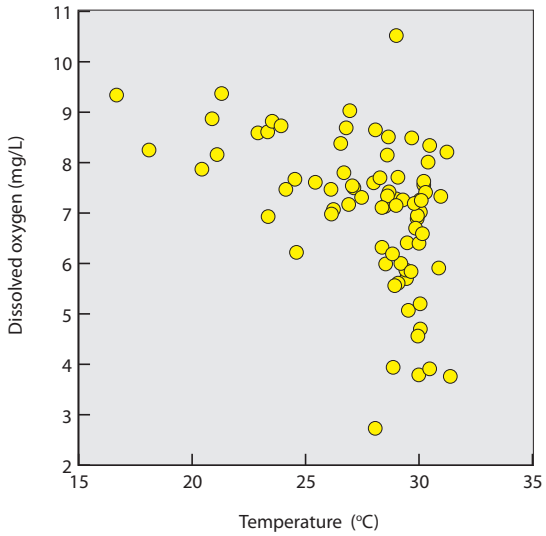

Figure 3.3 Relationship between average water temperature and average dissolved oxygen concentration.

The water was slightly alkaline at most of the sites, with pH varying between 5.2 and 8.6, with an overall average of 7.5. EC was generally low, varying from 3.9 to 76.7 mS/m with an average of 15.3 mS/m. Lower conductivity was found in tributary sites, whereas higher values were found at the sites in the main channel and those with human disturbance or in limestone catchments. Higher pH values tended to be associated higher EC (Figure 3.4).

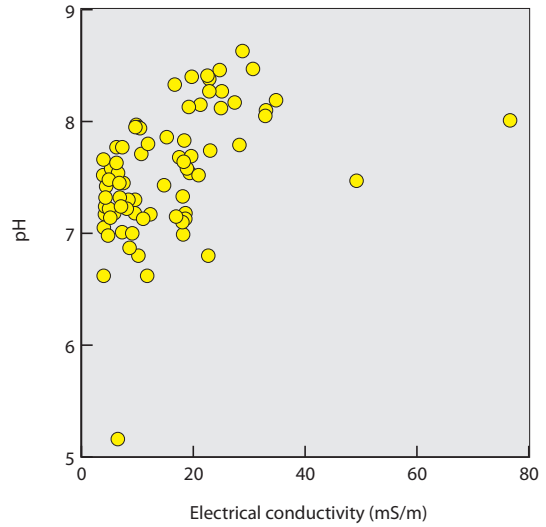

Figure 3.4 Relationship between average electrical conductivity and average pH.

Water transparency (Secchi depth) was variable, and ranged from 0.2 m to 3.4 m with an overall average of 1.0 m. Turbidity ranged from 2.4 to 71.1 NTU with an average of 15.3 NTU, and as expected was inversely related to transparency (Figure 3.5).

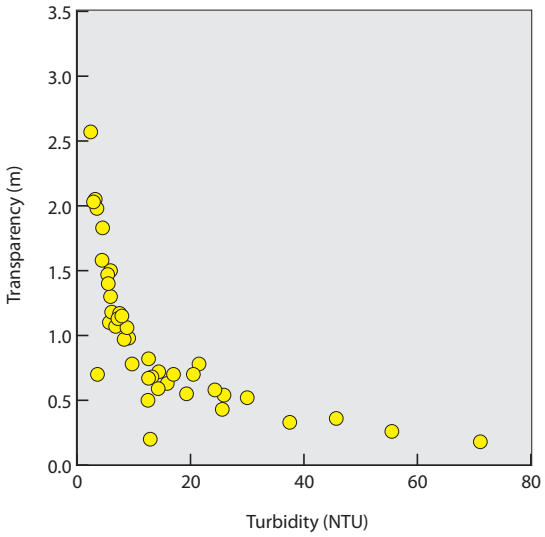

Figure 3.5 Relationship between average turbidity and average transparency. Turbidity was not measured in 2004 or 2005.

Chlorophyll-a concentrations were generally low, ranging between 0.2 and 4.0 µg/L, except for a value of 33.6 µg/L in Tonle Sap at Phnom Penh Port. Chlorophyll-a concentration was negatively related to transparency, suggesting that phytoplankton levels were limited by light availability (Figure 3.6).

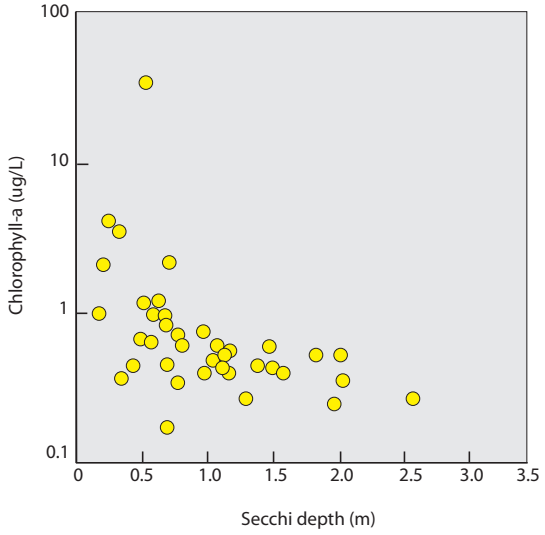

Figure 3.6 Relationship between average transparency (Secchi depth) and average chlorophyll-a concentration (plotted on a logarithmic scale). Chlorophyll a was not measured in 2004 or 2005.

Inter-annual changes

Twenty of the 55 sites were sampled in two or more years. Often, values of environmental variables were similar at the same site in different years, for example EC (Figure 3.7). Other variables such as DO varied more at a site between years (Fig. 3.8). However, DO typically fluctuates even within the same day, because of variations in sunlight and temperature and consequent differences in oxygen exchange with the atmosphere, the release of oxygen by aquatic organisms via photosynthesis, and uptake for respiration. The most notable inter-annual difference in pH was a low value of 5.2 at site CKM in 2006 compared to 7.5 and 7.8 in 2005 and 2007 respectively.

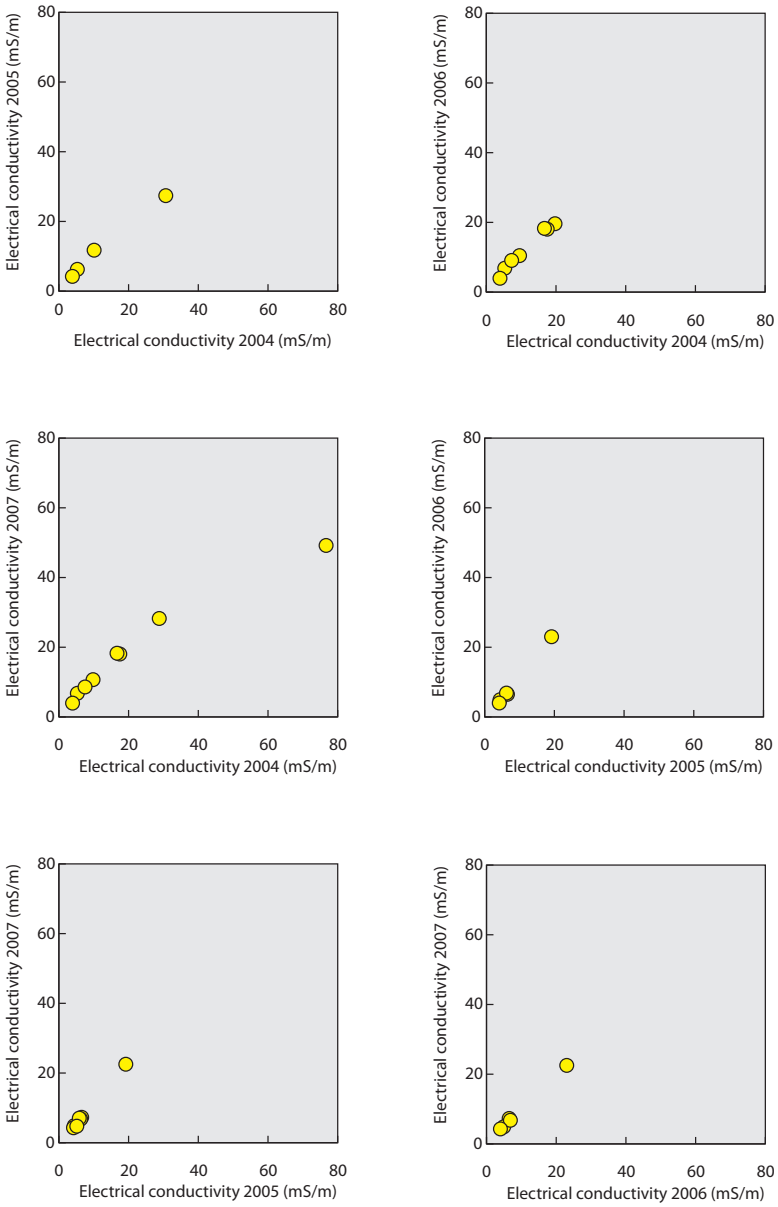

Figure 3.7 Relationships between electrical conductivity values measured at the same site in different years.

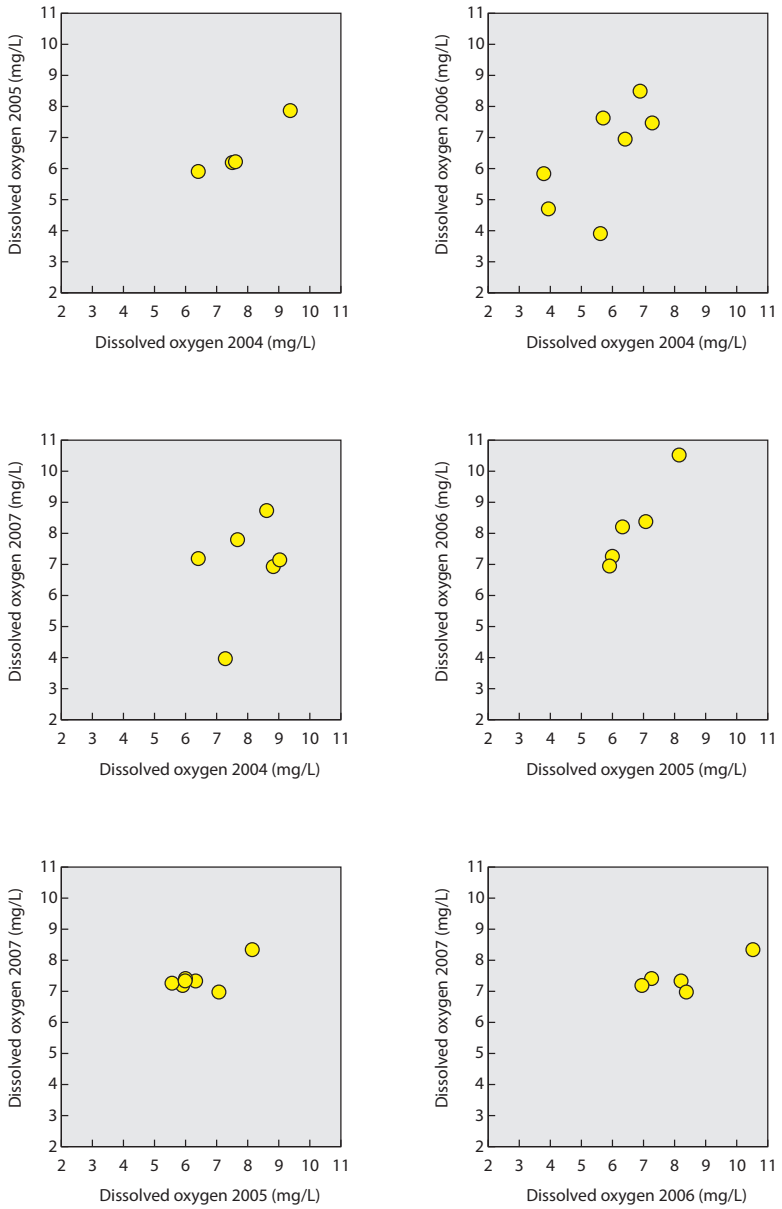

Figure 3.8 Relationships between dissolved oxygen values measured at the same site in different years.

Relationships with the site disturbance score

The average SDS did not exhibit any significant relationship with some of the environmental variables measured in this survey (Table 3.1). This would be expected for altitude, which is not affected by human activity. Other variables, such as pH and temperature, although potentially affected by human activities, are also subject to wide natural variations which may mask any human impact. However, water transparency and dissolved oxygen concentration both showed significant negative relationships with the SDS. Human disturbance often increases the rate of bank and catchment erosion, resulting in greater concentrations of suspended particles that

reduce transparency. It also frequently increases the loading of organic matter, which consumes dissolved oxygen as it decays.

It should be remembered that the SDS is a visual assessment by the survey team at a single point in time, and should not be expected to precisely reflect all human factors impacting on a site in the long term.

Table 3.1 *Probability and R^2 values resulting from linear regression analyses of selected environmental variables on the Site Disturbance Score (n=77).*

Variable	p value	R^2
Altitude	0.46	0.007
River width	0.35	0.012
Secchi depth	<0.001	0.212
Temperature	0.24	0.019
Dissolved oxygen	<0.001	0.141
pH	0.18	0.024
Electrical conductivity	0.45	0.008

3.4 Discussion

The environmental variables were mostly within the natural ranges expected for surface waters in the region. The temperature, DO, pH and EC values were generally within the acceptable ranges for protection of aquatic ecosystems according to the standards for surface water quality set by Cambodia, Thailand and Viet Nam (PCD, 2004; MRC, 2005).

DO values were mostly high, even at some sites showing evidence of human disturbance from villages, agriculture or dam construction. Out of 77 visits at 51 sites over the four years, there were only six occasions when DO was lower than the minimum concentration considered suitable for aquatic life by MRC (5 mg/L). However, it should be noted that DO was measured in daytime and concentrations are likely to be lower at night. The distinctly low pH value of 5.2 at site CKM may have been caused by recent activities upstream, and a high EC at site TSK (76.7 mS/m) may have been a result of contamination from saline land upstream.

High turbidity at some sites may have been a natural phenomenon related to the soil type and storms prior to sampling. However, high values at site VSR were apparently caused by sediments released from a dam construction site, located 6 km upstream.

4. Benthic diatoms

4.1 Introduction

Benthic diatoms are microscopic plants that are an important base for the pathway by which energy and nutrients enter the invertebrate and vertebrate food web in the Mekong River and other fresh waters. In the biomonitoring programme, the diatoms represent the primary producer trophic level; all of the other groups examined represent consumer levels. Primary consumers include the invertebrates that graze on the diatoms that are attached to hard surfaces; secondary consumers include the invertebrate and vertebrate predators that feed on the primary consumers. As a result of this connection, the diatoms provide an important link between the chemical and physical settings that ultimately determine primary productivity in the system and the secondary productivity of the invertebrates described in later chapters.

There are numerous scientific papers and publications that document the advantages of using diatoms in biomonitoring programmes (Table 1.1). In particular, diatoms are easy to sample, they are very diverse, and they respond in many ways to physical and chemical change. Because they have a short generation time, they respond quickly to environmental changes and recovery rapidly from most disturbances. They have more rapid responses to nutrient inputs than the other biological groups sampled in this project. As with all groups, there are some reported disadvantages in their use (Table 1.2). Identification requires specialist taxonomic skills that may require years of training to develop and analytical metrics for diatoms are not as firmly based in ecological theory or empirical studies as those for macroinvertebrates and fish.

Diatoms have been well studied in Southeast Asia, most recently through the extensive studies of the Algal Research Laboratory at Chiang Mai University and their collaborators. Broader application of diatoms in biomonitoring likely would result if an identification manual specific to Southeast Asia, including information on ecological tolerances and preferences, were available.

This chapter describes the diatom assemblages recorded in the biomonitoring programme from 2004 to 2007, and their relationships with environmental variables.

4.2 Methods

Sampling and sample processing

Locations for sampling of benthic diatoms were chosen where the water depth was less than 1 m and suitable substrata extended over a distance of 100 m. The most appropriate substrata were cobbles and other grades of stones with a surface area greater than 10 cm^2, but that were still small enough to fit in a sampling bowl of 20–30 cm diameter. At sites where the river bed was predominantly muddy or sandy and lacked suitable sized stones, samples were taken from

bamboo sticks, aquatic plants, and artificial materials. At each site, ten samples were taken at intervals of about 10 m. Samples were removed from stones chosen because they were coated with a thin brownish film or had a slippery feel. These characteristics are often indicative of the presence of an abundance of benthic diatoms. Where there were no suitable stones, the nearest hard substratum was sampled instead. To sample the diatoms, a plastic sheet with a square, 10 cm^2 'cutout' was placed on the upper surface of the stone or other substratum, and benthic diatoms were brushed and washed off into a plastic bowl until the cut-out area was completely clear. Each sample was transferred to a plastic container labelled with the site location code, date of sampling, and replicate number. The collector's name and the type of substratum were also recorded. Samples were preserved with Lugol's solution.

In the laboratory, the samples were cleaned by digestion in concentrated acid, and then centrifuged at 3500 rpm for 15 minutes. The diatom cells (the brown layer between the supernatant and solid particles) were siphoned into an 18 cm core tube. Strong acid (H_2SO_4, HCl or HNO_3) was added and the tubes were heated in a boiler (70-80 ºC) for 30 – 45 minutes. The samples were then rinsed with de-ionized water 4 – 5 times and adjusted to a volume of 1 mL. A sub-sample of each sample (a drop with a volume of 0.02 ml) was placed on a microscope slide and dried. A mounting agent such as Naphrax or Durax was added to make a permanent slide for diatom identification and counting, which were done under a compound microscope. Identification was based on frustule type, size, special characteristics, and structure, as described and illustrated in textbooks, monographs and other publications on tropical and temperate diatoms (Foged, 1971, 1975, 1976; Krammer & Lange-Bertalot, 1986, 1988, 1991a, 1991b; Pfister, 1992). In many cases identification to described species was not possible and presumptive species were designated by numbers. The total count of cells on the slide can be used to estimate density, i.e. the number of cells counted multiplied by five is the number per cm^2 sampled. The permanent slides are kept in the Applied Algal Research Laboratory Collection at Chiang Mai University.

Derivation of biological indicators

Three biological indicators were calculated for all diatom samples: richness (the number of taxa of diatoms identified from each sub-sample), abundance (the number of individual diatoms per sub-sample), and the average tolerance score per taxon (ATSPT).

Harm to the ecosystem is indicated by unnaturally low richness (low biodiversity), unnaturally low abundance (few organisms present), or an unnaturally high ATSPT. Taxa that are sensitive to stress, and tend to be absent at stressed sites, have low tolerance scores. Stress-tolerant species, which are hardy and survive at stressed sites, have high tolerance scores. Consequently, the average score is higher at sites with environmental stress.

Tolerance scores for individual taxa were derived from the relationship between the presence and absence of taxa in samples from each study site and the value of the Site Disturbance Score for that site (see Chapter 2). The tolerance of each species or variety was calculated as the average Site Disturbance Score for all sites at which that taxon occurred, weighted by the number of samples per site in which the taxon was recorded. The tolerance values were then

re-scaled so that their possible range was from 0 to 100, where 0 represents low tolerance and 100 represents high tolerance to human-generated stress, such as water pollution. The Average Tolerance Score per Taxon (ATSPT) was then calculated for each sample collected. ATSPT is simply the average tolerance of all taxa recorded in a sample. A higher value of ATSPT indicates a more tolerant biota, and hence a more stressed environment.

Linear regression analysis was used to test for statistically significant relationships between the environmental variables that were measured on all 77 sampling occasions and the average richness, abundance and ATSPT of the diatom flora. Abundance data were highly skewed and were therefore converted to logarithms before analysis.

4.3 Results

Biota collected

In total, 218,324 diatoms comprising 177 species and varieties were identified from 770 algal samples collected (Appendix 2 and 3).

Richness

Average richness per sub-sample ranged from 3.9 to 20.6 taxa (Appendix 2 and 3), and was significantly positively-related to site altitude and negatively related to water temperature (Figure 4.1).

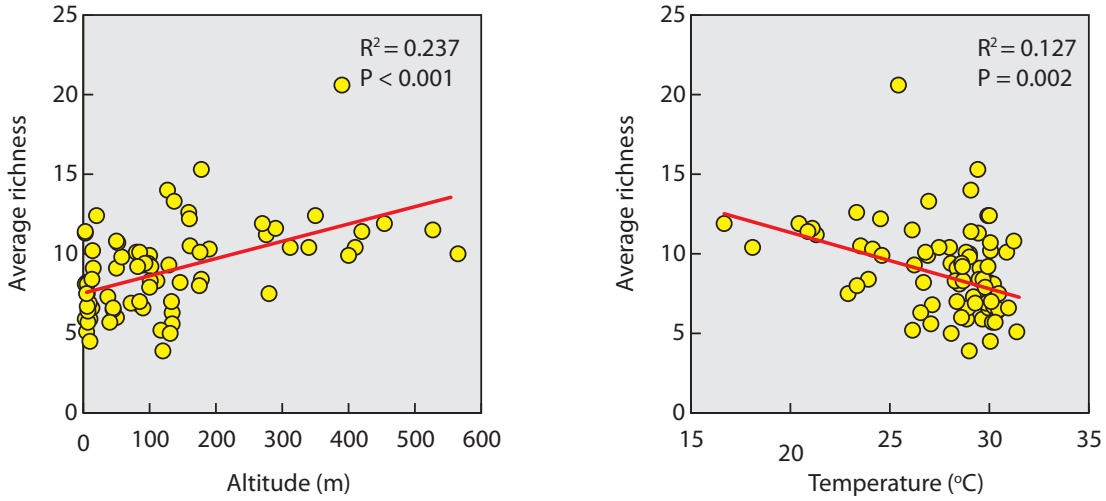

Figure 4.1 Statistically significant relationships of average richness of diatoms to environmental variables.

Abundance

The average number of diatoms per sub-sample ranged from 46 to 1338 (Appendix 2 and 3), and the logarithm of average abundance was significantly positively-related to water transparency (Secchi depth) (Figure 4.2).

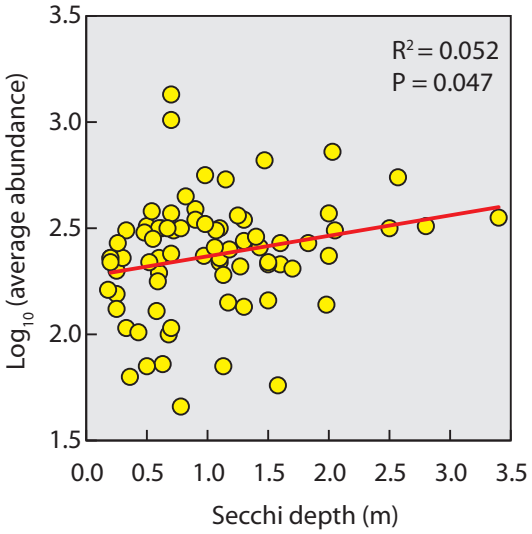

Figure 4.2 Statistically significant relationship of average abundance of diatoms to Secchi depth.

Average Tolerance Score Per Taxon

The tolerance scores for individual taxa of benthic diatoms varied from 4 to 81 (Appendix 2 and 3). The average ATSPT per sub-sample ranged from 30 to 51, and was significantly positively-related to the Site Disturbance Score, and significantly negatively-related to Secchi depth, dissolved oxygen concentration and pH (Figure 4.3).

4.4 Discussion

The significant positive relationship of diatom species richness with altitude and the significant negative relationship with temperature indicates that higher-elevation, and hence cooler sites, had a richer diatom flora. This might have been a result of greater human disturbance at lower altitudes, but since the Site Disturbance Score did not correlate significantly with altitude (Chapter 3), a more likely explanation is that richness was influenced by habitat suitability. The sites at higher altitude often had an abundance of stony substrata, which support a wide variety of diatom species, whereas less suitable sandy and muddy substrata predominated at lower elevations. A similar explanation may apply to the significant relationships for diatom abundance, whereby density was greater at cooler sites with greater water clarity, which tended to be upland sites with abundant stony substrata.

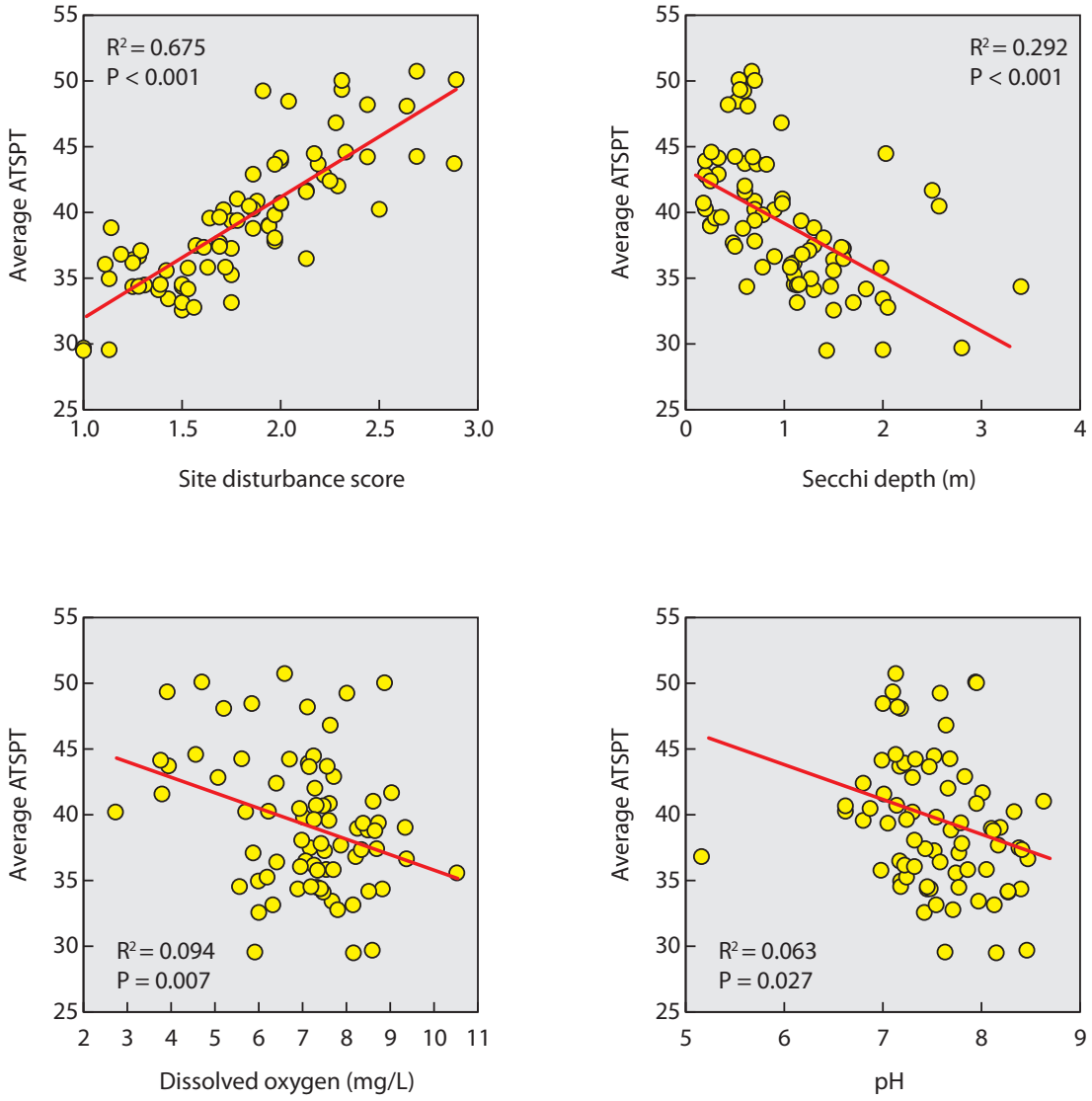

Figure 4.3 Statistically significant relationships of average ATSPT of diatoms to environmental variables.

The ATSPT for diatoms showed obvious relationships with human activity. Its strong relationship with the Site Disturbance Score was to be expected since the SDS was used to derive the tolerance values for individual diatom taxa. However, the strong relationships of ATSPT with Secchi depth and dissolved oxygen, which are well known to be affected by human disturbances such as wastewater disposal and removal of bank vegetation, provide independent corroboration of the sensitivity of ATSPT as an indicator of human impact. The negative association between ATSPT and pH suggests that this indicator will also reflect acidification, e.g. from acid sulphate soils.

5. Zooplankton

5.1 Introduction

Zooplankton are tiny, swimming, animals, that are represented mostly by crustaceans, rotifers, and protozoans. They include both primary consumers that feed on phytoplankton and secondary consumers that feed on other zooplankton. They are a major component of the diet of fishes, especially small fish and the larvae of larger fish, and are therefore essential to the productivity of the Mekong fishery.

Relative to diatoms and macroinvertebrates, zooplankton have not been widely used in biological monitoring studies. According to the scientific literature (Table 1.1), they do offer specific advantages for biomonitoring in that they are a diverse group with a variety of species having a range of responses to environmental changes. Like diatoms, they have a short generation time and thus a rapid response to environmental changes and recovery from disturbance. There are reported disadvantages associated with the use of zooplankton in biological monitoring programs as well (Table 1.2). There are sampling issues associated with their daily fluctuations in abundance and composition, and patchy spatial distributions related to current and depth. As with diatoms, there are few metrics or indices that have been proved to be consistently effective in biomonitoring programs.

Most research on zooplankton in the region has been on taxonomy and species distributions, and importance as fish food. An taxonomic key (which is currently unavailable) to assist the identification of zooplankton species, would help facilitate the use of these animals for biomonitoring on a regional scale.

This chapter describes the zooplankton assemblages recorded from 77 sampling events at 51 sites in 2004 – 07 and their relationships with environmental variables.

5.2 Methods

Sampling and sample processing

Three samples were collected at each site. One was taken near the left bank of the river, at a distance of about 4 – 5 m from the water's edge. A separate sample was taken at a similar distance from the right bank, and another in the middle of the river. The samples were taken at least 1 m from potentially contaminating substances such as debris and aquatic plants, and at least 2 m from vertical banks. At sites where the water current was too fast to sample exactly in the mid-stream, samples were collected closer to the left or the right bank, but not as close to the bank as where the 'side samples' were taken.

Before sampling at each site, the sampling equipment (a net, bucket, and plastic jar) was washed to remove any organisms and other matter left from the previous site. Quantitative samples were collected at a depth of 0 to 0.5 m in a bucket having a volume of 10 L. The 10 L of river water collected was filtered slowly through a plankton net (mesh size of 20 μm) to avoid any overflow. When the water volume remaining in the net was about 150 mL, the water was transferred to a plastic jar (250 mL volume). The samples were immediately fixed in the field with 4% formaldehyde. The sample jars were labelled with the site code, sampling date, and sampling position.

In the laboratory, large particles of debris were removed from the samples with forceps. Each sample was filtered via a net with a mesh size of 10 μm and rinsed with distilled water, and then settled in a graduated cylinder. Excess water was discarded until about 50 mL of water and settled material remained. This was transferred into a petri dish and examined under a stereomicroscope at a magnification of 40x to identify the large species of zooplankton (> 50 μm in diameter). The smaller species and details of larger species were examined on a microscope slide under a compound microscope at a magnification of 100 – 400x. All individuals collected were counted and identified to lowest level of taxonomy possible, generally species. Identification was based on morphology as described in Vietnamese and international references (e.g. Dang *et al*., 1980; Eiji, 1993) After analysis, samples were returned to the bottles and preserved. All specimens are kept at Ton Duc Thang University, Ho Chi Minh City, Viet Nam.

Derivation of biological indicators

Three biological indicators were calculated for all zooplankton samples: richness (the number of taxa of zooplankton identified from each sample), abundance (the number of individual zooplankton per sample), and the average tolerance score per taxon (ATSPT).

Harm to the ecosystem is indicated by unnaturally low richness (low biodiversity), unnaturally low abundance (few organisms present) or an unnaturally high ATSPT. Taxa that are sensitive to stress, and tend to be absent at stressed sites, have low tolerance scores. Stress-tolerant species, which are hardy and survive at stressed sites, have high tolerance scores. Consequently, the average score is higher at sites with environmental stress.

Tolerance scores for individual taxa were derived from the relationship between the presence and absence of taxa in samples from each study site and the value of the Site Disturbance Score for that site (see Chapter 2). The tolerance of each taxon was calculated as the average Site Disturbance Score for all sites at which that taxon occurred, weighted by the number of samples per site in which the taxon was recorded. The tolerance values were then re-scaled so that their possible range was from 0 to 100, where 0 represents low tolerance and 100 represents high tolerance to human-generated stress such as water pollution. The Average Tolerance Score per Taxon (ATSPT) was then calculated for each sample collected. ATSPT is simply the average tolerance of all taxa recorded in a sample. A higher value of ATSPT indicates a more tolerant biota, and hence a more stressed environment.

Linear regression analysis was used to test for statistically significant relationships between the environmental variables that were measured on all 77 sampling occasions and the average richness, abundance and ATSPT of the zooplankton fauna. Abundance data were highly skewed and were therefore converted to logarithms before analysis.

5.3 Results

Biota collected

A total of 86,076 individuals was recorded from 231 samples collected in 2004–2007, comprising 207 taxa (Appendix 2 and 3).

Richness

Average richness per sample ranged from 6.3 to 40.0 taxa (Appendix 2 and 3), and was significantly negatively-related to dissolved oxygen concentration and pH (Figure 5.1).

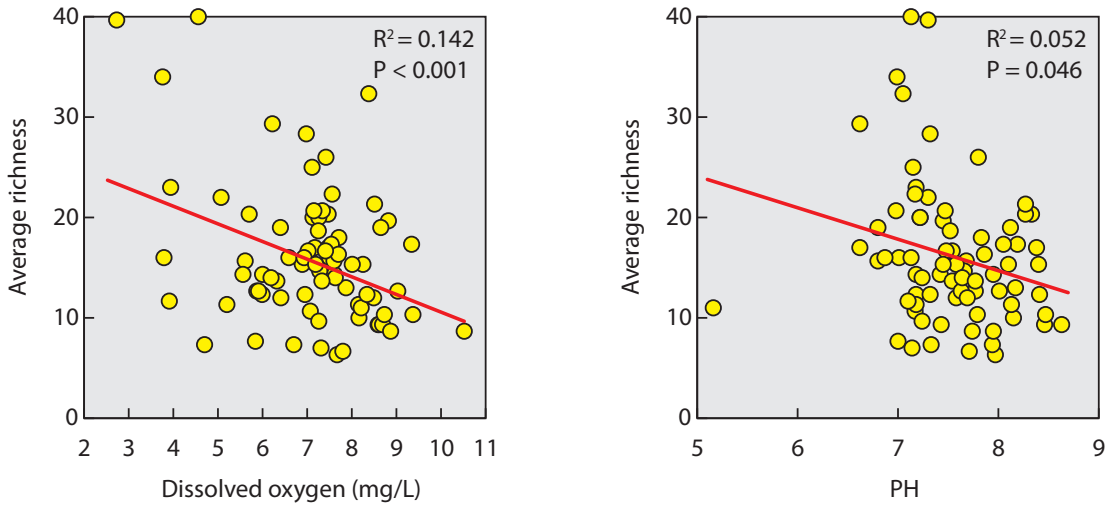

Figure 5.1 Statistically significant relationships of average richness of zooplankton to environmental variables.

Abundance

The average number of zooplankters per sample ranged from 8 to 8394 (Appendix 2 and 3), and the logarithm of average abundance was significantly positively-related to the Site Disturbance Score and electrical conductivity, and significantly negatively-related to the concentration of dissolved oxygen (Figure 5.2).

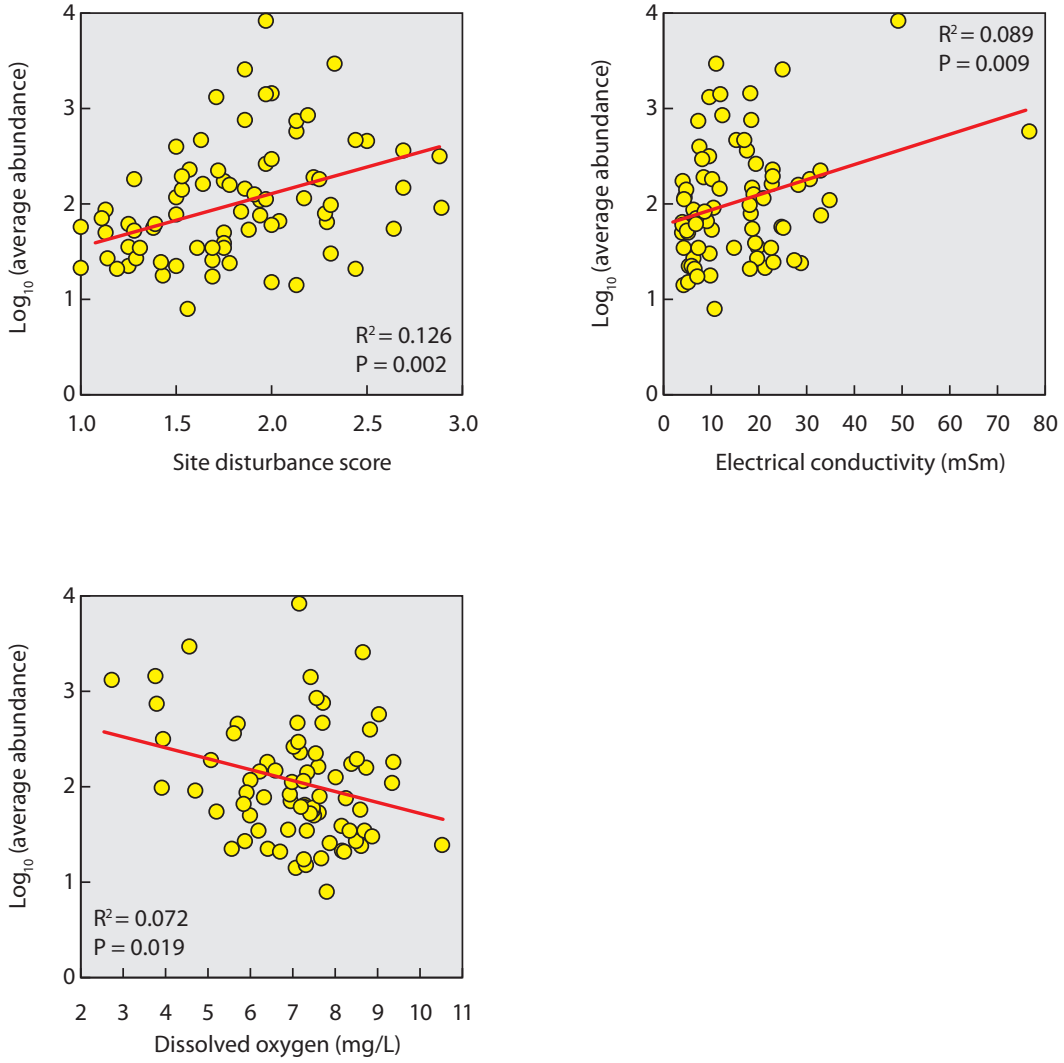

Figure 5.2 Statistically significant relationships of average abundance of zooplankton to environmental variables.

Average Tolerance Score Per Taxon

The tolerance scores for individual taxa of zooplankton varied from 0 to 94 (Appendix 2 and 3). The average ATSPT per sample ranged from 23 to 48, and was significantly positively-related to the Site Disturbance Score, river width and water temperature, and significantly negatively-related to altitude, Secchi depth and dissolved oxygen concentration (Figure 5.3).

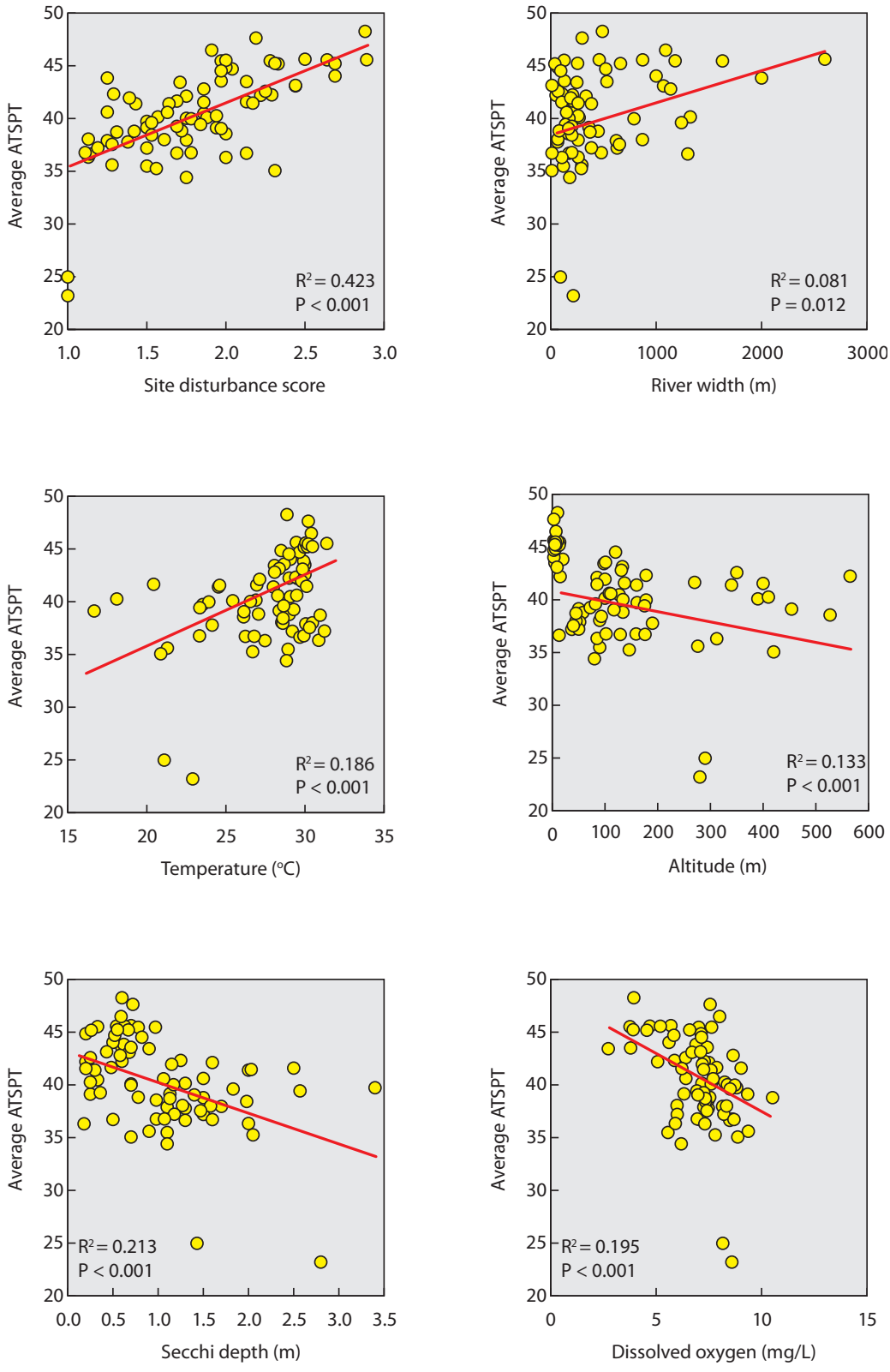

Figure 5.3 Statistically significant relationships of ATSPT of zooplankton to environmental variables.

5.4 Discussion

The tendency for zooplankton taxon richness and abundance to be lower at sites with high dissolved oxygen concentrations may seem surprising because higher oxygen levels are often associated with an absence of organic pollution. However, the relationship may be indirect. Dissolved oxygen concentrations are also often high at sites of higher elevation because of the increased solubility of oxygen in cooler water. The high-elevation sites were often on smaller streams (Chapter 3) with faster current velocities, which are habitat conditions that are not favoured by many zooplankton species.

Zooplankton abundance also tended to be higher at sites with higher Site Disturbance Scores. Some human-generated disturbances such as mild to moderate organic and nutrient enrichment may act as a stimulus to zooplankton by increasing the availability of food in the form of planktonic algae and small, non-living organic particles. Similar processes may underlie the positive relationship between zooplankton abundance and electrical conductivity.

The ATSPT for zooplankton had several significant relationships with environmental variables: the zooplankton fauna tended to be more tolerant of human-generated stress at sites with a high level of human disturbance (as expected from the use of the Site Disturbance Score to derive the tolerance values for individual zooplankton taxa), but also at wide, warmer, low-altitude sites with lower water clarity and lower dissolved oxygen concentrations. Sites with the latter two characteristics, in particular, are often those with a higher level of human influence.

6. Littoral macroinvertebrates

6.1 Introduction

Littoral macroinvertebrates occur in the near-shore areas of rivers and are mainly insects, crustaceans, molluscs, and worms. Some animals occupying this habitat live in or on the river bed while others swim in the water column. The littoral fauna comprises both primary and secondary consumers and includes grazers that scrape diatoms and other material from hard surfaces, shredders that break down leaves and other coarse organic materials, filterers that trap small organic particles moving through the water column, deposit feeders that ingest settled organic particles, and predators. These organisms are an important component of the food web.

Macroinvertebrates in general are the organisms that are most widely used in biomonitoring programs. The scientific literature indicates that macroinvertebrates offer many advantages in biomonitoring (Table 1.1), such as being abundant and widespread in littoral and other habitats, and highly diverse with many species that exhibit a variety of responses to environmental change. They have limited mobility and hence can be used to infer local conditions, and are easily sampled with little specialized training or effort. Because some species have long generation times, they can indicate transient stressors (e.g. periodic spills) that may not be chronic problems. Some disadvantages in their use have been reported, such as seasonal fluctuations in abundance and composition, and the training required for precise identifications (Table 1.2). However, an identification key to the macroinvertebrates of the Lower Mekong River has recently been prepared (Sangpradub and Boonsoong, 2004).

This chapter describes the littoral macroinvertebrate assemblages recorded from 77 sampling events at 51 sites in 2004 – 07 and their relationships with environmental variables.

6.2 Methods

Sampling and sample processing

Littoral macroinvertebrate samples usually were taken on only one side of the river at each site. In most instances this was the depositional side where sampling was easier because of the gradual shelving of the bottom that occurs in this setting in contrast to the steeper bottom that is characteristic of the erosional side. In addition, the depositional side tends to support more aquatic vegetation, which also provides more habitat suitable for invertebrates. Because the study area was large, a wide range of littoral habitat types was sampled. As far as possible, similar habitats were selected at each site to facilitate comparisons among sites.

A D-frame net with 30 cm x 20 cm opening and mesh size of 475 μm was used to take two types of samples: sweep and kick samples. Sweep samples were taken along the shore at intervals of about 20 m. To obtain each sweep sample, the collector stood in the river about

1.5 m from the water's edge and swept the net 10 times along the substrate toward the bank, in positions that did not overlap. Kick sampling was done off-riverbank in areas of rapid current, and involved kicking the substrate in an area of 30 x 30 cm, or using fingers to disturb this area, for about 20 seconds, with the net held downstream to catch dislodged animals. A range of substrates was sampled, including cobbles, gravel, sand, silt, mud, and aquatic plants.

Between five and ten sweep samples were taken per site for all 77 sampling events. Five kick samples per site were taken at those sites where suitable habitat was present, except in 2004 when no kick samples were collected.

After sample collection, the net contents were washed to the bottom of the net. The net was inverted and its contents were emptied into a metal sorting tray, with any material adhering to the net being washed off with clean water. Invertebrates were picked from the tray with forceps and placed in a jar of 70% ethanol. Small samples were kept in 30 mL jars and large samples were kept in 150 mL jars. During the picking process, the tray was shaken from time to time to redistribute the contents, and tilted occasionally to look for animals adhering to it. Sorting proceeded by working back and forth across the tray until no more animals were found. The sample jars were labelled with the site location code, date, and sample replicate number. The collector's name, the sampling site, and replicate characteristics (including substratum types sampled) were recorded in a field notebook.

In the laboratory, the samples were identified under a stereomicroscope with a 2-4x objective lens and a 10x eyepiece. Identification was done to the lowest taxonomic level that could be applied accurately, which was usually to genus. The references used for identification included Sangpradub and Boonsoong (2004), Nguyen *et al*. (2000), and Merritt and Cummins (1996). Specimens were divided into orders, kept in separate jars. All specimens were stored in the Department of Biology at the National University of Laos.

Derivation of biological indicators

Three biological indicators were calculated for all littoral macroinvertebrate samples: richness (the number of taxa of macroinvertebrates identified from each sample), abundance (the number of individual macroinvertebrates per sample), and the average tolerance score per taxon (ATSPT).

Harm to the ecosystem is indicated by unnaturally low richness (low biodiversity), unnaturally low abundance (few organisms present) or an unnaturally high ATSPT. Taxa that are sensitive to stress, and tend to be absent at stressed sites, have low tolerance scores. Stress-tolerant species, which are hardy and survive at stressed sites, have high tolerance scores. Consequently, the average score is higher at sites with environmental stress.

Tolerance scores for individual taxa were derived from the relationship between the presence and absence of taxa in samples from each study site and the value of the Site Disturbance Score for that site (see Chapter 2). The tolerance of each taxon was calculated as the average Site

Disturbance Score for all sites at which that taxon occurred, weighted by the number of samples per site in which the taxon was recorded. The tolerance values were then re-scaled so that their possible range was from 0 to 100, where 0 represents low tolerance and 100 represents high tolerance to human-generated stress such as water pollution. The Average Tolerance Score per Taxon (ATSPT) was then calculated for each sample collected. ATSPT is simply the average tolerance of all taxa recorded in a sample. A higher value of ATSPT indicates a more tolerant biota, and hence a more stressed environment.

Linear regression analysis was used to test for statistically significant relationships between the environmental variables that were measured on all 77 sampling occasions and the average richness, abundance and ATSPT of the littoral macroinvertebrate fauna. Abundance data were highly skewed and were therefore converted to logarithms before analysis.

6.3 Results

Biota collected

In total, 81,186 individuals and 361 taxa of littoral macroinvertebrates were collected in 2004-07 (Appendix 2 and 3).

Richness

Average richness per sweep sample ranged from 1.8 to 20.4 taxa (Appendix 2 and 3), and was significantly positively-related to water transparency (Secchi depth) and significantly negatively-related to the Site Disturbance Score (Figure 6.1). Average richness per kick sample was higher, ranging from 5.0 to 39.4 taxa.

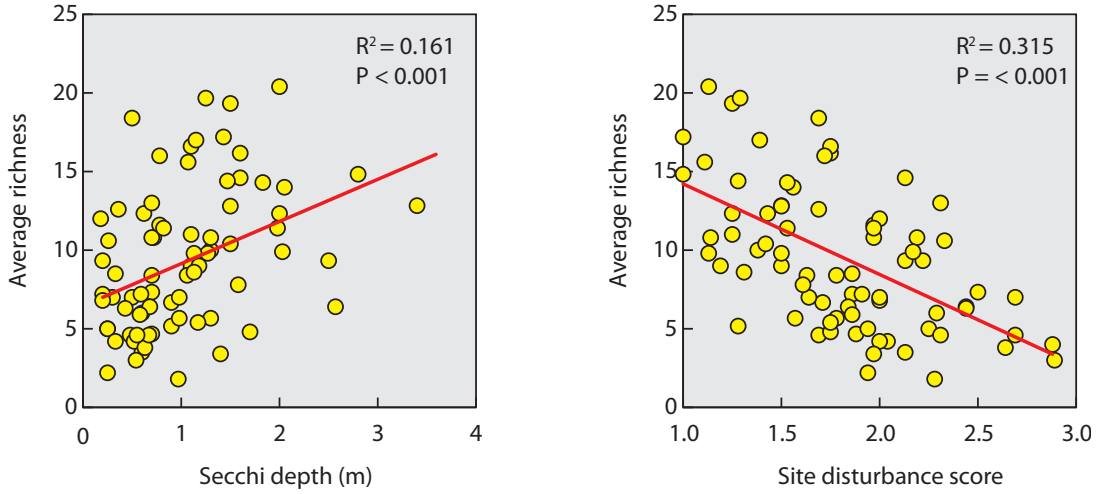

Figure 6.1 Statistically significant relationships of average richness of littoral macroinvertebrates (sweep samples) to environmental variables.

Abundance

The average number of littoral invertebrates per sweep sample ranged from 4 to 1627 (Appendix 2 and 3). The logarithm of abundance in sweep samples was significantly positively-related to water transparency (Secchi depth), dissolved oxygen concentration and pH, and significantly negatively-related to the Site Disturbance Score (Figure 6.2). The average number of individuals per kick sample had a narrower range from 13 to 466.

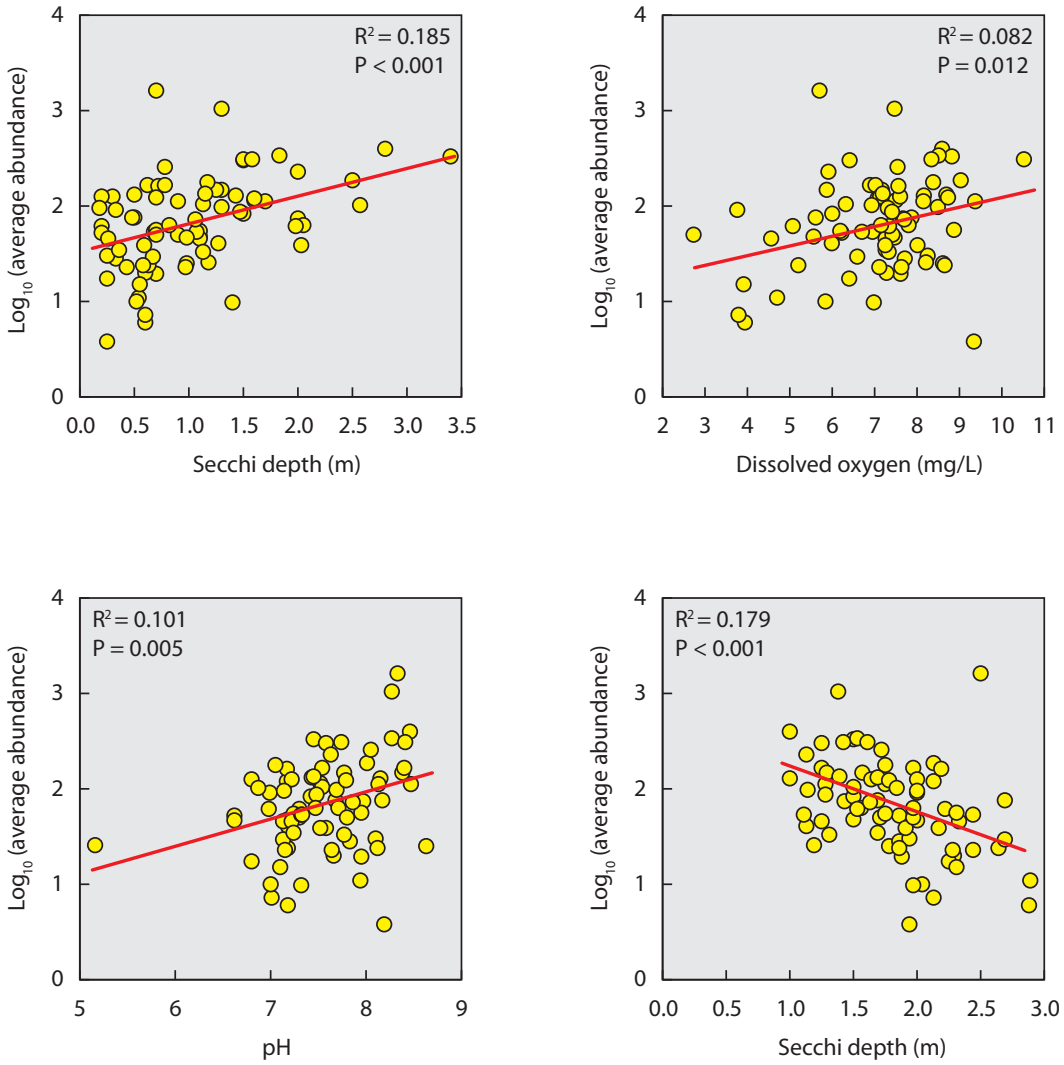

Figure 6.2 Statistically significant relationships of average richness of littoral macroinvertebrates (sweep samples) to environmental variables.

Average Tolerance Score per Taxon

The tolerance scores for individual taxa of littoral macroinvertebrates ranged from 0 to 84 (Appendix 2 and 3). The average ATSPT for sweep samples ranged from 24 to 46, and was significantly

positively correlated with the Site Disturbance Score and water temperature, and negatively correlated with altitude, water transparency (Secchi Depth) and dissolved oxygen concentration. (Figure 6.3).

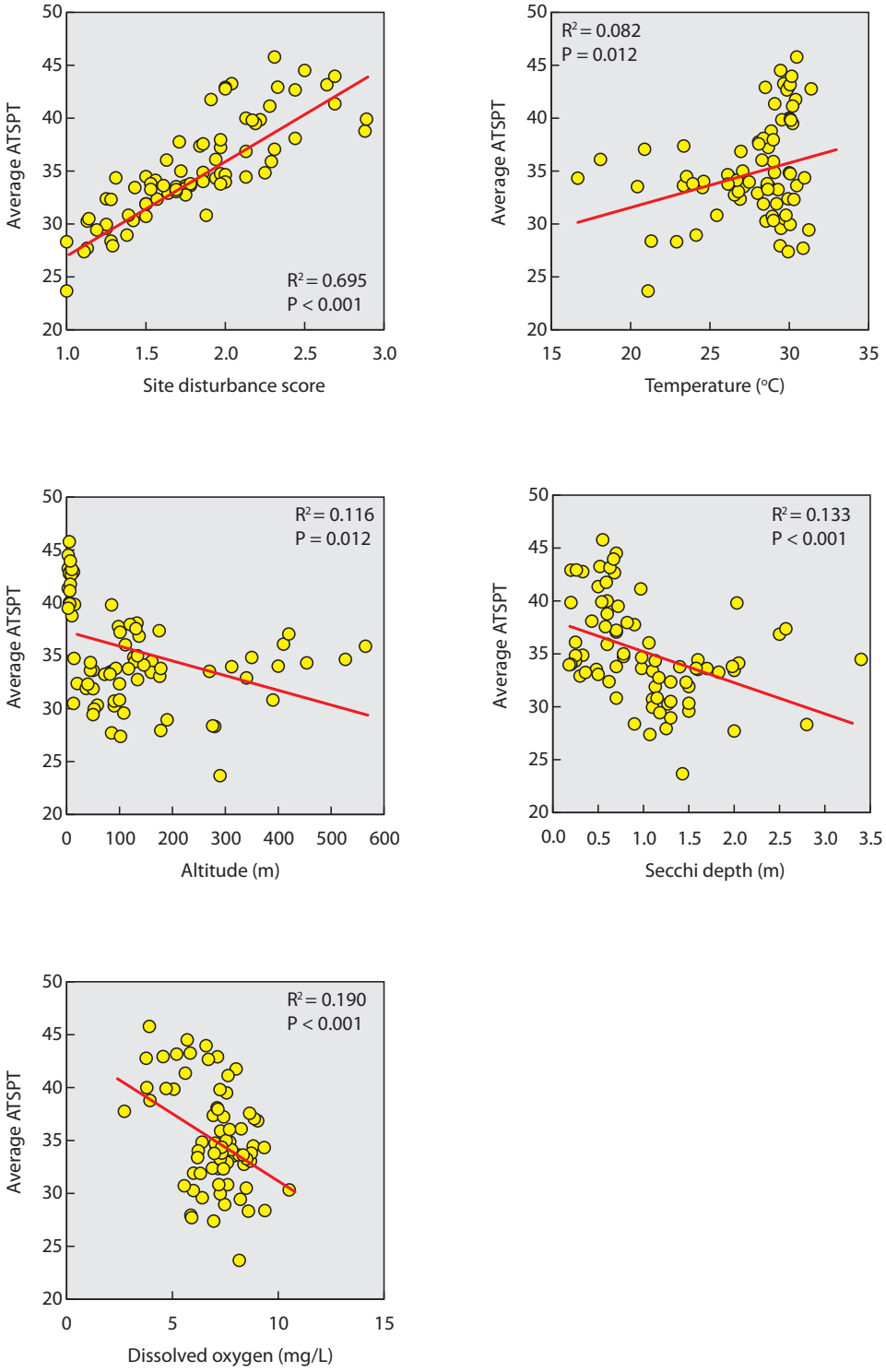

Figure 6.3 Statistically significant relationships of average ATSPT of littoral macroinvertebrates (sweep samples) to environmental variables.

6.4 Discussion

The negative relationships between the richness and abundance of littoral macroinvertebrates and the Site Disturbance Score suggest that the littoral fauna is particularly susceptible to the impact of local human activities. Richness and abundance also tended to be higher in clearer rivers, which may be partly a natural phenomenon but probably also reflects the tendency of human activities to increase soil erosion and therefore reduce water clarity. Reduced water clarity and associated higher levels of suspended particles can adversely affect the littoral fauna by clogging of the gills of sensitive species and by decreasing light penetration and hence reducing algal food sources. As reported in Chapter 4, the density of benthic diatoms, a common food source for macroinvertebrates, tended to be greater at sites with clearer waters.

The positive relationship of abundance of littoral macroinvertebrates with dissolved oxygen concentration was expected, because many littoral species are sensitive to low dissolved-oxygen concentrations resulting from organic pollution. The positive correlation between abundance and pH may have been related to food availability, since high algal production is typically associated with a higher pH.

As expected, the ATSPT was strongly correlated with the Site Disturbance Score that was used in the derivation of tolerance values for individual taxa, but it was also correlated with water transparency (Secchi depth) and dissolved oxygen, indicating that sensitive macroinvertebrate taxa favour clear, well oxygenated waters.

7. Benthic macroinvertebrates

7.1 Introduction

The benthic macroinvertebrates are those organisms that occur in or on the bed of rivers, including those parts in deep water away from the littoral zone. The deepwater benthos includes the same major groups as that of the littoral zone, but is usually less diverse. Most deepwater species are deposit feeders that consume small particles of organic matter or filter feeders that remove particles from the water column.

Of the biomonitoring advantages reported in the scientific literature, the ones that specifically apply to benthic macroinvertebrates are that they have limited mobility and reflect local conditions, and that because some species are long lived they may reflect conditions that are not chronic problems (Table 1.1). The disadvantage of benthic macroinvertebrates is that some of the species may be very difficult to identify to precise taxonomic levels, even more so than for the littoral macroinvertebrates (Table 1.2).

This chapter describes the benthic macroinvertebrate assemblages recorded from 77 sampling events at 51 sites in 2004–07 and their relationships with environmental variables.

7.2 Methods

Sampling and sample processing

Sample locations at each site were selected in each of the right, middle, and left parts of the river. Five locations were sampled at each of these parts of the river. At some sites, the middle of river could not be sampled because of the presence of hard beds or fast currents. Also, sites narrower than 30 m were sometimes not sampled in the middle portion. Prior to sampling, all the equipment to be used was thoroughly cleaned to remove any material left from the previous sampling site.

At each sampling location, a composite of four grabs was taken with a Petersen grab sampler, covering a total area of 0.1 m^2. If the sampler did not close properly because material such as wood, bamboo, large water-plants, or stones jammed its jaws, its contents were discarded and another grab was taken. The composite sample was washed through a sieve (0.3 mm) with care taken to ensure that macroinvertebrates did not escape. The contents of the sieve were then placed in a white sorting tray and dispersed in water. All the animals in the tray were picked out with forceps and pipettes, placed in jars, and fixed with formaldehyde. Samples of less experienced sorters were checked by an experienced sorter. The sample jar was labelled with the site location code, date, position within the river, and replicate number. The sampling location conditions, collector's name and sorter's name were recorded on a field sheet.

Sometimes, samples could not be sorted on site because the boat was poorly balanced, because a very large number of animals was collected, because there was insufficient time at a site, or because the presence of lumps of clay caused the samples to cloud continually. In these cases, samples were sorted in the laboratory.

All individuals collected were identified and counted under a compound microscope (with magnifications of 40 – 1200x) or a dissecting microscope (16 – 56x). Oligochaeta, Gastropoda, Bivalvia, and Crustacea were generally identified to species level. Insecta and Insecta larvae were classified only to genus level. The results were recorded on data sheets and specimens are kept at the Ton Duc Thang University, HCMC, Viet Nam.

Derivation of biological indicators

Three biological indicators were calculated for all benthic macroinvertebrate samples: richness (the number of taxa of macroinvertebrates identified from each sample), abundance (the number of individual macroinvertebrates per sample), and the average tolerance score per taxon (ATSPT).

Harm to the ecosystem is indicated by unnaturally low richness (low biodiversity), unnaturally low abundance (few organisms present) or an unnaturally high ATSPT. Taxa that are sensitive to stress, and tend to be absent at stressed sites, have low tolerance scores. Stress-tolerant species, which are hardy and survive at stressed sites, have high tolerance scores. Consequently, the average score is higher at sites with environmental stress.

Tolerance scores for individual taxa were derived from the relationship between the presence and absence of taxa in samples from each study site and the value of the Site Disturbance Score for that site (see Chapter 2). The tolerance of each taxon was calculated as the average Site Disturbance Score for all sites at which that taxon occurred, weighted by the number of samples per site in which the taxon was recorded. The tolerance values were then re-scaled so that their possible range was from 0 to 100, where 0 represents low tolerance and 100 represents high tolerance to human-generated stress such as water pollution. The Average Tolerance Score per Taxon (ATSPT) was then calculated for each sample collected. ATSPT is simply the average tolerance of all taxa recorded in a sample. A higher value of ATSPT indicates a more tolerant biota, and hence a more stressed environment.

Linear regression analysis was used to test for statistically significant relationships between the environmental variables that were measured on all 77 sampling occasions and the average richness, abundance and ATSPT of the benthic macroinvertebrate fauna. Abundance data were highly skewed and were therefore converted to logarithms before analysis.

7.3 Results

Biota collected

In total, 23,470 benthic macroinvertebrates belonging to 177 taxa were collected in 2004–2007 (Appendix 2).

Richness

Average richness per sample ranged from 0.3 to 12.0 taxa (Appendix 2), and was significantly positively correlated with water transparency (Secchi depth) and pH (Figure 7.1).

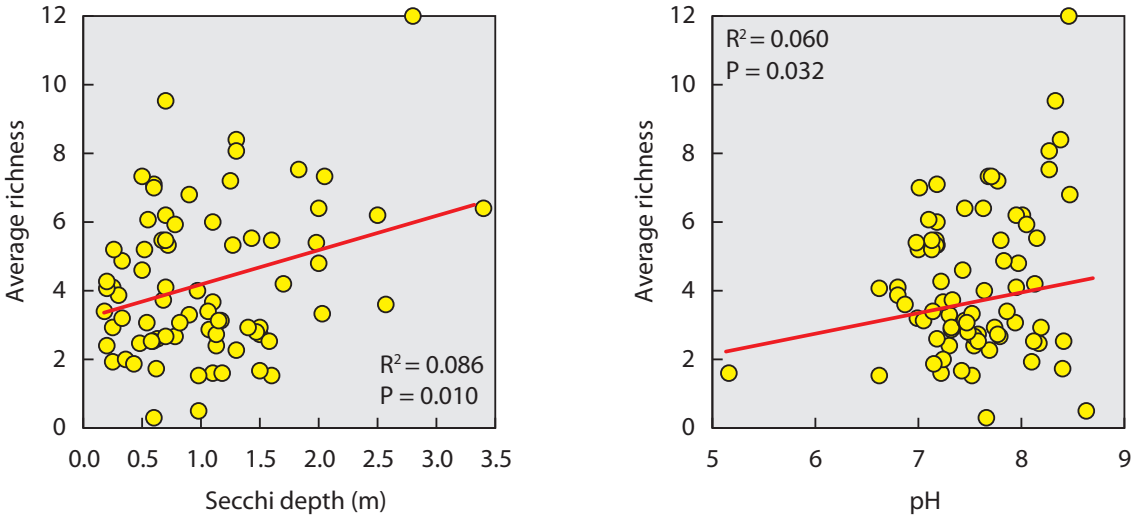

Figure 7.1 Statistically significant relationships of average richness of benthic macroinvertebrates to environmental variables.

Abundance

The average number of individual macroinvertebrates per benthic sample ranged from 1 to 219. The logarithm of abundance had a significant positive correlation with electrical conductivity (Figure 7.2).

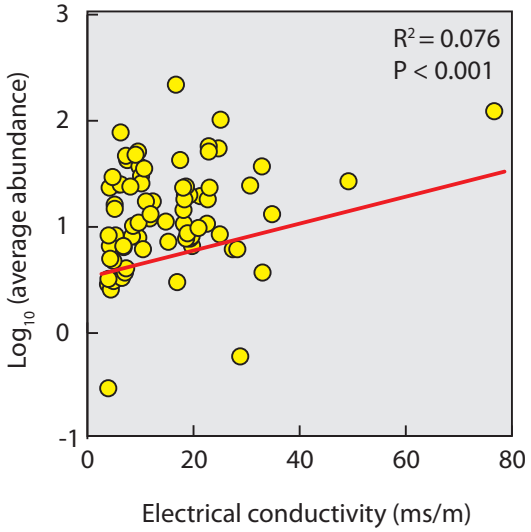

Figure 7.2 Statistically significant relationship of average abundance of benthic macroinvertebrates to electrical conductivity.

Average Tolerance Score Per Taxon

The tolerance scores for individual taxa of benthic macroinvertebrates varied from 0 to 94 (Appendix 2). The average ATSPT per sample ranged from 23 to 63 and had statistically significant positive correlations with the Site Disturbance Score, river width and water temperature, as well as statistically significant negative correlations with altitude, water transparency (Secchi depth) and dissolved oxygen (Figure 7.3).

7.4 Discussion

As for the littoral fauna, the positive relationships of benthic richness with water transparency and pH may represent a response to greater algal productivity. Reasons for the positive relationship between benthic abundance and electrical conductivity are not clear, but may have been an indirect consequence of relationships between EC and other factors, such as substratum suitability. The associations between the ATSPT of the benthic fauna and environmental variables were also very similar to those for the littoral fauna, and indicated that the most tolerant fauna tended to be found in large, warm, turbid, lowland rivers with low dissolved oxygen concentrations. This is a typical finding for benthic macroinvertebrates worldwide.

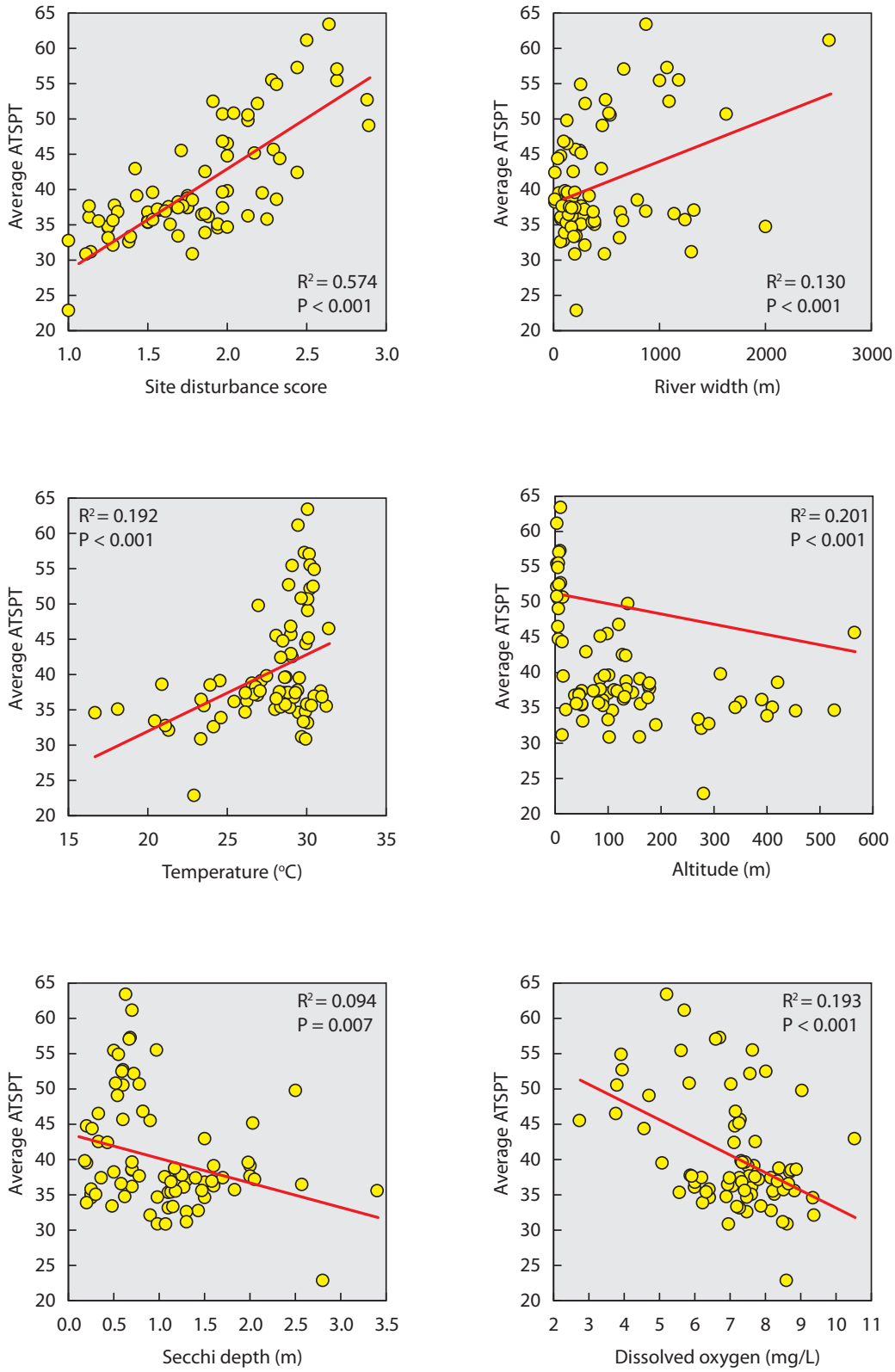

Figure 7.3 Statistically significant relationships of average ATSPT of benthic macroinvertebrates to environmental variables.

8. The use of biological indicators of harm to classify and rate sites

As described in previous chapters, three types of indicators of harm to the aquatic ecosystem were calculated for each of four groups of organisms included in the biomonitoring programme (diatoms, zooplankton, littoral macroinvertebrates and benthic macroinvertebrates). These indicators were richness (the number of taxa per standard sample), abundance (the number of individual organisms per sample), and tolerance (the average tolerance score per taxon calculated for each sample). Harm to the ecosystem is indicated by low richness (low biodiversity), low abundance (few organisms present) or a high average tolerance score (signifying a scarcity of pollution-sensitive species and a predominance of hardy species that are able to withstand pollution).

Each indicator was calculated for the individual samples of each group of organisms that were collected when a site was visited. The collection of multiple samples per site enables assessment of within-site variability of the indicators and also statistical testing of the significance of differences among sites and within the same site over multiple years. For overall assessment of a site, the values of each indicator from individual samples were averaged.

Interim guidelines for site-average values of each indicator were set according to the range of site-average values obtained at the reference sites. For indicators where low values indicate harm to the ecosystem (richness and abundance) the guideline was set at the 10th percentile of reference site values (the value that is lower than 90 percent of all reference values). For the indicator where a high value indicates harm to the ecosystem (tolerance) the guideline was set at the 90th percentile of reference site values (the value than is higher than 90 percent of all reference values). These percentiles are commonly used in biomonitoring programmes in other parts of the world. Interim guidelines are listed in Table 8.1.

Table 8.1 *Interim guidelines for biological indicators of harm to the ecosystem.*

Indicator	Indication of harm to the ecosystem	Biological group	Reference site values			Interim guideline
			10th percentile	50th percentile (median)	90th percentile	
Richness (average number of taxa per standard sample or sub-sample).	Low value	Diatoms	6.54	9.30	11.78	Greater than 6.54
		Zooplankton	9.80	12.67	20.20	Greater than 9.80
		Littoral macroinvertebrates	5.37	11.40	18.48	Greater than 5.37
		Benthic macroinvertebrates	1.84	3.87	7.85	Greater than 1.84
Abundance (average number of individual organisms per standard sample).	Low value	Diatoms	136.22	257.30	376.34	Greater than 136.22
		Zooplankton	22.33	52.33	174.07	Greater than 22.33
		Littoral macroinvertebrates	46.68	124.80	328.56	Greater than 46.68
		Benthic macroinvertebrates	4.13	18.33	56.34	Greater than 4.13
Tolerance (average of average tolerance score per taxon per standard sample).	High value	Diatoms	30.85	35.58	38.38	Less than 38.38
		Zooplankton	34.83	38.58	41.80	Less than 41.80
		Littoral macroinvertebrates	27.80	30.72	33.58	Less than 33.58
		Benthic macroinvertebrates	31.57	35.36	37.74	Less than 37.74

The sites were classified and grouped according to the number of the 12 indicators that met the guidelines. Table 8.2 gives definitions of the classification and some characteristics to be expected for sites in each class. Figure 8.1 and Table 8.3 give the assessment of all sites for each sampling occasion. Of the 77 sampling events over four years, 28 were in Class A, 32 in Class B, and 17 in Class C. None was in Class D. This rating suggests that the principal rivers of Lower Mekong Basin have not yet suffered severe harm from the development of water resources or waste disposal. However, some rivers are showing signs of stress.

Table 8.2 *Definition and characteristics of the classification system.*

Class	Rating criterion	Characteristic features
A: Excellent	10 – 12 of 12 indicators meet guidelines	Level of biodiversity is the same as reference site conditions. Species composition is dominated by taxa that are sensitive to pollution. Ecological capacity of the river to support production of fish and other biological products within the range of capacity of reference sites* Minimal disturbance from human activities.
B: Good	7 – 9 of 12 indicators meet guidelines	Level of biodiversity slightly reduced from reference site conditions. Species composition has many taxa that are sensitive to pollution. Ecological capacity of the river to support production of fish and other biological products slightly below the range of capacity of reference sites* Some disturbance from human activities.
C: Moderate	4 – 6 of 12 indicators meet guidelines	Level of biodiversity is notably less than under reference site conditions. Species composition is a mixture of taxa that are sensitive to pollution and taxa that are tolerant to pollution. Ecological capacity of the river to support production of fish and other biological products moderately below the range of capacity of reference sites* Some impacts from human activities.
D: Poor	0 – 3 of 12 indicators meet guidelines	Level of biodiversity significantly altered from reference site conditions. Species composition dominated by taxa that are tolerant to pollution. Ecological capacity of the river to support production of fish and other biological products far below the range of capacity of reference sites* Several negative to extensive adverse impacts from human activities.

* Ecological capacity to support production of fish means that the riverine food web that fish depend on (including algae, zooplankton, and macroinvertebrates) is maintained. However, even if ecological capacity is maintained, actual fish production may be detrimentally affected by other factors such as excessive harvesting, fish diseases, migration barriers such as dams, and loss of floodplain habitat during the wet season. These factors were not assessed in the biomonitoring programme.

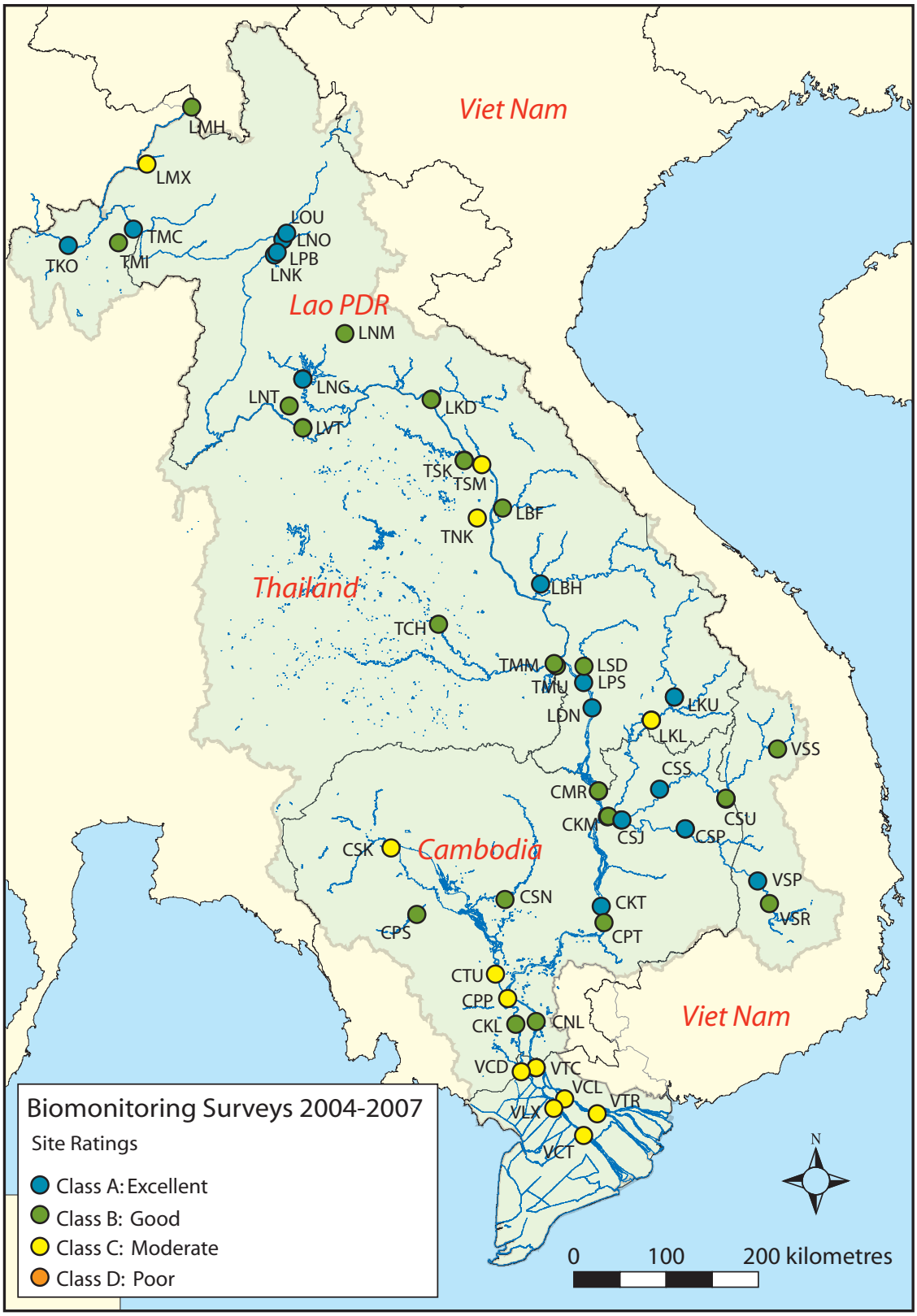

Figure 8.1 Ratings of sites in the Lower Mekong Basin sampled during 2004–2007. If a site was sampled more than once and had varying ratings, the most recent is shown.

Table 8.3 *Assessment of all sites against suggested guidelines. Y = meets guideline; N = does not meet guideline.*

Site	Sampling date	Diatom richness	Diatom abundance	Diatom tolerance	Zooplankton richness	Zooplankton abundance	Zooplankton tolerance	Littoral macroinvertebrate richness	Littoral macroinvertebrate abundance	Littoral macroinvertebrate tolerance	Benthic macroinvertebrate richness	Benthic macroinvertebrate abundance	Benthic macroinvertebrate tolerance	Number meeting guidelines	Class
CKL	07-March-2006	Y	Y	N	Y	Y	N	Y	Y	N	Y	Y	N	8	B
CKM	26-March-2005	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	N	Y	11	A
CKM	16-March-2006	Y	Y	Y	Y	N	Y	Y	N	Y	N	N	Y	8	B
CKM	18-March-2007	Y	N	Y	Y	Y	Y	Y	N	N	Y	N	Y	8	B
CKT	23-March-2004	Y	Y	Y	Y	Y	Y	Y	Y	Y	N	Y	Y	11	A
CKT	14-March-2006	Y	N	N	Y	Y	Y	Y	Y	Y	Y	Y	Y	10	A
CMR	24-March-2005	N	Y	Y	Y	Y	Y	N	Y	N	Y	Y	Y	9	B
CMR	15-March-2006	Y	Y	Y	N	Y	Y	Y	Y	Y	Y	Y	N	10	A
CMR	17-March-2007	N	N	Y	Y	Y	Y	Y	Y	N	Y	Y	Y	9	B
CNL	08-March-2006	Y	Y	N	Y	Y	N	Y	Y	N	Y	Y	N	8	B
CPP	17-March-2004	N	Y	N	Y	Y	N	N	N	N	Y	Y	N	5	C
CPP	06-March-2006	Y	Y	N	N	Y	N	N	N	N	Y	Y	N	5	C
CPS	18-March-2004	Y	Y	N	Y	Y	N	Y	Y	N	Y	Y	N	8	B
CPT	13-March-2006	Y	Y	N	Y	Y	N	Y	N	N	Y	Y	N	7	B
CSJ	25-March-2005	Y	Y	Y	Y	Y	Y	Y	Y	Y	N	N	Y	10	A
CSJ	16-March-2006	Y	Y	Y	Y	Y	Y	Y	N	Y	N	N	Y	9	B
CSJ	19-March-2007	N	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	11	A
CSK	11-March-2006	N	N	N	Y	Y	N	N	Y	N	Y	Y	N	5	C
CSN	10-March-2006	Y	Y	N	Y	Y	N	Y	Y	N	Y	Y	N	8	B
CSP	21-March-2004	Y	Y	Y	Y	N	N	Y	Y	Y	Y	Y	Y	10	A
CSP	29-March-2005	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	12	A
CSP	18-March-2006	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	12	A
CSP	21-March-2007	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	12	A
CSS	20-March-2004	Y	Y	Y	Y	Y	Y	Y	Y	Y	N	N	N	9	B
CSS	28-March-2005	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	12	A
CSU	27-March-2005	Y	Y	Y	Y	N	Y	Y	Y	N	Y	Y	Y	10	A
CSU	19-March-2006	N	Y	N	Y	Y	Y	Y	Y	Y	Y	Y	N	9	B
CSU	20-March-2007	N	Y	Y	Y	Y	Y	N	N	N	Y	Y	Y	8	B
CTU	17-March-2004	Y	Y	N	Y	Y	N	N	N	N	Y	Y	N	6	C
CTU	09-March-2006	N	Y	N	N	Y	N	N	N	N	Y	Y	N	4	C
LBF	10-March-2007	N	N	Y	Y	Y	Y	Y	Y	N	Y	Y	Y	9	B
LBH	11-March-2007	Y	Y	Y	Y	Y	Y	Y	Y	N	Y	Y	Y	11	A
LDN	16-March-2007	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	12	A
LKD	10-March-2004	Y	Y	Y	N	N	N	Y	Y	Y	Y	Y	N	8	B
LKD	09-March-2007	Y	Y	Y	N	N	Y	Y	Y	N	Y	Y	Y	9	B
LKL	21-March-2005	Y	Y	Y	Y	N	Y	Y	Y	Y	Y	Y	Y	11	A
LKL	14-March-2007	Y	N	N	N	N	Y	Y	N	Y	Y	N	Y	6	C
LKU	20-March-2005	Y	Y	Y	Y	Y	Y	Y	N	Y	Y	Y	Y	11	A

Site	Sampling date	Diatom richness	Diatom abundance	Diatom tolerance	Zooplankton richness	Zooplankton abundance	Zooplankton tolerance	Littoral macroinvertebrate richness	Littoral macroinvertebrate abundance	Littoral macroinvertebrate tolerance	Benthic macroinvertebrate richness	Benthic macroinvertebrate abundance	Benthic macroinvertebrate tolerance	Number meeting guidelines	Class
LKU	15-March-2007	Y	Y	Y	Y	Y	Y	Y	Y	N	Y	Y	N	10	A
LMH	12-March-2005	Y	Y	N	Y	Y	N	N	N	N	Y	Y	Y	7	B
LMX	13-March-2005	Y	N	N	Y	Y	Y	N	N	N	Y	N	Y	6	C
LNG	09-March-2004	Y	Y	Y	Y	Y	Y	Y	Y	N	Y	Y	Y	11	A
LNG	07-March-2007	Y	Y	N	Y	Y	Y	Y	Y	N	Y	Y	Y	10	A
LNK	10-March-2005	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	12	A
LNM	08-March-2007	Y	Y	N	N	Y	Y	Y	Y	N	Y	Y	N	8	B
LNO	07-March-2004	Y	Y	Y	N	Y	Y	Y	Y	Y	Y	Y	Y	11	A
LNT	05-March-2007	Y	N	Y	N	Y	Y	Y	Y	Y	Y	Y	N	9	B
LOU	09-March-2005	Y	Y	Y	Y	N	Y	Y	Y	Y	Y	Y	Y	11	A
LPB	07-March-2004	Y	Y	Y	Y	Y	Y	N	Y	Y	Y	Y	Y	11	A
LPB	10-March-2005	Y	Y	Y	Y	Y	N	N	Y	Y	Y	Y	Y	10	A
LPS	11-March-2004	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	12	A
LSD	12-March-2007	Y	N	Y	Y	Y	N	Y	Y	N	Y	Y	N	8	B
LVT	08-March-2004	Y	Y	N	N	Y	Y	Y	N	N	N	N	Y	6	C
LVT	06-March-2007	Y	Y	N	Y	Y	Y	Y	Y	N	Y	Y	N	9	B
TCH	13-March-2004	Y	Y	N	Y	Y	Y	Y	N	N	Y	Y	N	8	B
TKO	15-March-2004	Y	Y	N	Y	Y	Y	N	N	Y	Y	Y	Y	9	B
TKO	17-March-2005	Y	Y	N	Y	Y	Y	Y	Y	N	Y	Y	Y	10	A
TMC	16-March-2005	Y	Y	N	Y	Y	N	Y	Y	Y	Y	Y	Y	10	A
TMI	16-March-2005	Y	Y	N	Y	Y	N	N	N	N	Y	Y	Y	7	B
TMM	23-March-2007	Y	Y	N	Y	Y	N	Y	N	N	Y	Y	N	7	B
TMU	12-March-2004	Y	Y	N	Y	Y	N	Y	Y	N	Y	Y	N	8	B
TNK	24-March-2007	Y	N	N	Y	Y	N	Y	N	N	Y	N	N	5	C
TSK	14-March-2004	Y	Y	N	Y	Y	N	Y	Y	N	Y	Y	N	8	B
TSK	25-March-2007	N	Y	N	Y	Y	N	Y	Y	N	Y	Y	N	7	B
TSM	26-March-2007	N	N	N	Y	Y	N	Y	N	N	Y	Y	Y	6	C
VCD	26-March-2004	Y	Y	N	Y	Y	N	Y	Y	N	Y	Y	N	8	B
VCD	28-March-2006	Y	Y	N	Y	Y	N	N	N	N	Y	Y	N	6	C
VCL	26-March-2006	N	Y	N	Y	Y	N	Y	N	N	Y	Y	N	6	C
VCT	24-March-2006	N	N	N	Y	Y	N	N	N	N	Y	Y	N	4	C
VLX	25-March-2006	N	Y	N	Y	Y	N	N	N	N	Y	Y	N	5	C
VSP	29-March-2004	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	N	11	A
VSR	21-March-2006	Y	Y	N	N	N	Y	Y	Y	N	Y	Y	N	7	B
VSS	28-March-2004	Y	Y	N	Y	Y	N	Y	N	N	N	N	N	5	C
VSS	20-March-2006	Y	Y	N	Y	Y	Y	Y	Y	N	N	N	Y	8	B
VTC	25-March-2004	Y	Y	N	Y	Y	N	Y	Y	N	Y	Y	N	8	B
VTC	27-March-2006	Y	Y	N	Y	Y	N	N	N	N	Y	Y	N	6	C
VTR	23-March-2006	Y	N	N	N	N	N	Y	Y	N	Y	Y	N	5	C

9. Future directions

The biomonitoring programme to date has defined suitable biological groups and indicators for monitoring the aquatic ecosystems of the Mekong river system and developed a workable preliminary methodology for comparing, classifying and rating sites across the Lower Mekong Basin. This has allowed biomonitoring to be adopted by the member states of the MRC as a routine activity complementing physical and chemical monitoring, commencing in 2008.

The current biological groups, indicators, guidelines and rating scheme should be seen as a starting point for ongoing biomonitoring. All of these aspects should be subject to further testing and evaluation over time, so that they can be improved, refined and added to as required.

The absence of fish from the programme is a significant limitation because fish are clearly very important to the people of the Lower Mekong Basin. The most cost-effective method for sampling fish in large-scale biomonitoring is electric fishing, which temporarily stuns fish, allowing them to be easily captured, identified and released unharmed. This method might be included in future monitoring programmes in the Mekong, but to achieve this, the following matters would need to be resolved:

- Electric fishing in deep water requires a large boat specially built and equipped for the purpose. This boat would have to be either bought or rented and transported to each site. This is an expensive option when compared to other biological sampling that can be done from small boats hired locally.

- Electric fishing is hazardous to sampling personnel, and consequently requires a high level of training and rigorous safety measures.

- Other people, including local villagers, must to be excluded from the vicinity of sampling operations to avoid the risk of electric shock and electrocution.

- Local fishers may attempt to copy the method. This practice may be illegal and can lead to over-exploitation. Furthermore, electric fishing has caused serious injury to, and even the death of, fishers who use the gear carelessly.

At present, only 14 reference sites have been identified and data from each monitoring site are compared against guidelines based on the variation of indicators among all 14 sites. It is common in biomonitoring programmes worldwide to develop separate reference data for individual sites or types of sites, in order to take more account of natural variation in reference conditions. Such an approach would be a valuable future addition to the Mekong programme, but would require the identification and sampling of a large number of reference sites, and further studies to understand the causes of natural variation in indicators among reference sites. For example, natural variations in substratum types may account for some of the biological variability among reference sites. A particular need is to try to locate suitable reference sites in the delta region, to check whether the current interim guidelines are appropriate to this

distinctive part of the Lower Mekong Basin. Future monitoring data will also allow the further development and refinement of tolerance scores for individual taxa, especially those that have been seldom collected as yet.

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Appendix 1. Physical and chemical variables and site disturbance

Site Number	Year Sampled	Altitude (m)	River width (m)	Depth (m)	Secchi depth (m)	Temperature (°C)	DO (mg/L)	pH	EC (mS/m)	Chlorophyll-a (µg/L)	Turbidity (NTU)	Site disturbance score
CBS	2006	3	298	7.0	0.72	30.21	7.56	7.17	12.32	2.13	14.37	2.19
CKM	2005	50	363	1.7	1.13	28.37	6.32	7.54	6.54	-	-	1.50
CKM	2006	50	386	2.0	1.18	31.23	8.21	5.16	6.50	0.57	6.05	1.19
CKM	2007	45	373	1.7	1.13	30.96	7.33	7.77	7.30	0.43	7.24	1.31
CKT	2004	20	2000	-	0.62	29.92	6.89	8.40	19.72	-	-	1.25
CKT	2006	13	1300	8.0	1.30	29.68	8.49	7.69	19.62	0.27	5.87	1.14
CMR	2005	50	110	3.6	1.70	28.60	8.15	8.13	19.20	-	-	1.75
CMR	2006	58	450	8.0	1.50	29.00	10.52	7.74	23.02	0.42	5.89	1.42
CMR	2007	45	870	4.4	1.58	30.47	8.34	8.41	22.53	0.39	4.42	1.61
CNL	2006	14	1629	15.0	0.78	30.06	7.02	7.54	19.35	0.72	21.53	1.97
CPP	2004	10	490	-	0.60	28.86	3.94	7.18	9.54	-	-	2.88
CPP	2006	6	460	12.0	0.54	30.06	4.70	7.94	10.47	33.59	25.87	2.89
CPS	2004	15	50	-	0.20	29.53	5.07	7.30	8.40	-	-	2.22
CPT	2006	13	39	1.6	0.26	29.95	4.56	7.13	11.03	3.99	55.50	2.33
CSJ	2005	37	630	2.4	1.50	29.20	6.00	7.42	4.41	-	-	1.50
CSJ	2006	52	622	3.0	1.10	30.05	7.26	7.22	4.93	0.61	5.67	1.25
CSJ	2007	40	652	2.5	1.47	30.29	7.41	7.48	4.90	0.59	5.40	1.28
CSK	2006	5	127	2.0	0.33	31.38	3.76	6.99	18.18	3.45	37.50	2.00
CSN	2006	6	66	10.0	0.20	28.49	7.13	7.22	8.10	2.04	12.93	2.00
CSP	2004	108	215	-	1.50	29.48	6.41	7.58	5.30	-	-	1.25
CSP	2005	85	294	2.1	2.00	30.87	5.91	7.63	6.26	-	-	1.13
CSP	2006	102	200	2.8	1.07	29.93	6.95	7.32	6.85	0.61	6.77	1.11
CSP	2007	100	190	2.4	1.15	29.79	7.19	7.45	6.78	0.51	7.94	1.39
CSS	2004	85	335	-	1.60	27.13	7.50	7.52	3.88	-	-	1.75
CSS	2005	80	180	1.1	1.10	28.83	6.19	7.24	4.23	-	-	1.75

Site Number	Year Sampled	Altitude (m)	River width (m)	Depth (m)	Secchi depth (m)	Temperature (ºC)	DO (mg/L)	pH	EC (mS/m)	Chlorophyll-a (µg/L)	Turbidity (NTU)	Site disturbance score
CSU	2005	129	174	2.0	1.60	26.23	7.07	7.17	4.19	-	-	2.13
CSU	2006	134	173	15.0	1.17	26.55	8.38	7.05	4.00	0.39	7.51	1.75
CSU	2007	117	170	2.9	1.40	26.14	6.98	7.32	4.30	0.45	5.48	1.97
CTU	2004	5	533	-	0.60	29.99	3.79	7.01	7.27	-	-	2.13
CTU	2006	3	522	10.0	0.52	29.65	5.84	7.00	9.08	1.12	29.97	2.04
LBF	2007	134	80	3.8	0.78	27.06	7.54	8.05	32.88	0.34	9.69	1.72
LBH	2007	111	150	1.2	1.06	28.28	7.70	7.86	15.25	0.48	8.81	1.63
LDN	2007	82	1 240	4.8	1.83	28.65	8.51	8.27	22.87	0.51	4.47	1.53
LKD	2004	160	173	-	2.00	24.53	7.67	7.97	9.80	-	-	1.43
LKD	2007	146	290	5.8	2.05	26.69	7.80	7.71	10.70	0.35	3.24	1.56
LKL	2005	90	120	2.5	1.10	28.93	5.56	7.18	5.86	-	1.33	1.50
LKL	2007	72	200	2.4	0.36	29.29	7.26	7.24	7.07	0.37	45.70	1.69
LKU	2005	90	68	1.7	1.27	28.53	5.99	7.18	5.14	-	22.93	1.13
LKU	2007	93	200	2.6	1.98	28.61	7.34	6.98	4.75	0.25	3.49	1.53
LMH	2005	454	138	2.9	0.25	16.67	9.34	8.19	34.80	-	55.73	1.94
LMX	2005	410	260	3.3	0.25	18.10	8.25	8.10	33.00	-	55.06	1.94
LNG	2004	161	196	-	3.40	23.53	8.82	7.45	7.51	-	-	1.50
LNG	2007	175	140	2.7	2.57	23.35	6.93	6.87	8.60	0.27	2.38	1.84
LNK	2005	190	65	0.9	1.30	24.13	7.47	8.27	25.10	-	-	1.38
LNM	2007	420	11	0.7	0.70	20.88	8.87	7.95	9.65	0.17	3.55	2.31
LNO	2004	280	214	-	2.80	22.90	8.59	8.46	24.72	-	-	1.00
LNT	2007	176	12	0.5	0.50	26.79	8.69	7.43	14.77	0.67	12.47	1.69
LOU	2005	290	92	2.4	1.43	21.10	8.16	8.15	21.27	-	64.50	1.00
LPB	2004	276	295	-	0.90	21.30	9.37	8.47	30.67	-	-	1.28
LPB	2005	270	212	1.4	0.48	20.43	7.87	8.17	27.40	-	54.17	1.69
LPS	2004	100	1 324	-	1.30	26.90	7.17	8.38	22.86	-	-	1.57

Site Number	Year Sampled	Altitude (m)	River width (m)	Depth (m)	Secchi depth (m)	Temperature (ºC)	DO (mg/L)	pH	EC (mS/m)	Chlorophyll-a (µg/L)	Turbidity (NTU)	Site disturbance score
LSD	2007	101	130	1.7	0.70	28.68	7.42	7.80	11.90	0.84	17.03	1.97
LVT	2004	159	480	-	0.98	23.33	8.61	8.63	28.80	-	-	1.78
LVT	2007	178	790	2.6	0.70	23.92	8.73	7.79	28.25	0.44	20.46	1.78
TCH	2004	127	185	-	0.33	29.07	7.71	7.83	18.38	-	-	1.86
TKO	2004	390	270	-	0.70	25.43	7.61	7.95	10.11	-	-	1.88
TKO	2005	400	105	1.0	0.20	24.60	6.22	6.62	11.75	-	128.30	1.86
TMC	2005	340	386	1.6	0.30	28.00	7.60	6.80	22.68	-	-	1.64
TMI	2005	350	70	0.4	0.25	30.00	6.40	6.80	10.18	-	-	2.25
TMU	2004	98	248	-	0.90	28.07	2.73	7.30	9.59	-	-	1.71
TNK	2007	133	12	0.8	0.43	28.37	7.11	7.15	16.92	0.45	25.61	2.44
TSK	2004	137	125	-	2.50	26.95	9.03	8.01	76.66	-	-	2.13
TSK	2007	120	95	3.9	0.82	28.99	7.15	7.47	49.20	0.60	12.60	1.97
TSM	2007	131	1137	1.6	0.58	28.08	8.65	8.12	24.95	0.63	24.31	1.86
VCD	2004	3	1000	-	0.60	29.08	5.61	7.68	17.48	-	-	2.69
VCD	2006	5	255	7.4	0.55	30.47	3.91	7.10	18.05	0.63	19.32	2.31
VCL	2006	7	1,090	15.0	0.59	30.40	8.01	7.58	18.87	0.97	14.27	1.91
VCT	2006	10	872	6.9	0.63	30.05	5.20	7.18	18.60	1.20	15.93	2.64
VLX	2006	7	662	7.7	0.67	30.15	6.59	7.13	18.57	0.97	12.55	2.69
VSP	2004	178	110	-	1.25	29.42	5.87	7.77	6.26	-	-	1.29
VSR	2006	312	106	5.0	0.18	27.47	7.31	7.14	5.15	0.98	71.08	2.00
VSS	2004	565	207	-	0.60	29.00	7.28	7.66	3.93	-	-	2.29
VSS	2006	527	167	0.5	0.98	26.12	7.47	6.62	3.97	0.40	9.14	2.00
VTC	2004	3	2600	-	0.70	29.45	5.70	8.33	16.68	-	-	2.50
VTC	2006	6	1,180	12.0	0.97	30.21	7.63	7.64	18.28	0.73	8.26	2.28
VTR	2006	9	1,070	5.2	0.68	29.85	6.70	7.33	18.11	0.82	13.17	2.44

Appendix 2. Species lists and counts per site and sampling occasion

This appendix gives a full listing of the number of each taxa of each of the four biological indicator groups recorded at each site and sampling occasion. Most sites were sampled on more than one occasion, and at many sites samples were recovered at different locations or river settings at the site. As a result the full listing is too large to be presented in this paper and instead is available on the CD that is included in the back of this document.

Appendix 3. Summary of biological indicator values

Site code	Water body	Sampling date	Reference site	Diatom				Zooplankton			
				No. of samples	Mean No. taxa	Mean No. individuals	Mean ATSPT	No. of samples	Mean No. taxa	Mean No. individuals	Mean ATSPT
CBS	Bassac	07-March-2006	N	10	8	311	44	3	22	844	48
CKM	Se Kong	26-March-2005	N	10	9	191	33	3	14	78	39
CKM	Se Kong	16-March-2006	N	10	11	250	37	3	11	21	37
CKM	Se Kong	18-March-2007	N	10	7	71	34	3	14	35	39
CKT	Mekong	23-March-2004	Y	10	12	318	34	3	15	36	44
CKT	Mekong	14-March-2006	Y	10	8	134	39	3	12	27	37
CMR	Mekong	24-March-2005	Y	10	6	206	33	3	11	39	38
CMR	Mekong	15-March-2006	Y	10	10	217	36	3	9	24	39
CMR	Mekong	17-March-2007	Y	10	7	58	37	3	12	35	38
CNL	Mekong	08-March-2006	N	10	10	314	40	3	17	265	45
CPP	Tonle Sap	17-March-2004	N	10	6	197	44	3	23	318	48
CPP	Tonle Sap	06-March-2006	N	10	8	377	50	3	7	92	46
CPS	Por Sat	18-March-2004	N	10	9	231	43	3	22	192	42
CPT	Prek Te	13-March-2006	N	10	7	268	45	3	40	2965	45
CSJ	Se San	25-March-2005	Y	10	7	214	33	3	14	119	37
CSJ	Se San	16-March-2006	Y	10	11	314	36	3	20	62	38
CSJ	Se San	19-March-2007	Y	10	6	655	34	3	17	52	38
CSK	Stoeng Sangke	11-March-2006	N	10	5	107	44	3	34	1431	46
CSN	Stoeng Sen	10-March-2006	N	10	8	221	44	3	20	297	45
CSP	Sre Pok	21-March-2004	Y	10	8	144	36	3	12	22	41
CSP	Sre Pok	29-March-2005	Y	10	10	232	30	3	13	86	36
CSP	Sre Pok	18-March-2006	Y	10	9	308	36	3	12	70	37
CSP	Sre Pok	21-March-2007	Y	10	8	532	35	3	15	62	42
CSS	Se San	20-March-2004	N	10	7	214	37	3	16	50	42
CSS	Se San	28-March-2005	N	10	10	232	35	3	14	34	34
CSU	Se San	27-March-2005	N	10	9	269	36	3	11	14	37
CSU	Se San	19-March-2006	N	10	6	140	39	3	32	176	40
CSU	Se San	20-March-2007	N	10	5	287	38	3	28	113	39
CTU	Tonle Sap	17-March-2004	N	10	8	227	42	3	16	745	43
CTU	Tonle Sap	09-March-2006	N	10	6	219	48	3	8	66	45
LBF	Se Bang Fai	10-March-2007	N	10	6	46	36	3	17	222	39
LBH	Se Bang Hieng	11-March-2007	N	10	8	257	36	3	16	473	41
LDN	Mekong	16-March-2007	Y	10	9	266	34	3	21	194	40
LKD	Nam Ka Ding	10-March-2004	N	10	12	372	33	3	6	18	41
LKD	Nam Ka Ding	09-March-2007	N	10	8	309	33	3	7	8	35
LKL	Se Kong	21-March-2005	Y	10	7	219	35	3	14	22	35

Site code	Littoral Macro. sweep				Littoral Macro. kick				Benthic Macro			
	No. of samples	Mean No. taxa	Mean No. individuals	Mean ATSPT	No. of samples	Mean No. taxa	Mean No. individuals	Mean ATSPT	No. of samples	Mean No. taxa	Mean No. individuals	Mean ATSPT
CBS	5	11	163	39	0				15	5	17	52
CKM	5	10	104	32	5	20	115	30	15	2	4	35
CKM	5	9	26	29	5	14	67	28	15	2	3	36
CKM	5	9	33	34	5	14	72	27	15	3	4	37
CKT	6	12	165	32	0				15	2	7	35
CKT	5	11	97	30	5	12	62	30	15	2	8	31
CMR	5	5	112	34	5	7	219	33	15	4	20	37
CMR	5	10	311	30	5	8	102	28	15	3	24	43
CMR	5	8	311	34	5	8	57	31	15	3	11	37
CNL	5	12	166	35	0				15	3	8	51
CPP	6	4	6	39	0				10	7	51	53
CPP	5	3	11	40	0				15	3	6	49
CPS	6	9	62	40	0				10	2	8	40
CPT	5	11	46	43	0				15	5	17	44
CSJ	5	13	83	32	5	18	173	29	15	2	3	37
CSJ	5	11	46	30	5	18	95	28	15	2	3	33
CSJ	5	14	88	32	5	25	301	29	15	3	5	36
CSK	5	4	92	43	0				15	3	11	47
CSN	5	7	125	43	0				15	4	24	45
CSP	6	19	301	30	0				15	3	8	35
CSP	5	20	229	28	5	24	235	25	15	6	25	38
CSP	5	16	54	27	5	20	177	26	15	3	6	31
CSP	5	17	136	31	5	24	352	29	15	3	7	33
CSS	6	16	116	34	0				15	2	3	39
CSS	5	17	55	33	5	15	71	31	15	4	7	37
CSU	5	15	121	34	5	19	58	33	15	5	23	36
CSU	5	5	179	33	5	8	51	32	15	3	8	39
CSU	5	3	10	34	5	6	13	34	15	3	5	37
CTU	6	4	7	40	0				15	7	46	51
CTU	5	4	10	43	0				15	5	48	51
LBF	10	16	254	35	0				15	6	38	38
LBH	5	8	73	36	5	11	42	30	15	3	7	38
LDN	10	14	340	33	0				15	8	51	36
LKD	6	12	74	33	0				10	5	37	39
LKD	10	14	63	34	0				15	7	36	37
LKL	5	9	48	31	5	28	269	28	15	6	25	35

Site code	Water body	Sampling date	Reference site	Diatom				Zooplankton			
				No. of samples	Mean No. taxa	Mean No. individuals	Mean ATSPT	No. of samples	Mean No. taxa	Mean No. individuals	Mean ATSPT
LKL	Se Kong	14-March-2007	Y	10	7	63	40	3	10	17	39
LKU	Se Kong	20-March-2005	Y	10	9	209	35	3	12	51	38
LKU	Se Kong	15-March-2007	Y	10	9	139	36	3	21	142	38
LMH	Mekong	12-March-2005	N	10	12	155	39	3	17	111	39
LMX	Mekong	13-March-2005	N	10	10	133	39	3	15	76	40
LNG	Nam Ngum	09-March-2004	N	10	11	354	34	3	20	398	40
LNG	Nam Ngum	07-March-2007	N	10	8	544	40	3	16	83	39
LNK	Nam Khan	10-March-2005	Y	10	10	276	34	3	20	56	38
LNM	Nam Mo	08-March-2007	N	10	11	1019	50	3	9	30	35
LNO	Nam Ou	07-March-2004	Y	10	8	326	30	3	9	57	23
LNT	Nam Ton	05-March-2007	N	10	10	70	37	3	9	35	37
LOU	Nam Ou	09-March-2005	Y	10	12	257	29	3	10	21	25
LPB	Mekong	07-March-2004	Y	10	11	388	37	3	10	182	36
LPB	Mekong	10-March-2005	Y	10	12	305	38	3	13	26	42
LPS	Mekong	11-March-2004	Y	10	10	343	37	3	17	227	40
LSD	Se Done	12-March-2007	N	10	8	108	38	3	26	1408	44
LVT	Mekong	08-March-2004	N	10	13	563	41	3	9	24	37
LVT	Mekong	06-March-2007	N	10	8	1338	39	3	10	160	40
TCH	Nam Chi	13-March-2004	N	10	14	306	43	3	18	751	40
TKO	Nam Kok	15-March-2004	N	10	21	372	41	3	14	53	40
TKO	Nam Kok	17-March-2005	N	10	10	229	40	3	29	145	42
TMC	Mekong	16-March-2005	Y	10	10	229	40	3	16	162	41
TMI	Nam Mae Ing	16-March-2005	N	10	12	199	42	3	19	180	43
TMM	Nam Mun–Chi	23-March-2007	N	10	7	720	44	3	19	114	41
TMU	Nam Mun	12-March-2004	N	10	9	346	40	3	40	1327	43
TNK	Nam Kham	24-March-2007	N	10	7	101	48	3	25	473	43
TSK	Songkhram	14-March-2004	N	10	13	318	42	3	13	580	42
TSK	Songkhram	25-March-2007	N	10	4	451	44	3	21	8394	45
TSM	Songkhram	26-March-2007	N	10	5	128	39	3	19	2586	43
VCD	Bassac	26-March-2004	N	10	11	326	44	3	16	363	44
VCD	Bassac	28-March-2006	N	10	8	280	49	3	12	97	45
VCL	Cao Lanh	26-March-2006	N	10	6	180	49	3	15	127	46
VCT	Bassac	24-March-2006	N	10	5	72	48	3	11	55	46
VLX	Long Xuyen	25-March-2006	N	10	6	317	51	3	16	148	45
VSP	Sre Pok	29-March-2004	Y	10	15	359	37	3	13	27	42
VSR	Sre Pok	21-March-2006	N	10	10	161	41	3	7	15	36

Site code	Littoral Macro. sweep				Littoral Macro. kick				Benthic Macro			
	No. of samples	Mean No. taxa	Mean No. individuals	Mean ATSPT	No. of samples	Mean No. taxa	Mean No. individuals	Mean ATSPT	No. of samples	Mean No. taxa	Mean No. individuals	Mean ATSPT
LKL	5	13	35	33	5	24	139	31	15	2	4	37
LKU	5	10	41	30	5	24	287	28	15	5	16	36
LKU	5	11	62	34	5	22	137	31	15	5	30	40
LMH	5	2	4	34	5	5	18	35	15	3	13	35
LMX	5	5	30	36	5	6	13	37	15	2	4	35
LNG	6	13	329	34	0				10	6	42	36
LNG	5	6	101	37	5	8	39	34	15	4	10	36
LNK	5	10	1056	29	5	19	466	27	15	8	102	33
LNM	5	13	56	37	5	39	204	37	10	4	11	39
LNO	6	15	398	28	0				5	12	55	23
LNT	5	18	132	33	5	16	90	33	10	5	11	38
LOU	5	17	128	24	5	14	107	27	15	6	20	33
LPB	6	5	112	28	0				5	7	25	32
LPB	5	5	76	34	5	11	86	32	15	2	6	33
LPS	6	6	147	32	0				10	8	58	37
LSD	10	11	50	37	0				15	5	13	40
LVT	6	6	25	34	0				10	1	1	31
LVT	5	8	122	34	5	7	81	34	15	3	6	39
TCH	6	9	28	35	0				15	5	20	43
TKO	6	5	20	31	0				10	6	31	36
TKO	5	7	52	34	5	9	90	33	15	4	12	34
TMC	5	7	125	33	5	5	46	33	15	4	18	35
TMI	5	5	17	35	5	11	313	38	10	4	26	36
TMM	10	10	39	40	0				15	3	10	45
TMU	6	7	50	38	0				10	3	8	46
TNK	10	6	23	38	0				15	2	3	42
TSK	6	9	184	37	0				10	6	122	50
TSK	10	11	63	38	0				15	3	27	47
TSM	10	6	24	38	0				15	3	9	37
VCD	6	7	76	41	0				15	7	43	55
VCD	5	5	15	46	0				15	6	23	55
VCL	5	7	39	42	0				15	3	9	53
VCT	5	4	24	43	0				15	3	8	63
VLX	5	5	30	44	0				15	5	24	57
VSP	6	20	149	28	0				10	7	77	38
VSR	5	12	95	34	5	10	43	34	15	3	15	40

Site code	Water body	Sampling date	Reference site	Diatom				Zooplankton			
				No. of samples	Mean No. taxa	Mean No. individuals	Mean ATSPT	No. of samples	Mean No. taxa	Mean No. individuals	Mean ATSPT
VSS	Se San	28-March-2004	N	10	10	318	42	3	15	65	42
VSS	Se San	20-March-2006	N	10	12	334	41	3	17	60	39
VTC	Mekong	25-March-2004	N	10	11	239	40	3	20	459	46
VTC	Mekong	27-March-2006	N	10	7	234	47	3	14	79	45
VTR	Vinh Long	23-March-2006	N	10	7	100	44	3	7	21	43

Site code	Littoral Macro. sweep				Littoral Macro. kick				Benthic Macro			
	No. of samples	Mean No. taxa	Mean No. individuals	Mean ATSPT	No. of samples	Mean No. taxa	Mean No. individuals	Mean ATSPT	No. of samples	Mean No. taxa	Mean No. individuals	Mean ATSPT
VSS	6	6	20	36	0				10	0	0	46
VSS	5	7	47	35	5	19	66	32	15	2	3	35
VTC	6	7	1627	45	0				15	10	219	61
VTC	5	2	23	41	0				15	4	18	56
VTR	5	6	54	43	0				15	4	14	57

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Mekong River Commission
P.O.Box 6101,Vientiane 01000,
Lao PDR.Telephone: (856) 21 263 263 Facsimile: (856) 21 263 264
E-mail: mrcs@mrcmekong.org
Website: www.mrcmekong.org