ISBN No: 978-9932-08-060-1

Mekong River Commission

Biomonitoring Methods for the Lower Mekong Basin

April 2010

ISBN No: 978-9932-08-060-1

Mekong River Commission

Biomonitoring Methods for the Lower Mekong Basin

April 2010

Published in Vientiane in April 2010 by the Mekong River Commission

Cite this document as:

MRC (2010) Biomonitoring Methods for the Lower Mekong Basin. Mekong River Commission, Vientiane. 65 pp.

Editors: V.H. Resh, D.H. Giap

Graphic design: Technical Language Associates

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

Contents

Contributors

Vincent H. Resh
University of California, Berkeley, United States of America
resh@berkeley.edu

Bruce Chessman
Department of Environment, Climate Change and Water, Australia
Bruce.Chessman@environment.nsw.gov.au

Ian Campbell
Monash University, Melbourne, Australia
I.C.Campbell@bigpond.com

Dao Huy Giap
Mekong River Commission Secretariat, Lao PDR
daogiap@gmail.com

Sok Khom
Cambodia National Mekong Committee, Cambodia
khom@cnmc.gov.kh

Bounnam Pathoumthong

Chanda Vongsombath
National University of Laos, Lao PDR
vongsombath@yahoo.com

Do Thi Bich Loc
Institute of Tropical Biology, Viet Nam
bichlocdo@gmail.com

Nguyen Thi Mai Linh
Ton Duc Thang University, Viet Nam
ntmailinh@tdt.edu.vn

Pham Anh Duc
Ton Duc Thang University, Viet Nam
phamanhduc@tdt.edu.vn

Phan Doan Dang
Institute of Tropical Biology, Viet Nam
pddang@gmail.com

Narumon Sangpradub
Khon Kaen University, Thailand
narumon@kku.ac.th

Supatra Parnrong Davison
Prince of Songkla University, Thailand
supatra.d@psu.ac.th

Sutthawan Suphan
Chiang Mai Rajabhat University, Thailand
suttawan@hotmail.com

Tatporn Kunpradid
Chiang Mai Rajabhat University, Thailand
tkunpradid@hotmail.com

Yuwadee Peerapornpisal
Chiang Mai Rajabhat University, Thailand
scbio017@chiangmai.ac.th

Acknowledgements

We thank Pornsook Chongprasith, Vithet Srinetr, Hanne Bach, Monyrak Meng, Jane Lee and the staff of the Environment Programme of the Mekong River Commission for their assistance in coordinating the sampling programme during 2003-2008. The authors also express their appreciation for the support given to the study by representatives from the National Mekong Committees of Cambodia, Lao PDR, Thailand, and Viet Nam, particularly for the help they provided in the field. We especially thank Prum Somany, Niane Sivongxay, Chanthima Ponthalith for their contribution.

Abbreviations and acronyms

ATSPT:	Average Tolerance Score Per Taxon
LMB:	Lower Mekong Basin
MRC:	Mekong River Commission
MRCS:	Mekong River Commission Secretariat
NMC:	National Mekong Committee
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 abundance is sometimes a sign that the ecosystem has been harmed.

Average richness: This measurement refers to the mean number of taxa (types) of plants or animals belonging to a particular indicator group (e.g. diatoms, zooplankton) counted in a sample.

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.

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 common biological indicator groups used in 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.

Biological monitoring: The use of plants and animals to indicate the ecological health of an ecosystem. In fresh water environments, monitoring programmes typically are based on invertebrates and algae.

Biological Potential: Habitat conditions, such as substrate type and water flow, may limit occurrence of certain organisms at a site, regardless of the quality of the water. The biological potential refers to the level of richness, the species composition, and the abundance of particular groups of organisms that is attainable given these conditions.

Diatoms: These are microscopic algae with cell walls made of silica. They drift in river water (planktonic) or live on substrata such as submerged rocks and aquatic plants (benthic). They are important primary producers in aquatic food webs and are consumed by many invertebrate animals. Diatoms are a diverse group and respond in many ways to physical and chemical changes in the riverine environment. Diatom communities respond rapidly to environmental changes because diatoms have short generation times.

Environmental variables: These are chemical and physical parameters recorded at each sampling site at the same time as samples for biological indicator groups were collected. The parameters include altitude, water transparency, water temperature, concentration of dissolved oxygen (DO), electrical conductivity (EC), and activity of hydrogen ions (pH), 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. This group of animals is most widely used in biomonitoring exercises worldwide. They are often abundant and diverse and are found in a variety of environmental conditions.

Macroinvertebrates: An informal name applied to animals that do not have a vertebral column, and which includes snails, insects, shrimps, crabs, 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.

Metrics: These calculations are measurements that provide a summary of the information collected for the different indicator groups. They include Average Richness, Abundance, and Average Tolerance Score Per Taxon.

Primary producers: These are organisms at the bottom of the food chain, such as most plants and some bacteria (including blue-green algae), which can make organic material from inorganic matter.

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

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 activities, 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.

Sampling sites: These are 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 chosen provide broad geographical coverage of the basin and to sample a wide range of river settings along the mainstream of the Lower Mekong and its tributaries.

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 Campbell biomonitoring activities, the SDS is determined by a group of ecologists who attribute a score of 1 (No disturbance, best possible conditions) to 3 (substantial disturbance, worst possible conditions) to each of the sampling sites in the programme. A list of descriptors is evaluated after discussion of possible impacts and habitat quality in and near the river.

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

Total richness: This refers to the total number of taxa (types) of plants or animals belonging to a particular indicator group (e.g. diatoms, zooplankton) collected at a site.

Zooplankton: These are small or microscopic animals that drift or swim near the surface of rivers, lakes, and the sea. Some are single celled while others are multi-celled. They include primary consumers than feed on phytoplankton (including diatoms) and secondary consumers that eat other zooplankton. Zooplankton can be useful biological indicators of the ecological health of water bodies because they are a diverse group that has a variety of responses to environmental changes. Zooplankton communities respond rapidly to changes in the environment because zooplankton species have short generation times.

Summary

The aquatic resources of the Mekong River and its tributaries are essential for supporting the livelihoods of a large percentage of the 60 million or more people living in the Lower Mekong Basin. The sustainable management of these resources depends on maintaining the ecological health of the River. From 2003 – 2008 the ecological health was monitored by the Environment Programme of the Mekong River Commission (MRC) using biological metrics. This report provides a handbook of methods for future monitoring by the MRC and the National Mekong Committees (NMCs) of Cambodia, Lao PDR, Thailand, and Viet Nam.

Biological monitoring is used throughout the world for evaluating the ecological health of running water habitats. This report begins with a description of the concept of biological monitoring and how it relates to the physical and chemical monitoring of streams and rivers. The report then discusses the development phases of the monitoring programme, beginning with the early attempts of the Mekong Committee in the 1980s and up to the design of the activities under the MRC Environment Programme from 2002 through to 2010. The potential use of biological monitoring information by planning and management agencies is described.

Different indicator groups (benthic diatoms, zooplankton, littoral macro-invertebrates, and benthic macroinvertebrates) provide a broad spectrum of descriptors of the ecological health of the Lower Mekong, with each group offering distinct advantages. The various analytical approaches used (richness, abundance, and pollution tolerance) along with the measurement of selected physical and chemical factors, also provide valuable information.

The biological condition of any body of water depends on both the quality of the water and the quality of the available habitats. Five zones of the Lower Mekong are identified that have different substrate characteristics and will respond differently to alterations in flow. In each, factors that may influence the biological potential of each of the indicator groups is described. An approach for assessing the substrate characteristics at a site is also presented, as is a quantitative system for determining the Site Disturbance Score, which is the basis for the tolerance values for the various taxa in the biological groups, is presented.

The baseline data and criteria were established in the 2004 – 2007 studies. Because it is necessary to be able to compare any information collected in future sampling efforts in the Ecological Health Monitoring (EHM) Programme to these baseline data and criteria, this report provides detailed descriptions of equipment and materials needed, field sampling and laboratory techniques, analytical measures, and identification guides used to study the assemblages of benthic diatoms, zooplankton, littoral macroinvertebrates, and benthic macroinvertebrates. This report also explains how various physico-chemical variables (i.e. environmental variables) of the river were, and should continue to be, measured at each of the sampling sites.

The metrics used for each group include richness, abundance, and tolerance scores (as the Average Tolerance Score Per Taxon, or ATSTP) for each of the groups used. The calculation of each metric is described in detail and illustrated with an example presented. Criteria for evaluating the ecological health of sites being examined and designation of reference sites are also described.

Chapter 1
Introduction

Vincent H. Resh, Ian Campbell and Dao Huy Giap

Introduction to biological monitoring

Biological monitoring, the systematic use of biological responses to evaluate environmental changes (Rosenberg and Resh 1993), has proven to be a valuable water resource management tool in rivers (Hynes 1960, Bonada et al. 2006), and particularly for the management of rivers in developing countries (Resh 2007). This is because little capital-intensive laboratory equipment, which can also be expensive to calibrate, recalibrate and operate, is required. Moreover, biological samples are relatively easy to preserve or even to process in the field. However, in newly industrialised and developing countries in Asia, biological monitoring may be difficult to use because of the lack of background information and identification keys for the biota, and the lack of capacity of local institutions to conduct field and laboratory work. Gallacher (2001) highlighted some of these difficulties, which included the inability to correctly identify the organisms collected. Within the lower Mekong, however, some of these disadvantages have already been overcome (Campbell et al. 2009). For example, the Mekong River Commission (MRC) has supported the development of a key for the identification of freshwater invertebrates (Sangpradub and Boonsoong 2006), and plans are underway to produce other guides to the identification of benthic diatoms and zooplankton. There are also specialists in several regional universities and research institutions with expertise in identifying these potentially useful indicator taxa.

There has been an extensive chemical water quality monitoring programme in the Lower Mekong Basin (LMB) (Campbell 2007, Campbell et al. 2009, Ongley 2009), and since 2003, biological monitoring has been conducted as part of an ecological health monitoring programme. The combination of chemical quality and biological monitoring information is especially useful because the biota of rivers respond to a range of physical, chemical and biological factors. This combination of programmes also fits well into the context of the MRC mandate to maintain the ecological balance of the River.

In addition to the MRC monitoring programme (2008a, b), other studies of biological river assessments within the Lower Mekong River and the region have been undertaken. For example, Thorne and Williams (1997), Mustow (1999, 2002) and Mustow et al. (1997) used benthic invertebrates to successfully assess the condition of the Ping River (a tributary of the Chao Phraya River), which flows through Chiang Mai, in northern Thailand. Recently, Boonsoong et al. (2009) developed a practical rapid assessment method using benthic macroinvertebrates for stream assessment in Thailand.

In this introduction to biomonitoring methods, we review the development of biological monitoring in the Mekong region, provide the rationale for the choice of study organisms, provide a detailed description for the sampling of the different indicator groups selected, and describe methods for evaluating sites and assessing substrate types in terms of habitat assessment.

Development of the MRC biomonitoring activities

Early attempts

In the 1980's, the Mekong Committee, the forerunner of the present day MRC attempted to establish biological monitoring activities in the Mekong system (Campbell et al. 2009). Monitoring sites were selected in Thailand, Lao PDR and Viet Nam, staff from the regional government agencies were sent to Sweden for training, and at least two rounds of sampling and sample analysis were conducted (Grimås 1988, Eriksson and Smith undated, Smith undated). However, these activities were discontinued.

In 1995, the MRC was established under an international agreement with the mandate "to protect the environment, natural resources, aquatic life and conditions and ecological balance of the Mekong River Basin…" (MRC 2002), and in order to fulfil this mandate the MRC established a programme to monitor the ecological condition, or health, of the River. As a result, in 2002 the first steps were taken to initiate a biological monitoring regime.

Design of activities (2002 - 2003)

At a meeting in Phnom Penh, Cambodia, in July 2002, national experts from the Mekong region, members of the MRC staff, and international consultants discussed the possibility of developing an environmental heath monitoring programme for the Lower Mekong and its major tributaries. Permission to do preliminary studies was approved by the MRC Secretariat (MRCS).

In 2003, a pilot survey was conducted in the four riparian countries of the Mekong Region to test the potential for the use of five biological groups, and one ecological process, for routine ecological health monitoring of the Mekong River and its major tributaries. These groups and the process were selected after consideration of the existing international experience in freshwater biomonitoring. The process, the groups and the reasons for their selection are described below:

1. The process of planktonic primary production, i.e. the rate of accumulation of biomass by photosynthetic organisms such as diatoms and other algae, was selected because this process is critical to the well being of the Mekong fisheries;

2. Benthic algae, including microscopic diatoms, were selected because these are a food source for fish, macroinvertebrates, and macro-algae, such as 'river weed', are of economic value in that they are processed, sold and eaten by local people;

3. Zooplankton, microscopic animals floating and drifting in open water, were selected since because they are important as a food for fish;

4. Littoral macroinvertebrates, invertebrate animals visible to the naked eye living in the shallow water at the river's edge, were selected because they are important as a food for fish;

5. Benthic macroinvertebrates, invertebrate animals living in or on the sediments at the bottom of the river, were selected because they are important as a food for fish;

6. Fish, because of their economic value as food for the tens of millions of people living in the Mekong region.

The pilot study confirmed the practicality and the cost-effectiveness of the use of diatoms, zooplankton, and littoral and benthic macroinvertebrates in routine sampling and identification. The pilot study also recognised the need for the development of standardised protocols for sampling and laboratory analysis.

However, the pilot study also revealed that it was not practical for various reasons to adopt the use of planktonic primary production, macro-algae, and fish in the Mekong River system. With respect to the measurement of planktonic primary production, this requires the mooring of a boat on site for several hours through the middle part of the day, and the transporting of a large amount of equipment, including chemicals, from site to site. These logistical requirements mean that measuring primary production is a costly exercise in comparison to other components. The enumeration of macro-algae is not possible because they are not present in sufficient quantities to allow representative sampling at most sites. Finally, pilot studies involving the sampling of fish showed it was not possible to collect enough specimens in nets for reliable assessment, even when most of a day was spent in sampling one site.

Development and testing of methods (2004 - 2007)

A regular, annual, biomonitoring programme based on the four groups of organisms that proved most successful in the pilot study began in 2004, and continued through 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 sub-areas of the LMB;

2. Choose a set of reference sites to create a biological benchmark against which data from any site in the LMB could be compared;

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

4. Use the values of the biological indicators measured at the reference sites to develop a set of guidelines for rating and classifying the sites, and

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

The programme, undertaken by national experts from the Member Countries who were trained in biology and ecology, was supported by the MRCS and international biomonitoring experts. One or two team members were responsible for the sampling, identification, analysis and reporting of results for one of the four biological groups selected. These groups were sampled in all of the Member Countries. The sampling was confined to the dry season (March) because sampling in the wet season would have been too difficult logistically and too difficult and dangerous. However, because many of the organisms collected have a long-life span, the data collected reflected prior conditions as well as the conditions prevalent at the time of sampling. This is an advantage that biological monitoring has over physical and chemical monitoring.

A series of analytical approaches (univariate and multivariate) and biological metrics (e.g. abundance, richness, biotic indices, diversity indices) were considered for use and tested for appropriateness. The metrics finally chosen for analysis and for use in the report card based on the 2004 - 2007 collections were abundance, average richness and Average Tolerance Score Per Taxon (ATSPT).

Site evaluation involved the selection of reference sites and conditions using a combination of water quality criteria and human disturbance evaluations. Guidelines of ecosystem health were then determined from reference site conditions. An arbitrary site classification scheme was then developed involving the number of metrics for the biological groups that met the thresholds based on reference site conditions.

In total, 51 different sites were sampled in the LMB from 2004 – 2007. Many of these were sampled over several years. A complete report of the results of this study (MRC 2008b) and a listing of the flora and fauna collected (MRC 2008b Compact Disc) are available.

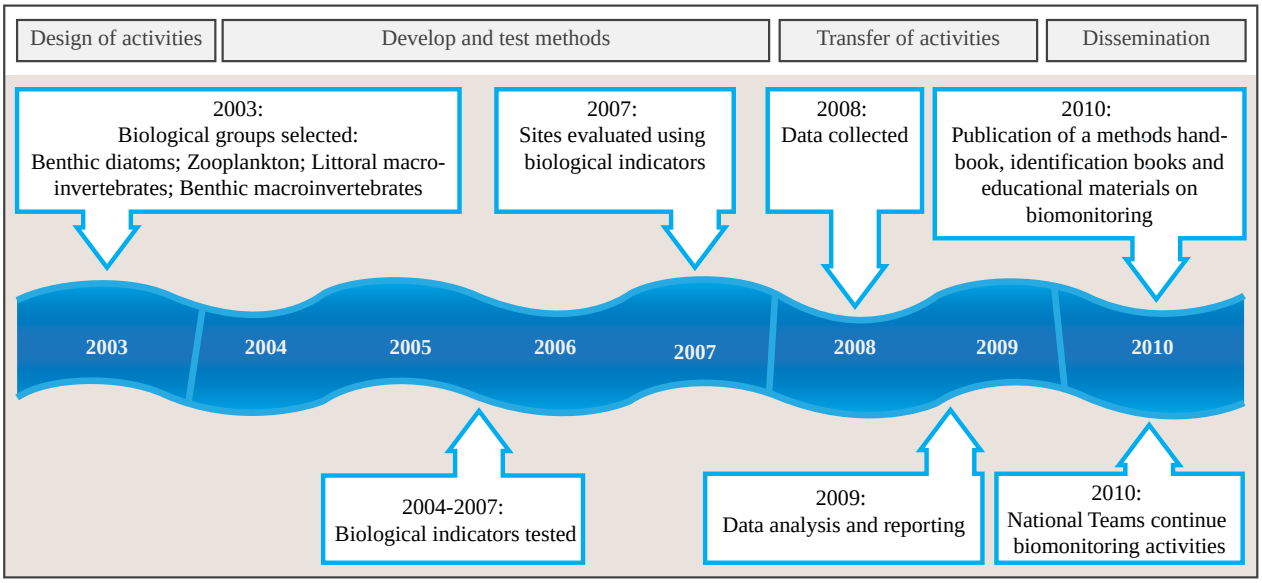


Figure 1.1. Timeline for biomonitoring in the Lower Mekong River and its tributaries

Handing-over of activities (2008 - 2009)

In 2008, the biomonitoring programme was handed-over to the line agencies of the Member Countries through their National Mekong Committees (NMCs). The MRCS continued to support the Programme and each Member Country sampled eight sites within their borders (making 32 sites in total). In contrast to the activities performed in 2004 - 2007, each of the national teams carried out all the processes of sampling, identification, analysis and reporting on the sites within their own countries.

In 2009, data analysis and reporting of the 2008 studies were supported by the MRCS and an international expert in the field of biomonitoring. Ten sites not previously evaluated in 2004 - 2007 were included in the analysis.

Dissemination of biomonitoring information (2010 - 2011)

In 2010, the MRC published the "Biomonitoring Methods for the Lower Mekong Basin", a handbook which includes detailed descriptions of the rationale behind the biomonitoring programme and the procedures to be followed for site evaluation, field sampling, laboratory processing, data analysis and reporting. The approach adopted was the use of photographic and graphic illustrations of procedures. It is expected that this handbook will be used in any regional training workshops or university courses in biological monitoring.

Three activities are planned to aid in the identification of the organisms found in the biomonitoring samples. These activities include:

1. The provision to the NMCs and to the MRCS of reference collections of identified individual littoral and benthic macroinvertebrates.

2. The preparation of a book for the identification of the zooplankton of the Lower Mekong. The book will include a dichotomous identification key and detailed photographs of all taxa.

3. The preparation of a book for the identification of benthic diatoms of the Lower Mekong. The book will include a dichotomous identification key and detailed photographs of all taxa.

In 2010, training for the national teams will be conducted and the "Biomonitoring Methods for the Lower Mekong Basin" will be translated into riparian languages.

Using Biological monitoring information in planning and management

Based on the 2004 – 2007 studies, a set of baseline conditions describing the ecological health of the Lower Mekong has been established through an MRC biomonitoring EHM Report Card (MRC 2008c). In 2008 and in subsequent years, sites previously studied and new sites will continue to be monitored using the methods described in this Handbook. In order to allow comparisons with the baseline conditions, field sampling methods, laboratory analyses, and data analysis must be performed in the same way as was used in the 2004 - 2007 baseline studies. Failure to do so may result in assigning an erroneous higher or lower score to a site.

Not only is biomonitoring information useful for the evaluation of current environmental impacts but it is also useful for providing new information on the trend of these impacts for management decisions. For example, as environmental regulations in the riparian countries in the Lower Mekong become laws, information from biomonitoring can provide the basis for developing standards and criteria similar to those available for chemical and physical monitoring. It is important to remember that biological assemblages are synthetic measures of the biological state of the environment being monitored. As a result, they can be used to state, in numerical terms, the biological condition of a water body relative to the targets set for that water body.

Future comparisons of sites examined in 2004 - 2007 may indicate improvements, reductions, or no change in the ecological health of a site. For example, the “Biomonitoring Methods for the Lower Mekong Basin” includes detailed methods for performing a visually based Site Disturbance Score Assessment at each site. Over time this assessment may provide the means of not only explaining any changes but also of possible mitigation of impacts or restoration procedures that may improve the conditions

References cited

Bailey, R.C., Norris, R.H. & T.B. Reynoldson (2004) Bioassessment of freshwater ecosystems: using the Reference Condition Approach. Kluwer Academic Publishers.

Bonada, N., Prat, N., Resh, V.H. & B. Statzner (2006) Developments in aquatic insect biomonitoring: a comparative analysis of recent approaches. Annual Review of Entomology 51: 495-523.

Boonsoong, B., Sangpradub, N. & M.T. Barbour (2009) Development of rapid bioassessment approaches using benthic macroinvertebrates for Thai streams. Environmental Monitoring and Assessment 155:129-147.

Campbell, I. C. (2007) Perceptions, data and river management - lessons from the Mekong River. Water Resources Research 43: doi: 10.1029/2006WR005130

Campbell, I. C., Chessman, B.C. & V.H. Resh (2009) The development and application of Biomonitoring in the Lower Mekong River System. In: I. Campbell (ed.). The Mekong. Biophysical environment of an international river basin. Elsevier Publishers.

Eriksson, L. & S. Smith (Undated) The chironomid fauna in the Mekong River and its tributaries in the Plain of Reeds. Report to the Interim Mekong Committee.

Gallacher, D. (2001). The application of rapid bioassessment techniques based on benthic macroinvertebrates in East Asian rivers (a review). Internationale Vereinigung für Theoretische Angewandte Limnologie Verhandlungen 27:3503-3509.

Grimås, U. (1988) Water quality investigations in the Lower Mekong Basin. Biological Monitoring – an Evaluation. Report to the Interim Mekong Committee. 37pp.

Hynes, H.B. N. (2007) The ecology of running waters. University of Toronto Press.

MRC (2002). Agreement on the cooperation for the sustainable development of the Mekong River Basin, 5 April 1995. Fourth Reprint. Mekong River Commission Secretariat, Phnom Penh.

MRC (2008a) An assessment of water quality in the Lower Mekong Basin. MRC Technical Paper No. 19. Mekong River Commission, Vientiane.

MRC (2008b) Biomonitoring of the lower Mekong River and selected tributaries 2004-2007, MRC Technical Paper No. 20, Mekong River Commission, Vientiane.

MRC (2008c) The Mekong River Report Card on aquatic ecological health (2004-2007).

Mustow, S.E. (2002) Biological monitoring of rivers in Thailand: use and adaptation of the BMWP score. Hydrobiologia 479:191-229

Mustow, S.E. (1999) Lotic macroinvertebrate assemblages in northern Thailand: altitudinal and longitudinal distribution and the effects of pollution. Natural History Bulletin of the Siam Society 47: 225-252.

Mustow, S.E., Wilson, R.S. & Sannarm (1997) Chironomid assemblages in two Thai water courses in relation to water quality. Natural History Bulletin of the Siam Society 45: 53-64.

Ongley, E.D. (1997) Matching water quality programs to management needs in developing countries: the challenge of program modernization. European Water Pollution Control 7:43-48

Resh V.H. (2007) Multinational, freshwater biomonitoring programs in the developing world: lessons learned from African and southeast Asian river surveys. Environmental Management 39:737-748.

Rosenberg, D.M. & V.H. Resh (editors) (1993) Freshwater biomonitoring and benthic macroinvertebrates. Chapman & Hall, New York.

Sangpradub, N. & B. Boonsoong (2006) Identification of freshwater invertebrates of the Lower Mekong River and its tributaries. Mekong River Commission, Vientiane.

Smith, S.H. (undated) Bottom fauna monitoring in the Lower Mekong Basin. January – March 1987. Mission Report to the Interim Mekong Committee.

Thorne, R. St. J. & W.P. Williams (1997) The response of benthic macroinvertebrates to pollution in developing countries: a multimetric system of bioassessment. Freshwater Biology 37:671-686.

Chapter 2

Biological, chemical and physical indicators of the ecological health of the Mekong

Vincent H. Resh

Choice of biotic assemblages to be used for biological monitoring

Many different types of organisms, from bacteria and viruses to higher plants and fish, have been proposed as being suitable for biological monitoring (Hellawell 1986, Bonada et al. 2006, Resh 2007, MRC 2008b). In Asia, 80% of programs surveyed use invertebrates. The next most popular are algae, particularly diatoms (Gallacher 2001). This order of preferences is also apparent throughout the world (Resh 2007).

The biomonitoring studies under the MRC's Ecological Health Monitoring (EHM) programme use three groups of invertebrates, namely those occurring in: (i) littoral zones of rivers (littoral macroinvertebrates); (ii) deep river channels (benthic macroinvertebrates); (iii) in the water column (zooplankton); and (iv) attached to substrates in the littoral zone (benthic diatoms) (MRC 2008b). Although it is unusual to use zooplankton in riverine biological monitoring programmes preliminary studies demonstrated that, in this particular programme, they are of value (Davison et al. 2006).

The use of diatoms and of these three groups of invertebrates living in the three different habitats has both advantages and limitations (Resh 2007, MRC 2008b). However, each of these groups offers unique information in the context of monitoring the ecological health, in understanding its ecology, and in management of the resources of the Mekong River.

Invertebrates are good indicators of local conditions. Firstly, because many are sessile and show limited migration, when abnormal increases in migration (e.g. unusually large numbers of organisms leaving the river bed, entering the water column and drifting downstream) do occur, these are often in response to disturbances such as pesticide inputs or increased siltation. Secondly, because of their complex life cycles and importance to higher trophic levels, invertebrates reflect the cumulative effects of short-term environmental variations. Finally, because their individual taxa demonstrate a complete range of pollution sensitivity, they are valuable indicators of both general and specific types of disturbance.

Littoral invertebrates respond to near shore changes, whether these are the result of human inputs, such as sewage, fertilizer or pesticide, or increases in silt such as those resulting from erosion and sedimentation. In addition, little equipment is needed for their sampling and access to littoral habitats is easy.

Benthic macroinvertebrates, i.e. those invertebrates present in the deep river channels, are valuable indicators of hydrologic stress, such as the increased shear stress resulting from fluctuating water releases from dams and wakes from boats. These organisms are less diverse than those found in the littoral habitats and their low abundance or absence may reflect changes in river bed conditions, such as the scouring of mid-channel areas. Sampling these taxa typically requires a boat for access to mid-river areas.

Zooplankton are important invertebrates for monitoring large rivers because unlike the littoral and mid-channel invertebrate taxa, these reflect changes occurring in the water column rather than in the sediment. Thus the inclusion of all three types of invertebrates results in coverage of both shoreline, mid-channel and water column habitats.

Benthic diatoms are valuable indicators of short-term impacts because they have rapid reproductive rates and very short life cycles. Moreover, because they are primary producers, they are responsive to both physical and chemical changes.

Therefore, the inclusion of invertebrates from three habitats and benthic diatoms in the monitoring program provides both short and mid-term indications of environmental changes resulting from a range of potential disturbances resulting from human activities. Fish, of course, are also good indicators of long-term changes (Resh 2007) and plans to incorporate data from other MRC programmes in this evaluation, along with water quality data may provide an integrated approach to monitoring, decision making and management.

Choice of analytical approaches to be used for biological monitoring

A variety of measures has been used in biological monitoring programs to describe the communities under examination (Rosenberg and Resh 1993, Bonada et al. 2006) and include:

1. Richness measures (the number of taxa of a particular group present in a sample or at a site),

2. Abundances (the number of individuals),

3. Diversity and evenness indices (the distribution of individuals among the various taxa),

4. Biotic indices (pre-established water-quality tolerance values averaged for the taxa).

Both univariate analyses (such as analysis of the measurements detailed above) and multivariate analyses (where each of the different taxa present is counted as a variable) have been used (Bailey et al. 2004). From 2004 to 2007, these approaches were evaluated in the EHM programme with the final measurements (or metrics) chosen being richness, abundance and a biotic index (the Average Tolerance Score Per Taxon (ATSPT)) for each of the four indicator groups (littoral invertebrates, benthic macroinvertebrates, zooplankton, and benthic diatoms) at a site. A detailed discussion of the process by which these metrics were chosen is presented in MRC (2008).

Choice of physical and chemical measurements for biological monitoring

In some areas (e.g. in Germany, Bonada et al. 2006), biomonitoring has been the basis for evaluating the ecological health of rivers for over 100 years, but in other parts of the world (e.g. North America, Carter et al. 2006), physical and chemical measurements have been the usual criteria. The approach used today is to include biological, physical and chemical measurements in assessment and management, in recognition of the different information that each can provide. Water chemistry and physical measurements, for example, are instantaneous, i.e. they provide information about the conditions existing when the samples are collected. In contrast, biological sampling can provide information about both past and present conditions as well because many of the organisms present have long-life spans. If, for example, only short-lived taxa that re-colonise easily are found rather than a mixture of short- and long-lived organisms, then this indicates that disturbances may occur frequently at the site being examined.

The physical and chemical characteristics measured at a site include water transparency and turbidity, water temperature, concentration of dissolved oxygen, pH and electrical conductivity.

Water transparency is measured by watching the depth at which a Secchi disk just disappears. All of the other measurements are made with electrical meters. For these, it is critical that the meter is calibrated correctly and that specific instructions for a particular brand and type of meter are followed carefully.

Light is a critical factor in river environments because solar radiation is necessary for plant photosynthesis. Decreased light transparency and increased turbidity may not only reduce primary productivity (i.e. the rate of accumulation of biomass by algae and plants) but also the predation rates on invertebrates. Human activities may reduce transparency and increase turbidity, which is why these measurements are taken.

Temperature affects the movement of molecules, the solubility of gases in water, the metabolic rates of the poikilothermous (i.e. cold blooded) animals that live in the Mekong, and many other factors. If there is a large amount of mixing of river water, the water temperatures are often uniform at the different parts of a site. However, such differences can occur from both natural and human activities.

The dissolved oxygen concentration affects aquatic life because oxygen is required for an organisms' metabolism. Aquatic organisms typically obtain the oxygen that they need from the water, for example through their gills or by cutaneous respiration. Oxygen production may be limited in areas where transparency is low and turbidity is high, and also where temperature is increased because the solubility of oxygen decreases as temperature increases. Dissolved oxygen concentrations may be similar or different in the various parts of the river examined, depending on turbulence, human activities and other factors. Organic pollution may significantly reduce dissolved oxygen concentrations while microbial activity within accumulations of leaves may reduce it on a very local scale. Although dissolved oxygen is typically measured during the day in the Mekong River EHM programme, rivers with luxuriant algal growth may experience broad ranges of dissolved oxygen concentrations as photosynthesis increases oxygen concentration during the day and respiration reduces it at night.

The pH measurement describes the acidity (pH <7), neutrality (pH=7) or basic levels (pH >7) of the water. Because the cellular components, body fluids and organs of many organisms are influenced by the homeostasis of acids and bases in their body, this factor often serves as an environmental filter determining whether an organism may occur in a particular area of a river.

Electrical conductivity (also called specific conductance) is a measure of a solution's ability to conduct an electrical current; the purer the water, the greater will be the resistance to electrical current and the lower the conductivity. The specific conductance of water in the Mekong system is proportional to the major cations present (e.g. calcium, magnesium, sodium, and potassium).

Conductivity is expressed in various different units, with:

1 milliSiemen/centimetre (mS/cm) = 100 milliSiemen/metre (mS/m) = 1000 microSiemens/centimetre (µS/cm)[1]

The conductivity of water samples is usually expressed in µS/cm or mS/m. The conductivity of most freshwater is between 5 to 150 mS/m, while sea water has a specific conductance of about 5,000 mS/m.

[1] The siemens (S) is the SI derived unit of electric admittance or, in the direct current case, electric conductance. Siemens denote the reciprocal of impedance or resistance: one siemens is equal to the reciprocal of one ohm, and is sometimes referred to as the mho.

References cited

Bailey, R.C., Norris, R.H. & T.B. Reynoldson (2004) Bioassessment of freshwater ecosystems: using the Reference Condition Approach. Kluwer Academic Publishers.

Bonada, N., Prat, N., Resh, V.H. & B. Statzner (2006) Developments in aquatic insect biomonitoring: a comparative analysis of recent approaches. Annual Review of Entomology 51: 495-523.

Carter, J.L., Resh, V.H., Rosenberg, D.M., & T.B. Reynoldson (2006) Biomonitoring in North American rivers: A comparison of methods used for benthic macroinvertebrates in Canada and the United States. In: Biological monitoring of rivers: applications and perspectives. Pp. 203-228, In: G. Ziglio, M. Siligardi, and G. Flaim (eds.). John Wiley & Sons, NY.

Davidson, S.P., Kunpradid, T., Peerapornisal Y., Linh, N.T.M., Pathoumthong, B., Vongsambath, C. & P.A. Duc (2006) Biomonitoring of the Lower Mekong and selected tributaries. MRC Technical Paper No.13, Mekong River Commission, Vientiane.

Gallacher, D. (2001). The application of rapid bioassessment techniques based on benthic macroinvertebrates in East Asian rivers (a review). Internationale Vereinigung für Theoretische Angewandte Limnologie Verhandlungen 27:3503-3509.

Hellawell, J.M. (1986) Biological indicators of freshwater pollution and environmental management. Elsevier, New York.

MRC (2008) Biomonitoring of the lower Mekong River and selected tributaries 2004-2007, MRC Technical Paper No. 20, Mekong River Commission, Vientiane.

Resh V.H. (2007) Multinational, freshwater biomonitoring programs in the developing world: lessons learned from African and southeast Asian river surveys. Environmental Management 39:737-748.

Rosenberg, D.M. & V.H. Resh (editors) (1993) Freshwater biomonitoring and benthic macroinvertebrates. Chapman & Hall, New York.

Chapter 3

Habitat assessment and the calculation of a site disturbance score

Vincent H. Resh

Habitat assessment and the biological potential of a site

The physical habitat of the Mekong and other riverine environments serves as the underlying template determining the presence and abundance of the benthic diatoms and invertebrates used in the MRC EHM Programme for biological monitoring. Together, the quality of the physical habitat and the quality of the water determine the types of biological communities present at a site (Carter et al. 2006). Because improvements in water quality, but not in habitat quality, may not result in the improvement of biological conditions (Hannaford and Resh 1995) habitat quality is an essential element of the Mekong EHM biomonitoring programme.

The interaction of hydrological and geo-morphological features of the Mekong gives rise to the physical habitats, the characteristics of which, in turn, may affect the water quality of a site thus impacting on the biological communities that could be present. Any failure to take into account the effect of the quality of the physical habitat may result in the assumption that the observed difference from the reference sites is the result of human disturbances to the water quality.

Physical habitat varies along the course of the Lower Mekong. Two recent reports (IBF Phase 2/3 Pilot-Specialist Report on Aquatic Invertebrates and the Integrated Basin Flow Management Specialist Report Phase 2: Geomorphology and Sedimentology) prepared for the MRC relate invertebrates and benthic algae to five geomorphic areas of the Lower Mekong. These zones, their extent, indicators of flow and substrate characteristics, and the response of some common organisms to flow change are summarised in Table 3.1. This summary represents the range of conditions that may influence the distribution of invertebrates and benthic diatoms in the Lower Mekong.

Table 3.1 presents indications of possible low-induced changes in substrate and may be useful in analysing biological information collected in the course of EHM monitoring, and the detection of possible changes from flow alterations. For example, changes in the abundance of river weed (information that can be obtained by the national teams from interviews with local people) or additions or deletions of various species of aquatic insects, molluscs and other organisms in the samples collected, particularly in the littoral zones, may indicate flow-induced effects operating through substrate changes.

The information in Table 3.1 may also be of use in examining the biological potential of the communities at each of the sites examined in particular zones. For invertebrates, for example, substrate characteristics can result in greater diversity and complexity of habitats and therefore a higher diversity of different types of aquatic organisms. Sometimes these substrate characteristics can be influenced by human activities but at other times they mainly reflect the underlying geology of the area, the morphological influences of the river channel and the location of a site within the drainage area. For example, the substrate present in sites in the Mekong in northern Lao PDR in Zone 1 typically have the substrate characteristics that are described in Appendix 3.1 for “optimal” biological condition while substrates in the Viet Nam Delta in Zone 5 do not, simply

Table 3.1. Five zones of the Lower Mekong, their extent, flow effects and substrate characteristics, and response to flow changes

Zones	Extent	Flow effects and Substrate	Possible Flow-Alteration Biological Indications	Invertebrate and Algal Response to Flow Changes
Zone 1 (Chiang Saen to Vientiane)	Extends from the Chinese border to upstream of Vientiane. There are extensive areas of bedrock channel and large rock outcrops. Further downstream sandy-gravel areas are more common than bedrock.	Upper reaches influenced by highly variable flows, especially in the dry season, and perhaps influenced by operation of hydroelectric generating plants in China. Changes in flow regime could lead to increases or decreases in the extent of the cobble sand, mud and bedrock habitats present.	Baetid mayflies (Baetis sp. and Centroptilum sp.) currently abundant in the reach and associated with sand habitats. If these habitats become more abundant the relative abundance of baetids would increase. Heptageniid mayflies are flattened and only occur on stone substrates. If bedrock or cobble habitats become more abundant these would also become more abundant. River weed, consisting of a mix of Cladophora and other filamentous algal species and requiring stony substrates for attachment, will be deleteriously impacted if water is too turbid or too deep because of insufficient light.	The baetid and heptageniid indicators will respond to changes in their habitat. River weed will respond to changed levels of rock surface and sediment transport.
Zone 2 (Vientiane to Mun River Confluence)	River channel largely sand and finer sediment. Bedrock is scarce, becoming scarcer downstream. The zone has numerous sandy islands, some with rock cores. Major habitats are sandy and muddy substrate, aquatic plants and riparian vegetation.	With change in flow, changes in substrate and fauna are likely to occur.	Snails and mussels can be found on a range of substrates. With changes in substrate and current the composition of the mollusc assemblage would change, with larger species possibly being replaced by smaller species less palatable for humans. Palingeniid mayflies use mud substrates and may indicate changes in habitat.	Mayflies and snails will be sensitive to changes in substrate, in particular to change that increases sand or decreases mud substrates. This may influence aquatic-insect assemblage composition and production. Palingeniid mayflies have a 12 month life cycle so any factors influencing their abundance at other times of the year will also impact their dry season emergence.
Zone 3 (Mun River Confluence to Stung Treng)	Encompassing the Siphandone or Four Thousand Iislands, the river here is braided with many silt and sand islands formed around rock cores. Much of the stream bed is bedrock or scoured hard clay.	Diversity of habitats is high here, which results in high species diversity.	The major invertebrates affected by flow changes are baetid mayflies, the Bilharzia host Neotricula aperta and shrimps. Impact on invertebrates through this reach is likely to arise through changes in dry season depth and current. An increase in dry season water depth will decrease light reaching the rocks, and thus decrease the amount of algae available as food for snails and decrease their abundance.	A decrease in Neotricula would probably have little impact on health of local people who need prophylactic drugs to remain bilharzia free. Decreases in baetid mayflies and shrimp would likely result from flow-induced substrate changes.
Zone 4 (Stung Treng to Phnom Penh and Cambodian Floodplain)	Extending from Stung Treng to Phnom Penh, this zone encompasses the Cambodian flood plain, the Tonle Sap River and the Great Lake.	The river through this stretch still has occasional rocky bars, especially from Sambor Rapids upstream, and numerous sandy islands in both the main channel and Tonle Sap River.	The species found in this reach are predominantly mussels, snails, shrimps and chironomid midges, all of which could be affected by substrate related flow changes.	Bivalves are sensitive to changes in suspended solids loads, while snails are likely to respond to changes in turbidity, which can decrease light and algal food availability, and changes in substrate.
Zone 5 (Phnom Penh to the Mekong Delta)	Extending from Phnom Penh to the South China Sea, it includes the flow bifurcations of the delta region of Viet Nam.	The river flows between silt and clay banks and has a silty bed. This section of the river is tidally influenced with a salt water wedge extending well in to Cambodia.	Snails, shrimp, polychaete worms, odonates and chironomids are common.	An increase in saline water from less water entering this zone would increase the relative abundance of polychaete worms and shrimps while decreasing the abundance of most aquatic insects.

Note: Table 3.1 is based on information provided in the reports of the IBF Phase 2/3 Pilot-Specialist Report on Aquatic Invertebrates and the Integrated Basin Flow Management Specialist Report Phase 2: Geomorphology and Sedimentology.

as the result of location within the Mekong basin. These geo-morphological influences should also be considered in the interpretation of data collected from samples in the different zones of the Lower Mekong.

Benthic diatoms are also strongly influenced by the amounts and types of substrate present, and also by the substrate texture and the degree to which they are covered with silt. These factors can result in differences in the colonisation and establishment of different species. Moreover, because diatoms need light for photosynthesis, water turbidity may limit light penetration and the consequent establishment of some invertebrates that feed on diatoms and other algae, and the diatoms themselves.. Wave action may produce disturbances (e.g. turning over rocks) that eliminate light, and shoreline exposure (e.g. resulting from fluctuating water level) and other factors may affect the assemblage from reaching its biological potential relative to the water quality present at that site.

It is important that these habitat characteristics for flow and substrate in Table 3.1 be considered in evaluating the potential for invertebrate occurrence and abundance at a site. In addition, the general patterns presented may aid in interpreting changes that occur in the benthic invertebrate and diatom fauna and flora. Sites that have substrate characteristics that can result in moderate or reduced biological condition may be limited in their overall and average richness and abundance. Therefore these substrate factors should be taken into consideration when considering the implications of the results of collections at a site, particularly in terms of possible remedial action at these sites.

In Appendix 3.1, we present an approach for examining the suitability of the habitat at a site for littoral and benthic macroinvertebrates. This approach involves a visually based physical habitat assessment, which was originally developed by Barbour et al. (1999) and which is now an approach widely used to provide a numerical evaluation of the quality of a habitat. With training, high rates of consistency among different scorers can be achieved (Hannaford et al.1997).

The first three factors evaluated in this scoring sheet may also be important in influencing the presence and abundance of benthic diatoms. The fourth does not apply because light typically does not extend to the depths at which benthic macroinvertebrates are sampled.

In contrast to the littoral and benthic macroinvertebrates and benthic diatoms, zooplankton respond largely to flow characteristics in the water column. This, of course, is the advantage of including this assemblage in biological monitoring. For example, in Zone 1 (Chiang Saen to Vientiane) and some other areas, high flows may result in reduced or heterogeneous zooplankton communities.

Calculation of a site disturbance score using a habitat assessment scoring sheet

The Site Disturbance Score (SDS) was used in studies conducted by the EHM programme from 2004 through 2007 and determined at each site examined. It also served as the basis for the tolerance score for each of the diatom, zooplankton, and invertebrate taxa collected. In 2008, these scores were also determined at each new site examined and each previously examined site to determine whether conditions had changed at a site over time.

The SDS in 2004 - 2008 of samples was reached through a verbal discussion of factors with potential impacts. In this Handbook, we provide the method for this along with a scoring sheet. At each site examined, an SDS is determined by each member of the national team (which typically consists of 8 to 10 ecologists/biologists) involved in measuring the environmental variables and collecting the biological samples. First, details of the site are completed in the space provided at the top of the Substrate Characteristics Scoring Sheet (see Appendix 3.1). Then, while still at the site and after sampling is completed, the members of the team discuss each of the 12 descriptors listed on the Site Disturbance Scoring Sheet (Appendix 3.2).

To complete the Scoring Sheet, the members of the team compare and discuss the actual characteristics of the habitat just sampled with the descriptions of habitats that would be awarded a score of 1 - 1.5, 1.5 - 2.5, or 3 on the Scoring Sheet. Each member of the team then individually scores each of these descriptors in terms of how the site conditions matches the various descriptions. A score of 1 is the highest score, representing the best possible habitat conditions in terms of biological potential, and 3 the lowest score, representing the worst possible habitat conditions. Scores using decimals (e.g. 1.2, 2.4) can be used to better delineate the site characteristics. Each score is then written in the far right column of each team member's Scoring Sheet.

Each team member decides individually on an overall SDS for that site. This overall score is based on their observations of the habitat quality and the combination of stressors generated by human activities. Light stress is rated 1, medium stress 2, and heavy stress 3.

The team members then discuss the results, and, if necessary, scores are adjusted. The scores selected by the team members are averaged to obtain the overall SDS for each site. This average SDS recorded represents the consensus of the group. Notes used in reaching the decision are provided on the final Substrate Characteristics Scoring Sheet, which is submitted to the appropriate NMC. Each team member retains his or her own initial, individual scoring-sheet.

The overall SDS must take into account all 12 of the descriptors but some characteristics may be considered as being more important in an individual's determination of the overall SDS. Discussions by the group on the significance of the 12 different descriptors are the basis for the determination of the final Site Score.

While the overall score was the basis for determining the tolerance of organisms based on their distribution (see Chapter 4), the individual descriptors may provide insights into the source of the disturbance at a site. For example, bank stability, vegetation protection, or riparian width alterations along one bank may explain a change in the biota at a site over time.

References cited

Barbour, M.T., Gerritsen J., Snyder B. D. & J.B. Stribling (1999) Rapid Bioassessment Protocols for Use in Streams and Wadable Rivers: Periphyton, Benthic Macroinvertebrates and Fish. Washington, DC: US EPA.

Hannaford, M.J. & V.H. Resh (1995) Variability in macroinvertebrate rapid bioassessment surveys and habitat assessments in a northern California stream. Journal of the North American Benthological Society:14:430-439.

Hannaford, M.J., Barbour, M. & V.H. Resh (1997) Training reduces observer variability in visual-based assessments of stream habitat. Journal of the North American Benthological Society 16:853-860.

Carter, J.M., Myers, M.J., Hannaford, M. & V.H. Resh (2006) Macroinvertebrates as biotic indicators of environmental quality. Pp. 805-833. In: F. R. Hauer and G. A. Lamberti (eds.) Methods in Stream Ecology. 2nd Ed. Academic Press, San Diego.

Appendix: Field data sheets

Appendix 3.1. Substrate characteristics scoring sheet

Site name: _____ Site code[2]: _____

Date: _____ Time: from _____ to _____

Members of team: _____

Member responsible for data collection: _____

General observations (water, substrate, bank appearance; weather; tide; other): _____

_____

_____

_____

Habitat characteristics	Resulting in optimal biological condition Score of 1-1.5 (1 is the best condition possible)	Resulting in moderate biological condition Score of 1.5-2.5	Resulting in reduced biological condition Score of 2.5-3 (3 is the worst condition possible)	Comments and score
1. Substrate cover in littoral zone	>50% of substrate favourable for colonisation by invertebrates or diatoms; mixture of materials such as submerged logs or wood, cobble or other stable substrates.	10 - 50% mix of stable habitat; some substrate frequently disturbed or removed.	<10% stable habitat; lack of habitat for invertebrate or diatom colonisation is obvious; suitable substrate for colonisation is unstable or lacking entirely.	
2. Embeddedness of littoral zone substrate	<25% of rocks are buried or surrounded by fine sediment. Rocks can be easily picked off of the bottom substrate	25 - 75% of rocks are buried or surrounded by fine sediment. Rocks must be pulled out of the bottom substrate	>75% of rocks are buried or surrounded by fine sediment. Rocks must be pried off of the bottom substrate with some force	
3. Sediment deposition	<20-50% of the bottom affected by sediment deposition.	Moderate deposition of new gravel, sand or fine sediment; 50 - 80% of the bottom affected by deposition; sediment deposits at obstructions, constrictions, and bends present.	Heavy deposits of fine material; >80% of the bottom changing frequently; pools almost absent due to substantial sediment deposition.	
4. Substrate of deep water channel	Mixture of different-sized substrate materials.	All mud or clay bottom.	Hard clay or bedrock, or all sand.	

[2] The site code consists of three letters; the first denotes the country and the second and third letters denote the river system or town. The same site code is used for repeated sampling at the same site.

Appendix 3.2. Site disturbance scoring sheet

Site name: _____ Site code: _____

Date: _____ Time: from _____ to _____

Members of team: _____

Member responsible for data collection: _____

General observations (water, substrate, bank appearance; weather; tide; other): _____

Descriptor	Site Disturbance Score of 1-1.5 (1 is the best condition possible)	Site Disturbance Score of 1.5-2.5	Site Disturbance Score of 2.5-3 (3 is the worst condition possible)	Comments and score (1-3)
1. Are there water diversions?	Upstream dams and other diversions of water are absent or have minimal effect; the amount of water in the channel and the amount of substrate exposed are typical for the season.	Water fills 25 - 75% of the available channel, and/or riffle substrates are mostly exposed.	Very little water in channel and mostly present as standing pools.	
2. Is there channel alteration from dredging of substrate?	Channelisation or dredging (e.g. sand or gravel removal) are absent	Channelisation may be extensive, and 20 - 50% of stream reach is channelised and disrupted. Dredging affects ~1% of habitat	Banks shored with gabion or cement; >50% of the stream reach channelised and disrupted. Dredging affects >1% of habitat	
3. How is stable is the left bank[3]?	Bank is stable; evidence of erosion or bank failure absent or minimal; <5 - 30% of bank affected by erosion	Moderately unstable; 30 - 60% of bank has areas of erosion; bank has high possibility of erosion during floods.	Unstable; many eroded areas; exposed areas frequent along straight sections and bends; with obvious bank sloughing; 60 - 100% of bank has erosion scars.	
4. How stable is the right bank?	See 3.	See 3.	See 3.	
5. What is the state of the protection provided by vegetation on the left bank?	>90% of the stream bank surfaces and immediate riparian zone is covered by native vegetation, including trees, understory shrubs, or non-woody plants; vegetative disruption through grazing, clearing, tree farms (e.g. teak) or agriculture is minimal (<5%) or not evident; >50% of plants allowed to grow naturally	50 - 90% of the stream bank surfaces are covered by native vegetation; some disruption of native vegetation is obvious, with patches of bare soil or closely cropped vegetation covering 5 - 10%; 10 - 50% of the plants are at the expected height.	<50% of the stream bank surfaces are covered by vegetation; disruption of stream bank vegetation is very high; at least 70% of the vegetation has been removed and either cleared or replaced by agriculture, grazing, or tree plantations (>10%); <10% of the plants are at the expected height.	

[3] Looking down stream, the left bank is to your left and the right bank is to your right.

Descriptor	Site Disturbance Score of 1-1.5 (1 is the best condition possible)	Site Disturbance Score of 1.5-2.5	Site Disturbance Score of 2.5-3 (3 is the worst condition possible)	Comments and score (1-3)
6. What is the state of the protection provided by vegetation on the right bank?	See 5.	See 5.	See 5.	
7. How extensive is the riparian vegetation width (left bank)?	Width of riparian zone >15 m; human activities (i.e., roadbeds, clear-cuts of forests, or crops) have not impacted riparian zone.	Width of riparian zone 6 - 15 m; human activities have impacted this zone	Width of riparian zone <6 m: little or no riparian vegetation remains because of human activities.	
8. How extensive is the riparian vegetation width (right bank)?	See 7.	See 7.	See 7.	
9. How large are water level fluctuations?	Discussions with local people indicate that water level only fluctuates seasonally and from natural phenomena, such as the amount of rainfall.	Discussions with local people indicate that water level fluctuates occasionally (<1 per month) from upstream water diversions but never > 20 cm.	Discussions with local people indicate that water level fluctuates daily to weekly, sometimes as much as 1 m, from upstream dam or water diversion operations.	
10. What are the human activities at the site? How extensive are they?	Little evidence of human activities, such as little to no agriculture, animal grazing, sand and gravel removal, inputs of domestic sewage or rubbish, boating activities, etc.	Moderate evidence of human activities, such as some (<5%) agriculture, animal grazing, inputs of domestic sewage or rubbish, boating activities, etc.	Obvious evidence of human activities, such as (>5%) agriculture, animal grazing inputs of domestic sewage or rubbish, boating activities, etc.	
11. What are the human activities up to 2km upstream of the site?	Low levels of human activities, such as little to no agriculture, animal grazing, inputs of domestic sewage or rubbish, boating activities, etc.	Moderate levels of human activities, such as (<5%) agriculture, animal grazing, inputs of domestic sewage or rubbish, boating activities, etc.	High levels of human activities, such as (>5%) agriculture, animal grazing inputs of domestic sewage or rubbish, boating activities, etc.	
12. What are the human activities >2 -10km upstream of the site?	Little levels of human activities, such as large-scale mining or other activities that may have strong downstream effects.	Moderate levels of human activities, such as large-scale mining or other activities that may have strong downstream effects.	High levels of human activities, such as large-scale mining or other activities that may have strong downstream effects.	

Overall Site Disturbance Score: _____

Note: This sheet is used to calculate a Site Disturbance Score. Characteristics of Habitats Having Different Site Disturbance Scores. Each descriptor is scored from 1-3. Decimals can be used to reflect the characteristics of a particular site.

Chapter 4

Environmental variables

Supatra Parnrong Davison and Sok Khom

Objective

The objective in studying the physical and chemical factors is to describe selected characteristics of the study sites in the Lower Mekong River by collecting data on altitude, river width, depth, Secchi depth (water transparency), water temperature, dissolved oxygen (DO), pH, electrical conductivity (EC).

Because the information collected by you will be compared to the baseline data and criteria established from the 2004-2007 studies, it is critical that you use **EXACTLY THE SAME METHODS** as used during that period, which are described below. Failure to follow these procedures may result in assignment of better or worse scores to a particular site. This requirement to follow the same procedures used in the 2004-2007 studies is an important responsibility for each of the National Mekong Committee teams doing the biomonitoring sampling.

Materials and supplies needed

- Garmin GPS unit
- Newcon Optik LRB 7x50 laser rangefinder
- Secchi disk
- Electronic meters (and instruction sheets) to measure temperature, dissolved oxygen, conductivity, and pH
- Weighted rope, marked in lengths, to determine water depth
- Solutions of manganese sulphate solution and alkali-iodide-azide to fix dissolved oxygen samples

Field procedures

A field data sheet for the environmental variables is provided for recording site information and should be filled in completely and accurately (see Appendix 4.1).

1. Before (and after) sampling at each site, the equipment is washed to remove any material left from the previous site.

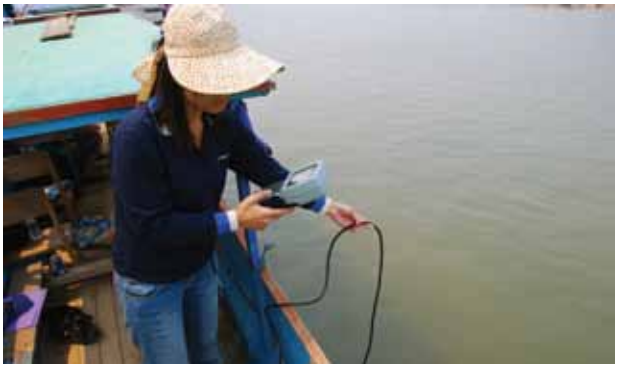
2. The map coordinates and altitudes of the sampling sites are determined with a Garmin GPS 12XL.

3. The stream or river width is measured with a Newcon Optik LRB 7x50 laser rangefinder, by Google Earth maps or GPS measurements at both banks.

4. At each site, environmental measurements in the water are made in three sections of the river, namely: (i) near the left bank; (ii) near the right bank, and (iii) in the centre. If a site is on a national border, then the three sections to be sampled should be within that Member Country's border.
   - A Secchi disk is used to determine water transparency. The disk is slowly lowered into the water to a depth at which it can no longer be seen. The disk is then slowly pulled up until it reappears. The depth is then recorded from the depth mark on the rope. In a fast current, additional weights may have to be added to the rope to have the disk sink in a straight line to the bottom.

Physical characteristics are measured at the site, including altitude, GPS coordinates, and river width

Light penetration is recorded by slowly lowering the Secchi disk into the water until it just disappears



Temperature, DO, pH, EC are recorded using an electrical metre

River width is measured using a laser rangefinder

Water depth determined by dropping a weighted rope directly to the river bottom

Altitude and coordinates of the sampling site are measured using a GPS device

Figure 4.1. Illustration of the equipment used to measure environmental variables

- Temperature, DO, EC, and pH are measured with an electronic meter. Because different meters are used in the different Member Countries, they should be calibrated before each use according to the manufacturer's instructions. Three readings at each location of the river are taken at a depth of 0.5m.
- Water depth is determined by dropping a weighted rope directly to the river bottom and is reported in m. In a fast current, additional weights may have to be added to the rope to have the line sink straight to the bottom. Alternatively, the depth mark on the rope used for the Petersen grab also can be used to determine water depth.

5. In some situations, for determination of chemical variables such as DO and EC, water samples can be collected from the water surface and examined later. To do this, one litre per sample or three separate litres per site is collected. Bottles are labelled, kept in an ice box and transferred to the water quality laboratory within 24 – 36 hours for analysis. If DO is measured later in the laboratory, the "Azide Modificaton Method" is to be used. The details of the method can be found in "Standard Methods for the Examination of Water and Wastewater" (APHA 1998).

6. All measured environmental variables are reported as average values. DO is reported as mg/L (ppm); electrical conductivity as mS/m and temperature in degrees Celsius. Water transparency, elevation and river width are measured in m.

References cited

APHA (1998) Standard Methods for the Examination of Water and Wastewater. American Public Health Association, American Water Works Association, Water Pollution Control Federation, 20th Edition.

Appendix: Field data sheets

Appendix 4.1. Field data sheet for environmental variables collections

Site name: _____ Site code: _____

Date: _____ Time: from _____ to _____

Members of team: _____

Member responsible for data collection: _____

General observations (water, substrate, bank appearance; weather; tide; other): _____

_____

_____

_____

Coordinate (UTM): _____

River width (m): _____ Altitude (m): _____

Measurements	Water depth (m)	Secchi depth (m)	Temperature (°C)	DO (mg/L)	pH	EC (mS/m)	Remarks
Left 1							
Left 2							
Left 3							
Middle 1							
Middle 2							
Middle 3							
Right 1							
Right 2							
Right 3							
Average							

Chapter 5

Benthic diatoms

Tatporn Kunpradid, Yuwadee Peerapornpisal, and Sutthawan Suphan

Diatoms are a group of algae that are dominant members of the community living on submerged strata where light can penetrate and stimulate plant growth through photosynthesis (i.e. the photic zone) in streams and rivers. They are recognisable in the field as tan to brown to gold coloured films on submerged objects.

Objective

The objective in studying the benthic diatoms is to quantitatively describe the characteristics of the diatom community. Diatoms provide a rapid response to environmental changes.

Because the information collected will be compared to the baseline data and criteria established from the 2004 - 2007 studies, it is critical that **EXACTLY THE SAME METHODS** are used as those used then. These methods are described below. Failure to follow these procedures may result in assignment of misleading better or worse scores to a particular site. The requirement of following the same procedures as used in the 2004 - 2007 studies is important and is the responsibility of each of the NMC teams performing the biomonitoring sampling.

Materials and supplies needed

Field:

- Small sampling bowl (such as a plastic soup bowl)
- Plastic sheet with a 10 cm^2 square cut out
- toothbrush,
- 10 collection bottles (10 - 50 mL, preferably plastic) that can be labelled
- Lugol's Solution (which consists of 5 g iodine (I_2) and 10 g potassium iodide (KI) mixed with 300 mL of distilled water
- Waterproof field notebook and pencil

Laboratory:

- Hot plate and various sizes of glassware
- Strong acid (H_2SO_4, HCl or HNO_3)
- De-ionized water
- Compound microscope
- Slides and cover slips
- Naphrax or Durax mounting agents

Field procedures

A field data sheet for benthic diatoms is provided to record site information and should be filled in completely and accurately (see Appendix 5.1)

1. The sampling of benthic diatoms within a site should be performed where the water is less than 1m deep and suitable substrata extends over a 100m distance. The most appropriate substrata are cobbles and other grades of stones with a surface area greater than 10 cm^2, but that are still small enough to fit in a 20 – 30 cm diameter sampling bowl. At sites where the river bed is predominantly muddy or sandy and lacks suitably sized stones, samples can be taken from bamboo sticks, aquatic plants, and artificial materials.

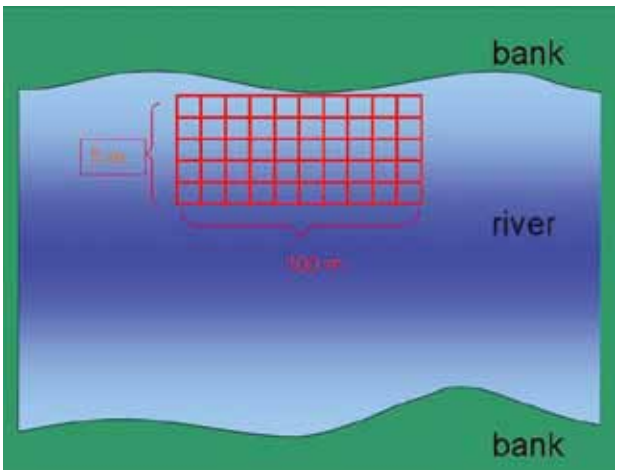
Lugol's solution:

- Dissolve 5.0g of iodine crystals and 10g of potassium iodide in 300 mL of water.
- Use three drops of this solution on a 100 mL sample.

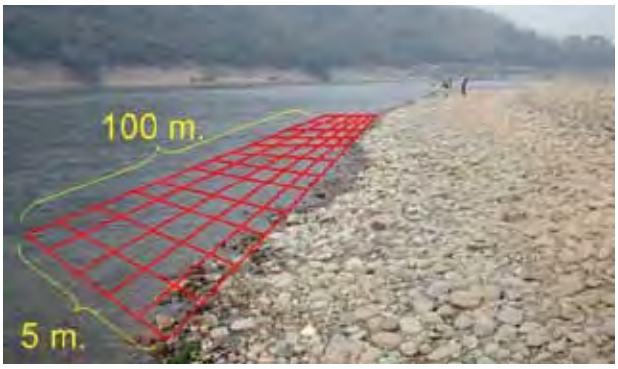
2. At each site, ten samples are collected, one at a time, at about 10m intervals. Samples are collected from stones coated with a thin brownish film or which have a slippery feel. These characteristics are often indicative of the presence of benthic diatoms. Where there are no suitable stones, the nearest hard substratum can be sampled.

3. To sample the diatoms, a plastic sheet with a 10 cm^2 square cut out is placed over the upper surface of the stone or other substratum, and benthic diatoms are brushed and washed off into a plastic bowl until the cut out area is completely clear. Each sample is then transferred to a plastic container and labelled with the name of the site, the location code, the date of sampling, and the sample-replicate number. The collector's name and substratum type are also noted. Samples are preserved with Lugol's Solution.

4. The name of the site, the location code, the date of sampling, the sample-replicate number, the collector's name and substratum type are also noted in the field notebook, as is any information about the site that could be influencing the presence or abundance of different types of diatoms.

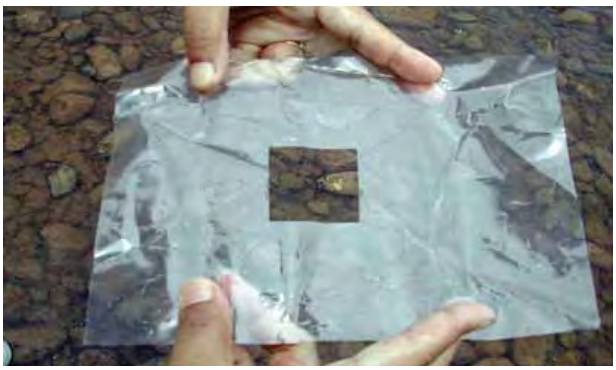


A diagram illustrating how benthic diatoms are sampled. The sampling area is a 100m length of shoreline, with a width that extends 5m from the river bank (and is less than 1m deep).



Area for collecting samples in the Mekong River.

Step 1. Benthic diatoms on the surface of a substrate.



Step 2. Plastic sheets, each with a 10 cm^2 (3.16 x 3.16 cm) square cut-out used to cover the substrate and delineate the sampling area on the rock.

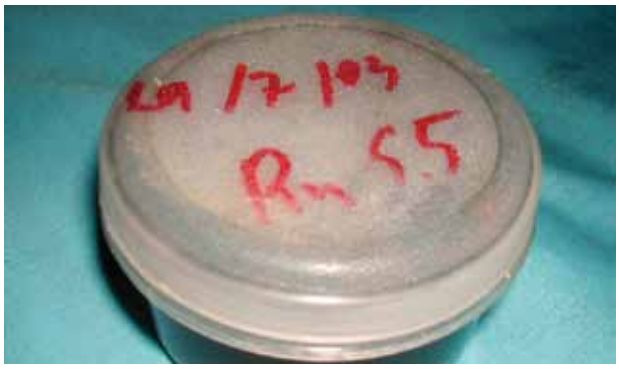
Step 3. Toothbrushes, plastic bowl, plastic containers with sealable lids, Lugol's Solution, labels and a marker pen used for sampling.

Step 4. A plastic sheet with a 10 cm^2 cut-out is placed on the upper surface of the selected stone.

Step 5. Benthic diatoms on the surface of the selected stone are brushed off.

Step 6. The scraped area is rinsed with water until the cut-out area is completely clear.

Step 7. Each sample is poured into a plastic bowl and then into a plastic container.



Step 8. The label includes the site name, the site code, the date, and the replicate number, written on the plastic container.

Figure 5.1. Illustration of the field procedures for sampling benthic diatoms

Laboratory procedures

1. In the laboratory, the samples are cleaned by digestion in concentrated acid. The raw samples are centrifuged at 3,500 rpm for 15 minutes. The diatom cells (which are the brown layer between the supernatant and solid particles) are pipetted off into an 18-cm core tube.

2. 2mL of strong acid (H_2SO_4, HCl or HNO_3) is added and the tubes are heated in a boiler (70 – 80ºC) for 30 - 45 minutes. The samples are then rinsed with de-ionized water 4 – 5 times and adjusted to a volume of 1mL by adding distilled water.

3. A drop of each sample (0.02 mL) is placed on a microscope slide and dried. One drop of a mounting agent such as Naphrax or Durax is added, followed by the addition of a cover slip, to make a permanent slide for diatom identification and counting.

4. Identifications are made under a compound microscope and are based on the frustule type, size, special characteristics, and structure, as described and illustrated in various textbooks, monographs and other publications on tropical and temperate diatoms (see the list of identification aids). In many cases identification to species-level is not possible and presumptive species are designated by numbers (e.g. Navicula sp.1). This designation must be applied to that particular morphological type over all the years of the study.

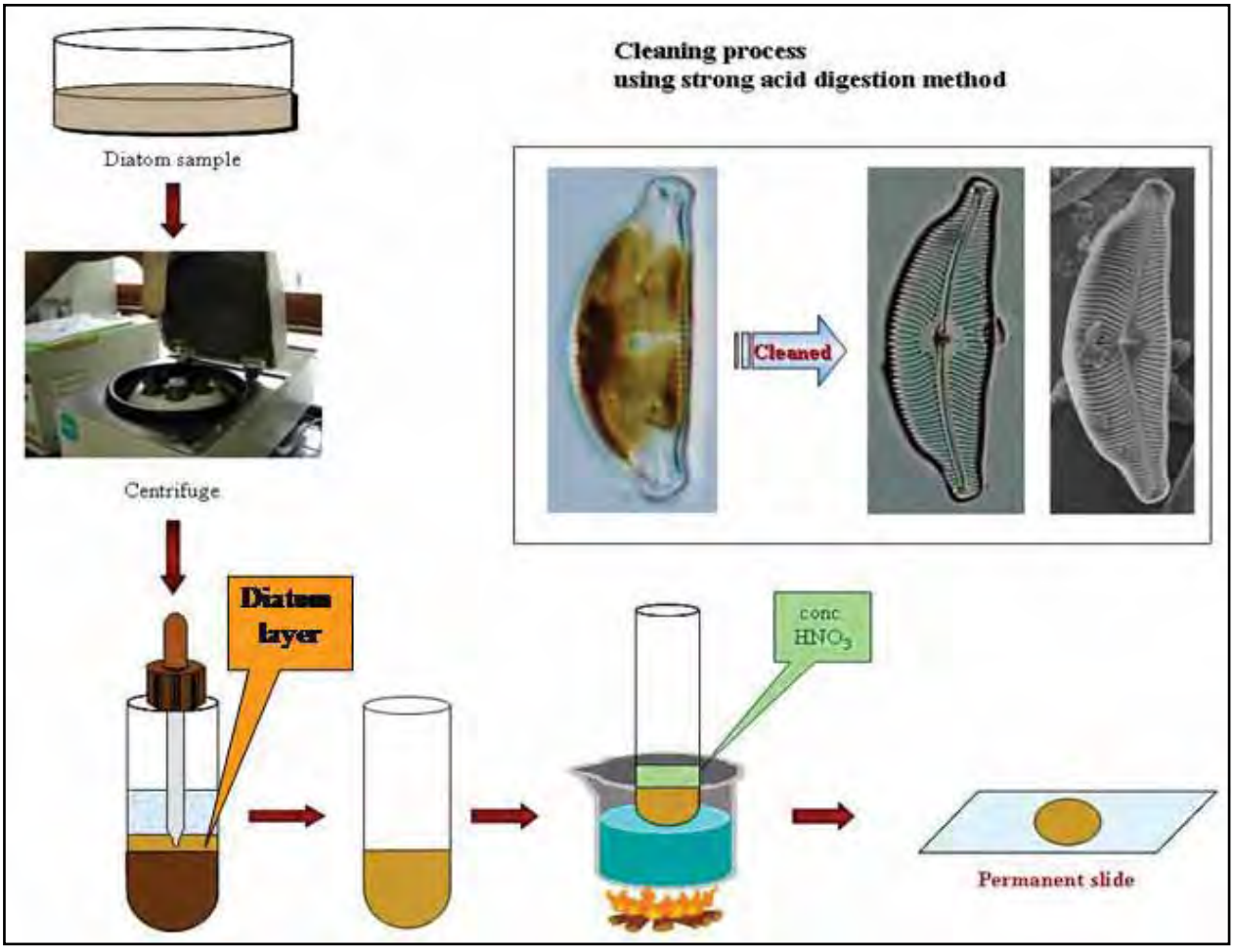
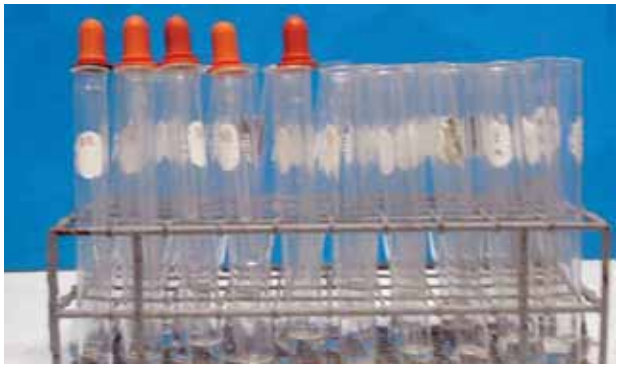


Figure 5.2. Summary of laboratory procedures for benthic diatoms

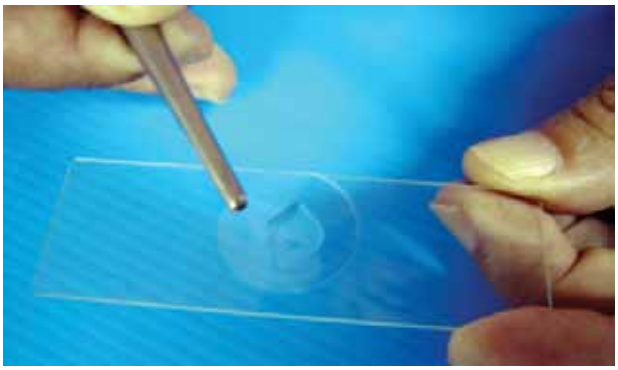
Step 1. The raw samples are centrifuged at 3,500rpm for 15 minutes.

Step 2. The diatom cells are the brown layer between the supernatant and solid particles.

Step 3. Strong acid (H_2SO_4, HCl or HNO_3) is added and the tubes are heated (70 – 80ºC) for 30 - 45 minutes.



Step 4. The diatom cells are rinsed with de-ionized water 4 – 5 times and adjusted to a volume of 1mL with distilled water.



Step 5. A drop of each sample (0.02mL) is placed on a microscope slide and dried.

Step 6. A drop of a mounting agent is added, followed by the addition of a cover slip, to make a permanent slide.

Step 7. A permanent slide for diatom identification and counting.

Figure 5.3. Illustration for benthic-diatom laboratory procedures

Analytical methods

1. Determine the average richness, abundance, and ATSTP value for each sample collected at a site. An average value is then obtained.

2. Average richness is the number of taxa per 0.2cm^2 sampled.

3. The total count of cells on the slide (0.02 mL) is used to estimate total number of individuals per sample, which is the abundance. The number of cells counted, when multiplied by 5, is the number per cm^2.

4. Calculate the ATSPT for that site

5. Richness, abundance and ATSPT scores always are reported per sample (which is 0.2 cm^2).

Identification aids

Foged (1971, 1975, 1976), Krammer & Lange-Bertalot (1986, 1988, 1991a, 1991b), Pfister (1992). A book on the identification of benthic diatoms for the Lower Mekong is in preparation.

References cited

Foged, N. (1971) Freshwater diatoms in Thailand. Odense Publisher, Lehre.

Foged, N. (1975) Some littoral diatoms from the coast of Tanzania. Odense Publisher, Lehre.

Foged, N. (1976) Freshwater diatoms in Sri Lanka (Ceylon). Odense Publisher, Lehre.

Krammer, K. & H. Lange-Bertalot (1986) Bacillariophyceae. Teil 1. Gustav Fischer Verlag: Stuttgart, Jena.

Krammer, K. & H. Lange-Bertalot (1988) Bacillariophyceae. Teil 2. Gustav Fischer Verlag: Stuttgart, Jena.

Krammer, K. & H. Lange-Bertalot (1991a) Bacillariophyceae. Teil 3. Gustav Fischer Verlag: Stuttgart, Jena.

Krammer, K. & H. Lange-Bertalot (1991b) Bacillariophyceae. Teil 4. Gustav Fischer Verlag: Stuttgart, Jena.

Pfister, V.P. (1992) Phytobenthos communities from 2 Tyrolean mountain streams. Arbeitsgemeinschaft Limnologie, Telfs, Austria.

Appendix: Field data sheets

Appendix 5.1. Field data sheet for benthic diatom collections

Site name: _____ Site code: _____

Date: _____ Time: from _____ to _____

Members of team: _____

Member responsible for data collection: _____

General observations (water, substrate, bank appearance; weather; tide; other): _____

_____

_____

_____

Samples are collected at: left side ○ OR right side ○

Replicates	Depth of collected substrate (m)	Type of substrate			Remarks
		Cobble (64-56mm)	Detritus (sticks, wood, trash)	Artificial (concrete, plastic…)	
1					
2					
3					
4					
5					
6					
7					
8					
9					
10					

Chapter 6

Zooplankton

Nguyen Thi Mai Linh, Phan Doan Dang, and Do Thi Bich Loc

Zooplankton are the uni- or multi-cellular animals that occupy the water column of lakes and large rivers. They are consumers of phytoplankton and food for fish.

The objective in studying the zooplankton is to quantitatively describe the characteristics of the zooplankton community. Zooplankton provide a biological reflection of the environment and chemistry of the water column whereas the other indicators that we use tend to reflect the influences of water chemistry and substrate characteristics.

Because the information collected will be compared to the baseline data and criteria established from the 2004 - 2007 studies, it is critical that **EXACTLY THE SAME METHODS** are used as those used then. These methods are described below. Failure to follow these procedures may result in assignment of misleading better or worse scores to a particular site. The requirement of following the same procedures as used in the 2004 - 2007 studies is important and is the responsibility of each of the NMC teams performing the biomonitoring sampling.

Materials and supplies needed

Field:
- 10L bucket
- Plankton net with a mesh of size 20 µm
- Collection jar with a 250mL volume
- 10% formaldehyde
- Hand-held straw pipette
- Waterproof notebook and pen
- Material for labelling

Laboratory:
- Forceps
- Petri dish
- Hand-held straw pipette
- Water sprayer
- Filtering net with a mesh size of 10 µm
- Distilled water
- Compound stereo-microscope with a magnification of up to 400x
- Compound dissecting stereo-microscope with a magnification of 40x
- 250mL graduated cylinder

Field procedures

A field data sheet for zooplankton is provided to record site information and should be filled in completely and accurately (see Appendix 6.1)

1. Three sets of samples are collected at each site. One sample is taken near the left bank of the river, at a distance of about 4 – 5 m from the water's edge. A second sample is taken 4 – 5m from the right bank, and the third sample is taken in the middle of the river. If a site is on a national border, the three sections to be sampled should be within that Member Country's border.

2. Samples are taken at least 1m from potential contaminants such as debris and aquatic plants, and at least 2m from vertical banks. At sites where the water current is too fast to sample exactly in the mid-stream, samples are collected closer to the left or the right bank, but not as close to the bank as where the sets of near-bank samples are taken.

3. Before (and after) sampling at each site, the equipment is washed to remove any organisms and other matter left from the previous site.

4. 10L of river water at a depth of 0 - 0.5 m is collected in a bucket.

5. The 10L of river water are filtered slowly through a plankton net (with a mesh size of 20 µm) to avoid any overflow from the net. Water is splashed on the outside of the net to wash down any zooplankton adhering to the inner parts of the net.

6. When the water volume remaining in the net is only about 150mL, the water (which contains the zooplankton sample) is transferred to a 250mL plastic jar. The sample is immediately fixed in the field by adding ~ 75mL of 10% formaldehyde to achieve a final concentration of 4 - 5% formaldehyde. The sample jars are labelled with the site name, the site code, the sampling position (left bank, middle, right bank), and the sampling date.

The site name, the site code, the sampling position (left bank, middle, right bank), the sampling date, the sample number and the collector's name are also noted in the field notebook, as is any information about the site that could be influencing the presence or abundance of different types of zooplankton.

Step 1. A plankton net.

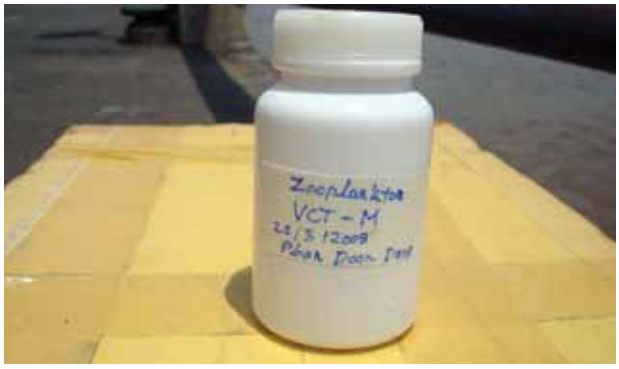
Step 2. Collect 10L of water and filter it slowly through the plankton net.

Step 3. Wash the net by splashing water on the outside.

Step 4. Rinse the bottom of the net to result in a water volume of about 150mL.

Step 5. Transfer the sample to a 250mL jar.

Step 6. Add 75mL of 10% formaldehyde as a preservative.



Step 7. Label the sample jar (site name, site code, date, location in the river).

Step 8. Pack and transport to the laboratory for identification.

Figure 6.1. Illustration of field procedures for sampling zooplankton.

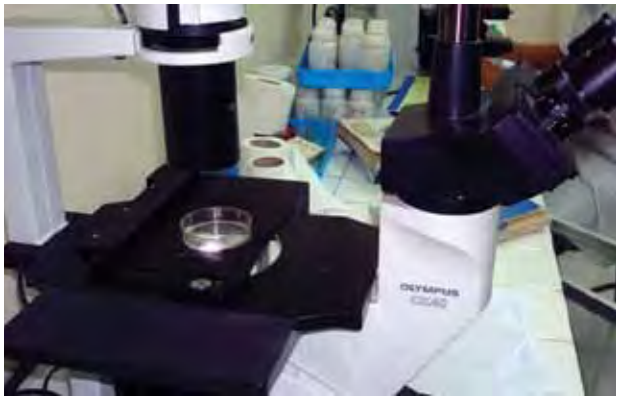
Laboratory procedures

1. In the laboratory, large particles of debris are removed from the samples with forceps and shaken to remove any attached zooplankton. Each sample is filtered through a net with a mesh size of 10µm, rinsed with distilled water, and then allowed to settle to the bottom of a graduated cylinder and left for 1 hour. Excess water is discarded until about 50mL of water and the settled material (which contains the zooplankton) remain.
2. This 50mL of water and the settled material is then transferred into a Petri dish and examined under a stereo-microscope at a magnification of 40x to identify the large species of zooplankton (> 50µm in diameter). The smaller species and details of larger species are examined with a compound microscope at a magnification of 100 – 400x. All individuals collected are counted and identified to the lowest taxonomic level possible, generally that of species. After analysis, samples are returned to the bottles and preserved.

Step 3. Filter the sample through a net with a mesh size of 10µm, to result in a total volume of 50mL.

Step 1. Use forceps to remove large debris from sample.

Step 2. Allow the sample to settle in a cylinder for about 1 hour.



Step 4. Transfer the sample to a Petri dish and examine it under a stereo-microscope at a magnification of 40x to identify the larger specimens of zooplankton.

Step 5. The smaller species and morphological details of large species are examined with a compound microscope at a magnification of 100 – 400x .

Figure 6.2. Illustration of the laboratory procedures for zooplankton.

Analytical methods

Determine the average richness, abundance and ATSPT values for each sample, which always are reported per sample (which is 10L) and averaged for the site.

Identification aids

Dang et al. (1980), Eiji (1993). A book on the identification of zooplankton for the Lower Mekong is in preparation.

References cited

Dang, N.T, Thai, T.B. & V.M. Pham (1980) Classification of freshwater invertebrate zoology in North Viet Nam. Science and Technology Publisher, Ho Chi Minh City.

Eiji, T. (1993) An illustrated guide to fresh water zooplankton. Togai University Publisher.

Appendix: Field data sheets

Appendix 6.1. Field data sheet zooplankton collections

Site name: _____ Site code: _____

Date: _____ Time: from _____ to _____

Members of team: _____

Member responsible for data collection: _____

General observations (water, substrate, bank appearance; weather; tide; other): _____

_____

_____

_____

Samples are collected at: left side ○ AND middle ○ AND right side ○

Chapter 7

Littoral macroinvertebrates

Chanda Vongsombath, Bounnam Pathoumthong, and Narumon Sangpradub

Macroinvertebrates are those animals that lack a backbone and are just visible to the naked eye >0.2 mm. Littoral macroinvertebrates occur in areas near the shoreline.

Objective

The objective of studying the littoral macroinvertebrates is to quantitatively describe the characteristics of the macroinvertebrate community living in the shallow near-shore areas. These reflect the quality of the areas near the riparian zone and reflect the influence of many human activities that occur there.

Because the information collected will be compared to the baseline data and criteria established from the 2004 - 2007 studies, it is critical that **EXACTLY THE SAME METHODS** are used as those used then. These methods are described below. Failure to follow these procedures may result in assignment of misleading better or worse scores to a particular site. The requirement of following the same procedures as used in the 2004 - 2007 studies is important and is the responsibility of each of the NMC teams performing the biomonitoring sampling.

Materials and supplies needed

Field:

- A D-frame net with 30 cm x 20 cm opening and mesh size of 475µm
- White sorting trays with at least a 0.1m^2 (25 cm x 40 cm) surface area and raised sides
- Forceps
- Plastic pipette
- Collecting jars
- 90% Ethanol
- Labelling materials
- Waterproof field notebook and pencil

Laboratory:

- Dissecting microscope
- 70% ethanol
- Vials to store specimens
- Labelling materials

Field procedures

At each site, littoral macroinvertebrate samples usually are taken on only one side of the river. In most instances this is done on the depositional, rather than the erosional side. Sampling is easier on the depositional side (the side of the river where the bend is depositing material) 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 habitats suitable for invertebrates. Because the study area is usually large, a wide range of littoral habitat types are typically sampled. As far as possible, similar habitats should be selected at each site to facilitate comparisons among sites.

A field data sheet for littoral macroinvertebrate is provided to record site information and should be filled in completely and accurately (see Appendix 7.1).

1. Before (and after) sampling at each site, the equipment is washed to remove any organisms and other matter left from the previous site.

2. At each site, sweep sampling methods are used. A D-frame net with 30 cm x 20 cm opening and mesh size of 475µm is used. Sweep samples are taken along the shore at about 20m intervals.

3. To obtain each sweep sample, the collector stands in the river about 1.5 m from the water's edge and sweeps the net towards the bank, near the substrate surface. The collector should use an up-and-down motion, making contact with the substratum on each down stroke, while moving steadily toward the water's edge. This motion will disturb the animals living at the water-substrate interface and result in their being swept into the net. Each sweep is done for about 1m at right angles to the bank, in water no deeper 1m, from downstream to upstream and not overlapping the previous sweep. Be careful not to bring sediment into the net because it will make sorting difficult. In water deeper than 1m, the sample should be obtained from a boat, as illustrated below.

4. Ten sweep samples are taken per site, and the 10 sweeps make up a sample. Therefore, 100 sweeps are collected in the 10 individual samples collected at each site.

5. After sample collection, the D-frame net contents are washed to the bottom of the net by splashing the outside of the net with water. The D-frame net is then inverted and its contents emptied into a hand net or sieve, with any material adhering to the net being washed off with clean water. The material now in the hand net or sieve is rinsed to remove silt. The contents of this hand net are then transferred to a metal or plastic sorting tray, with any material adhering to the hand net or sieve being washed with clean water.

6. Invertebrates can be sorted in the field or returned to the laboratory for sorting.
   - 6a. In the field, invertebrates are picked from the tray with forceps and placed in a jar of 80 - 90% ethanol. It is crucial that the final alcohol concentration after specimens or substrate are added never falls below 70% or the specimens will deteriorate and not be identifiable. Samples with a small number of individuals are kept in 30mL jars and large samples are kept in 150mL jars. During the picking process, the tray is shaken from time to time to redistribute the contents, and tilted occasionally to look for animals adhering to it. Sorting proceeds by working back and forth across the tray until no more animals are found.
   - 6b. If sorting is to be done in the laboratory, the entire sample is placed in a plastic container, with the sample drained of water through a fine sieve with a mesh of 475µm or smaller. The jars should be no more than half-full of substrate material. 90% alcohol is then added to the container. It is crucial that the final alcohol concentration after specimens or substrate are added never falls below 70% or the specimens will deteriorate and not be identifiable.

7. The sample jars are labelled with the site name, the site code, the date, and the sample replicate number.

8. The appropriate information is filled in on the field data sheet. Information about substrate types sampled as well as any information or characteristics about the site that could be influencing the presence or abundance of different types of littoral macroinvertebrates is included.

A D-frame net with 30cm x 20cm opening and mesh size of 475µm is used for sampling.

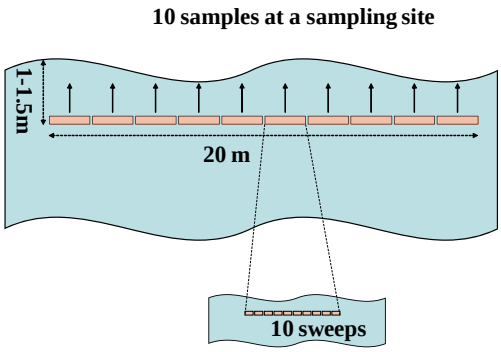


Diagram for collecting samples in the Mekong River.

Step 1a. In shallow water, the collector stands in the river about 1 - 1.5m from the water's edge and sweeps the net towards the bank, near the substrate surface OR

Step 1b. In deep water, the collector stands on a boat about 1 - 1.5 m from the water's edge and sweeps the net towards the bank, near the substrate surface.

Step 2. The D-frame net is then inverted and its contents emptied into a hand net or sieve.

Step 3. The material adhering to the net is washed off with clean water.

Step 4. The net is then inverted and its contents emptied into a metal or plastic sorting tray.

Step 5. Invertebrates can be sorted in the field with forceps and plastic pipette.

Step 6. Small invertebrates can be picked with plastic pipette.

Step 7. The sample jar is labelled with the site name, site code, date, and sample replicate number.

Figure 7.1. Illustration of the field procedures for littoral macroinvertebrate sampling.

Laboratory procedures

1. In the laboratory, the samples are identified under a stereo dissecting microscope with a 2x – 4x objective lens and a 10x eyepiece. Identification is done to the lowest taxonomic level that could be applied accurately, which is usually to genus.

2. Specimens are divided into orders, and kept in separate jars with 70% ethanol and labelled by site.

Analytical methods

Richness, abundance and ATSPT scores are calculated and reported per sample (which is 10 sweeps or approximately 3m^2 of substrate surface).

Identification aids

Dudgeon (1999), Morse et al. (1994), Merritt et al. (2008), Sangpradub and Boonsoong (2006), Yule and Sen (2004). Sangpradub and Boonsoong (2006) specifically covers the Lower Mekong macroinvertebrate fauna.

References cited

Dudgeon, D. (1999) Tropical Asian streams. Zoobenthos, ecology and conservation. Hong Kong University Press.

Merritt, R.W., Cummins, K.W. & M.B. Berg (2008) An introduction to the aquatic insects of North America. 4th edition. Kendall Hunt, Dubuque, Iowa.

Morse, J.C., Liangfang, Y. & T. Lixin (1994) Aquatic Insects of China Useful for Monitoring Water Quality. Hohai University Press, Nanjing, China.

Sangpradub, N. & B. Boonsoong (2006) Identification of freshwater invertebrates of the Lower Mekong River and its tributaries. Mekong River Commission, Vientiane.

Yule, C.M. & Y.H. Sen (2004) Freshwater invertebrates of the Malaysian region. Academy of Sciences Malaysia, Kuala Lampur.

Appendix: Field data sheets

Appendix 7.1. Field data sheet for littoral macroinvertebrate collections

Site name: _____ Site code: _____

Date: _____ Time: from _____ to _____

Members of team: _____

Member responsible for data collection: _____

General observations (water, substrate, bank appearance; weather; tide; other): _____

_____

_____

_____

Samples are collected at: left side of the river ○ OR right side of the river ○

Sample sorting: Field ○ OR Laboratory ○

Notes on sorting complications: _____

Preservation solution: _____

Composition and surface cover of substrate	Replicates									
	1	2	3	4	5	6	7	8	9	10
Approximate composition of substrate as percentage of total (100%)										
• Bedrock										
• Boulder (>256mm)										
• Cobble (64 - 256mm)										
• Pebble (16 - 64mm)										
• Gravel (2 - 16mm)										
• Sand (0.06 - 2mm)										
• Silt (0.04 - 0.06mm)										
• Clay (<0.04mm)										
• Detritus (leaves, sticks, wood, trash)										
• Muck/mud (black, very fine organics)										

Approximate surface cover of plant materials as percentage of total (100%)
• Moss
• Filamentous algae
• Macrophytes (roots, submerged and floating plants)
• No vegetation

Chapter 8

Benthic macroinvertebrates

Pham Anh Duc and Narumon Sangpradub

Macroinvertebrates are those animals that lack a backbone and are just visible to the naked eye >0.2 mm. Benthic macroinvertebrates occur in the deeper-water areas away from the shoreline.

Objective

The objectives of the benthic macroinvertebrates study are to quantitatively describe the characteristics of the macroinvertebrates that occur in the bottom substratum in deeper waters away from the littoral zone of the river.

Because the information collected will be compared to the baseline data and criteria established from the 2004 - 2007 studies, it is critical that **EXACTLY THE SAME METHODS** are used as those used then. These methods are described below. Failure to follow these procedures may result in assignment of misleading better or worse scores to a particular site. The requirement of following the same procedures as used in the 2004 - 2007 studies is important and is the responsibility of each of the NMC teams performing the biomonitoring sampling.

Materials and supplies needed

Field:

- Petersen grab sampler with a sampling area of 0.025m^2
- A large sieve (~50 cm in diameter) with a 0.3mm mesh
- A small kitchen sieve
- Sorting trays with at least a 1m^2 surface area and raised sides
- Forceps
- Collecting jars
- 90% ethanol
- 10% formaldehyde
- Labelling materials
- Waterproof field notebook and pencil

Laboratory:

- Dissecting microscope
- Compound microscope
- 95% alcohol
- Vials to store specimens

Field procedures

A field data sheet for benthic macroinvertebrates is provided to record site information and should be filled in completely and accurately (see Appendix 8.1)

1. Before (and after) sampling at each site, the equipment is washed to remove any organisms and other matter left from the previous site.

2. Sampling locations at each site are selected in the right, middle, and left parts of the river. If a site is on a national border, the three sections to be sampled should be within that Member Country's border.

3. Samples are taken at a minimum of three to a maximum of five locations at each of the three parts of the river. More samples are required at sites with higher inter-sample variability, such as in the Viet Nam Delta, than in sites with lower variability.

4. At each sampling location, four sub-samples are taken with a Petersen grab sampler and composited into a single sample, covering a total area of 0.1m^2.

5. At some sites, the middle of the river cannot be sampled because of the presence of hard bed material (which the grab sampler cannot penetrate) or fast currents. In these cases, a third area where samples can be appropriately collected should be selected and sampled. Also, sites where the middle portions of the river are narrower than 30m are not sampled.

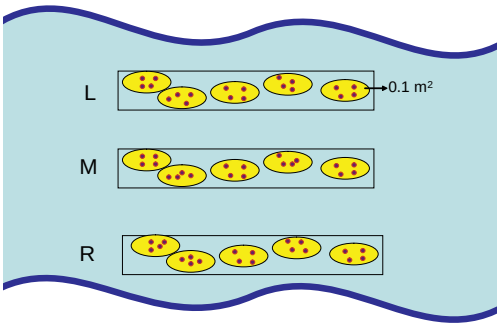
6. Grab contents are discarded if the grab did not close properly because material such as wood, bamboo, large waterplants, or stones jammed the grab's jaws. In these cases the sample is retaken.

7. Each sample is washed through a sieve (0.3mm mesh) with care taken to ensure that macroinvertebrates did not escape over the sides of the sieve.

8. The contents of the sieve are then placed in a white sorting tray and the material (including the benthic macroinvertebrates) is dispersed in water. All the animals in the tray are picked out with forceps and pipettes, placed in jars, and fixed with 10% formaldehyde to a final concentration of 5%. Alternatively, 95% ethanol can be used. It is crucial that the final alcohol concentration after specimens are added never falls below 70% or the specimens will deteriorate and not be identifiable. Samples of less experienced sorters are checked by an experienced sorter.

9. The sample jar is labelled with the site name, the location code, the date, the position within the river, and the sample replicate number. The sampling location conditions, collector's name and sorter's name are recorded on a field sheet. Sometimes, samples cannot be sorted on site because the boat is poorly balanced, a very large number of animals are collected, there is insufficient time at a site, or because the presence of lumps of clay cause the samples to cloud continually. In these cases, the entire sample is preserved and sorted in the laboratory. Preservation is with 10% formaldehyde at a final concentration of 5%. Alternatively, 95% ethanol can be used. It is crucial that the final alcohol concentration after specimens are added never falls below 70% or the specimens will deteriorate and not be identifiable.

10. The collector's name, the sampling site name, the location code and the replicate sample number are recorded in a field notebook. Information about substrate types sampled as well as any information or characteristics about the site that could be influencing the presence or abundance of different types of benthic macroinvertebrates are included.



Three to five samples (represented by ovals), each consisting of four sub-samples (represented by dots) are collected in Left (L), Middle (M) and Right (R) parts of the river.

Step1. Check the operation of the Petersen grab sampler.

Step 2. Lower the grab into the water, making sure that it catches on the river bottom.

Step 3. Release the contents from the grab into a stack of sieves with the largest mesh size on the top, and the finest at the bottom.

Step 4. Wash the sample, aquatic plants, and substrate, etc. by shaking the sample. Repeat many times.

Step 5. Remove some of the sample and place it in a smaller-sized sieve with a 0.3mm mesh for further washing.

Step 6. Invertebrates can be sorted in the field with forceps.

Step 7. The sample jar is labelled with the site name, site code, date, and sample replicate number.

Figure 8.1. Illustration of the field procedures for benthic macroinvertebrate sampling.

Laboratory procedures

All individuals collected are identified and counted under a compound microscope (with a magnification of 40 – 1200x) or a dissecting microscope (16 – 56x). Oligochaeta, Gastropoda, Bivalvia, and Crustacea are generally identified to species level. Insects are usually identified only to genus level.

Analytical methods

Richness, abundance and ATSPT scores always are calculated and reported per sample (which is 0.1m^2).

Identification aids

Dudgeon (1999), Morse et al. (1994), Merritt et al. (2008), Sangpradub and Boonsoong (2006), Yule and Sen (2004). Sangpradub and Boonsoong (2006) specifically cover the Lower Mekong macroinvertebrate fauna.

References cited

Dudgeon D. (1999) Tropical Asian streams. Zoobenthos, ecology and conservation. Hong Kong University Press.

Merritt, R.W., Cummins, K.W. & M.B. Berg (2008) An introduction to the aquatic insects of North America. 4th edition. Kendall Hunt, Dubuque, Iowa.

Morse, J.C., Liangfang, Y. & T. Lixin (1994) Aquatic Insects of China Useful for Monitoring Water Quality. Hohai University Press, Nanjing, China.

Sangpradub, N. & B. Boonsoong (2006) Identification of freshwater invertebrates of the Lower Mekong River and its tributaries. Mekong River Commission, Vientiane.

Yule, C.M. & Y.H. Sen (2004) Freshwater invertebrates of the Malaysian region. Academy of Sciences Malaysia, Kuala Lampur.

Appendix: Field data sheets

Appendix 8.1. Field data sheet for benthic macroinvertebrate collections

Site name: _____ Site code: _____

Date: _____ Time: from _____ to _____

Members of team: _____

Member responsible for data collection: _____

General observations (water, substrate, bank appearance; weather; tide; other): _____

_____

_____

_____

Samples are collected at: left side ○ AND middle ○ AND right side ○

Sample sorting: Field ○ OR Laboratory ○

Notes on sorting complications: _____

Preservation solution: _____

Approximate composition of substrate as percentage of total (100%)	Left	Middle	Right
• Bedrock			
• Boulder (>256mm)			
• Cobble (64 - 256mm)			
• Pebble (16 - 64mm)			
• Gravel (2 - 16mm)			
• Sand (0.06 - 2mm)			
• Silt (0.04 - 0.06mm)			
• Clay (<0.04mm)			
• Detritus (leaves, sticks, wood, trash)			
• Muck/mud (black, very fine organics)			

Chapter 9

Biological metrics calculation

Bruce Chessman and Dao Huy Giap

Objective

Metrics are those measurements that provide a summary of the information collected. The objective of this chapter is to review the calculation and analysis of the different metrics.

Because the information collected will be compared to the baseline data and criteria established from the 2004 - 2007 studies, it is critical that **EXACTLY THE SAME METHODS** are used as those used then. These methods are described below. Failure to follow these procedures may result in assignment of misleading better or worse scores to a particular site. The requirement of following the same procedures as used in the 2004 - 2007 studies is important and is the responsibility of each of the NMC teams performing the biomonitoring sampling.

For all sites, the metrics calculated are those of abundance (the number of individual organisms collected per sample, unit area or volume), average richness (the mean number of taxa counted in a sample), and the average tolerance score per taxon (ATSPT) for each site. ATSPT is an indicator of the presence of environmental stressors such as water pollution. Species that are sensitive to stress, tend to be absent at stressed sites and 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.

Calculation of abundance

Abundance is a measurement of the number of individual plants or animals belonging to a particular biological indicator group counted in a sample. Low abundance is sometimes a sign that the ecosystem has been harmed. Abundance can be measured as the number of individuals per unit of areas, volume or sample.

Calculation of average richness

Average richness refers to the mean number of taxa (types) of plants or animals belonging to a particular indicator group (e.g. diatoms, zooplankton) counted in a sample.

Calculation of ATSPT

A tolerance value was calculated for each taxon collected during the baseline studies conducted in 2004, 2005, 2006 and 2007. Tolerance values for new taxa collected in 2008 were determined from the average Site Disturbance Scores at the sites where these new taxa were found. Tolerance values are derived by assessing the relationship between the presence and absence of species in samples from each study site and the value of an independently measured ‘Site Disturbance Score’ (SDS) for each site. A visual method for determining the SDS is described in Chapter 3.

The tolerance of each species (or higher taxon where identification to species is not possible) is calculated as the average Site Disturbance Score for all sites at which that species occurs weighted by the number of samples per site in which the species is recorded. The tolerance values are then re-scaled so that they range 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) is then calculated for each sample collected. ATSPT is the average tolerance of all taxa recorded in a sample, calculated without regard to their abundances. A worked example[4] on the calculations is given in Figure 9.1.

Using biological indicators to evaluate sites

Three types of indicators of the health of the aquatic ecosystem are calculated for each of four groups of organisms included in the biomonitoring programme (benthic diatoms, zooplankton, littoral macroinvertebrates and benthic macroinvertebrates). These indicators are abundance (mean number of individual organisms per sample), average richness (mean number of taxa per sample), and tolerance (the average tolerance score per taxon). Signs of poor ecosystem are indicated by low abundance (few organisms present), low average richness (low biodiversity), 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), relative to the conditions found at the reference sites.

Each indicator is calculated for the individual samples of each group of organisms that are collected at a site. 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 are averaged.

Guidelines for site-average values of each indicator are set according to the range of site-average values obtained at the reference sites. For indicators where low values indicate harm to the ecosystem (abundance and average richness) the guideline was set at the 10th percentile of reference site values (the value that is lower than 90% 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% of all reference values). These percentiles are commonly used in biomonitoring programmes in other parts of the world. Interim guidelines are listed in Table 9.1.

The sites are classified and grouped according to the number of the 12 indicators that met the guidelines. It is important to remember that while each of the rating criteria has a scientific basis, the classification system is subjective, and being a policy decision, can be changed. Table 9.2 gives definitions of the classification and some characteristics expected for sites in each class.

[4] This worked example was extracted from the 2004 zooplankton survey. For detail demonstrative purposes, it has been simplified by considering only three taxa (*Ceratium* spp., Chironomidae, and Copepoda (Nauplius) and only four sites (LNO, LPB, LVT, and LNG).

Zooplankton were sampled at four different sites. Three samples of zooplankton were collected at each site (at Left, Middle and Right). Data in the table is number of individual found per sample.

Taxa Name	Site 1			Site 2			Site 3			Site 4		
	L	M	R	L	M	R	L	M	R	L	M	R
Taxon A			1	196	149	145	1	8		13	7	6
Taxon B	2	1		1	2	1	2	3	2			
Taxon C		2	1	3		1	1		5	42	38	78

Step	Example	Calculation
Step 1: Calculation of SDS for each site 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.	Eight participants gave the following scores:	
	for Site 1: 1, 1, 1, 1, 1, 1, 1, 1	→ SDS1 = (1+1+1+1+1+1+1+1)/8 = **1.00**
	for Site 2: 1, 1, 2, 1, 1, 1, 1, 2	→ SDS2 = (1+1+2+1+1+1+1+2)/8 = **1.25**
	for Site 3: 1, 1, 2, 1, 2, 2, 2, 3	→ SDS3 = (1+1+2+1+2+2+2+3)/8 = **1.75**
	for Site 4: 3, 3, 3, 3, 3, 2, 3, 3	→ SDS4 = (3+3+3+3+3+2+3+3)/8 = **2.88**
Step 2. Calculation of the Tolerance Score for each taxon This is calculated as the average of the SDSs for all samples in which the particular taxon was collected.	Taxon A was found in: 1, 3, 2, 3 samples from Sites 1, 2, 3, 4 respectively.	→ The tolerance score of taxon A would be: (1.00*1+1.25*3+1.75*2+2.88*3)/(1+3+2+3) = **1.88**
	Taxon B was found in: 2, 3, 3, 0 samples from Sites 1, 2, 3, 4 respectively.	→ The tolerance score of taxon B would be: (1.00*2+1.25*3+1.75*3+2.88*0)/(2+3+3+0) = **1.38**
	Taxon C was found in: 2, 2, 2, 3 samples from Sites 1, 2, 3, 4 respectively.	→ The tolerance score of taxon C would be: (1.00*2+1.25*2+1.75*2+2.88*3)/(2+2+2+3) = **1.85**
Step 3. Re-scaling of Tolerance Scores Tolerance scores were then re-scaled to range from 0 – 100 instead of 1 – 3, in order to make a more sensible range.	The re-scaling is done by subtracting 1 from the average tolerance score and then multiplying the remainder by 50.	→ Re-scaling of Tolerance Score (taxon A) = (1.88-1.00)*50 = **43.75** Re-scaling of Tolerance Score (taxon B) = (1.38-1.00)*50 = **18.75** → Re-scaling of Tolerance Score (taxon C) = (1.85-1.00)*50 = **42.36**
Step 4. Calculation of the Average Tolerance Score Per Taxon for each individual sample from a site	Site 1, sample 1: taxa B was found	→ =(43.75*0+18.75*1+42.36*0)/(0+1+0) = **18.75**
	Site 1, sample 2: taxa B, C were found	→ =(43.75*0+18.75*1+42.36*1)/(0+1+1) = **30.56**
	Site 1, sample 3: taxa A, C were found	→ =(43.75*1+18.75*0+42.36*1)/(1+0+1) = **43.06**
	Site 2, sample 1: taxa A, B, C were found	→ =(43.75*1+18.75*1+42.36*1)/(1+1+1) = **34.95**
	Site 2, sample 2: taxa A, B were found	→ =(43.75*1+18.75*1+42.36*0)/(1+1+0) = **31.25**
	Site 2, sample 3: taxa A, B, C were found	→ =(43.75*1+18.75*1+42.36*1)/(1+1+1) = **34.95**
	Site 3, sample 1: taxa A, B, C were found	→ =(43.75*1+18.75*1+42.36*1)/(1+1+1) = **34.95**
	Site 3, sample 2: taxa A, B were found	→ =(43.75*1+18.75*1+42.36*0)/(1+1+0) = **31.25**
	Site 3, sample 3: taxa B, C were found	→ =(43.75*0+18.75*1+42.36*1)/(0+1+1) = **30.56**
	Site 4, sample 1: taxa A, C were found	→ =(43.75*1+18.75*0+42.36*1)/(1+0+1) = **43.06**
	Site 4, sample 2: taxa A, C were found	→ =(43.75*1+18.75*0+42.36*1)/(1+0+1) = **43.06**
	Site 4, sample 3: taxa A, C were found	→ =(43.75*1+18.75*0+42.36*1)/(1+0+1) = **43.06**
Step 5. Calculation of the mean Average Tolerance Score Per Taxon for each site	ATSPT for Site 1	→ =(18.75+30.56+43.06)/3 = **30.79**
	ATSPT for Site 2	→ =(34.95+31.25+34.95)/3 = **33.72**
	ATSPT for Site 3	→ =(34.95+31.25+30.56)/3 = **32.25**
	ATSPT for Site 4	→ =(43.06+43.06+43.06)/3 = **43.06**

Figure 9.1. Illustration of the calculation of ATSPT

Table 9.1. Guidelines for biological indicators of ecosystem health based on 2004-2007 baseline studies

Indicator	Biological group	Reference site values		Guideline of healthy ecosystem
		10th percentile	90th percentile	
Abundance (mean number of individual organisms per sample).	Diatoms	136.22	376.34	Greater than 136.22
	Zooplankton	22.33	174.07	Greater than 22.33
	Littoral macroinvertebrates	46.68	328.56	Greater than 46.68
	Benthic macroinvertebrates	5.37	56.34	Greater than 5.37
Average richness (mean number of taxa per sample).	Diatoms	6.54	11.78	Greater than 6.54
	Zooplankton	9.80	20.20	Greater than 9.80
	Littoral macroinvertebrates	5.37	18.48	Greater than 5.37
	Benthic macroinvertebrates	1.87	7.88	Greater than 1.87
Average tolerance Score per taxon (ATSPT).	Diatoms	30.85	38.38	Less than 38.38
	Zooplankton	34.83	41.80	Less than 41.80
	Littoral macroinvertebrates	27.80	33.58	Less than 33.58
	Benthic macroinvertebrates	31.57	37.74	Less than 37.74

Table 9.2. 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 et season. These factors were not assessed in the biomonitoring programme.

Chapter 10

Designation of reference sites

Vincent H. Resh and Bruce Chessman

Objective

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 as reference sites are usually selected on the basis of good water quality and habitat, and minimal disturbance from human activities. They are commonly those sites that are in a most natural, or pristine, state but at other times they are the sites with the best attainable condition. Reference sites for the Mekong provide benchmark data against which all sites in the system can be compared.

Characteristics of reference sites

Accordingly, reference sites are 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 are based on those proposed for the MRC's Environment Programme Water Quality Index (MRC 2008). Site disturbance is scored by the national and international experts present on each sampling occasion (Chapter 3), with regard to site-scale activities such as the following (e.g. Figure 10.1):

1. Unnatural bank erosion;
2. Fishing intensity;
3. Dredging and mining;
4. Sand and gravel extraction;
5. Waste disposal from villages, farms, towns etc.;
6. Village activities such as bathing and washing of clothes;
7. Removal of natural riparian vegetation for agriculture or housing;
8. Agricultural cultivation;
9. In-stream aquaculture;
10. Road building;
11. Cattle and buffalo grazing;
12. Boat traffic;
13. Unnatural fluctuations in water level.

The determination of a Site Disturbance Score is described in Chapter 3. Briefly, the SDS calculated from 2004 - 2007 were reached by discussions among the team members at a site. In future, a visual assessment can be used (Chapter 3). SDS scores can range from 1 (little or none of any of these types of disturbance) to 3 (substantial disturbance of one or more types).

Visual assessment is used because it is 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. The 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 has 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 70mS/m.

3. The dissolved oxygen concentration at the time of biological sampling was greater than 5mg/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 urbanisation, intensive agriculture or waste disposal.

5. There was no major dam or city within 20km 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.

i

ii

iii

iv

v

vi

vii

viii

ix

x

Note: From left to right (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) urbanisation, (viii) aquaculture, (ix) agricultural cultivation, and (x) dredging .

Figure 10.1. Illustration of anthropogenic impacts that can occur at sites in the Lower Mekong

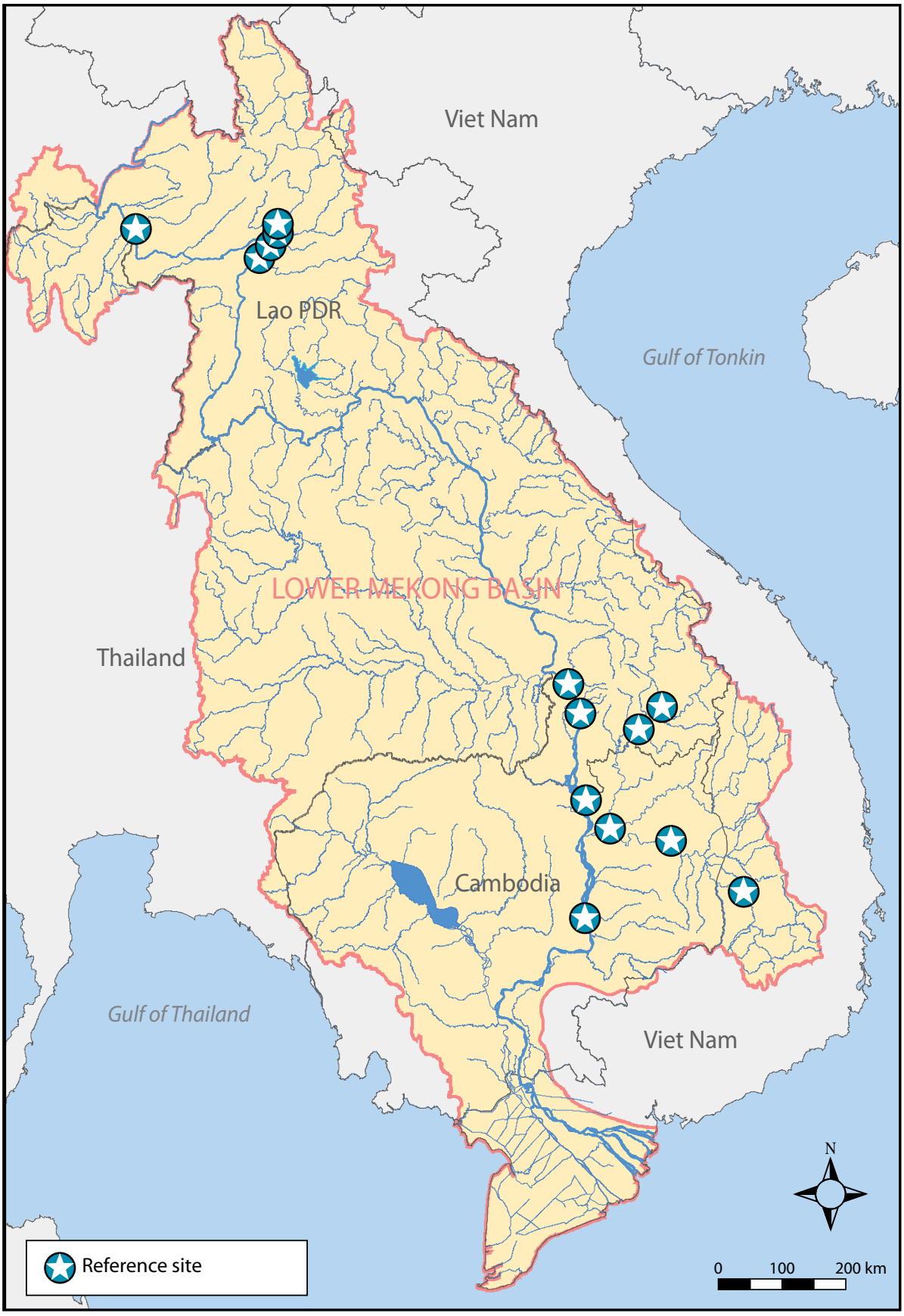


Figure 10.2. Map of the fourteen reference sites selected during 2004-2007 surveys

References cited

MRC (2008) An assessment of water quality in the Lower Mekong Basin. MRC Technical Paper No. 19. Mekong River Commission, Vientiane.

Other MRC publications on biomonitoring:

Sangpradub, N. & B. Boonsoong (2006) Identification of freshwater invertebrates of the Mekong River and its tributaries. Mekong River Commission, Vientiane. 274 pp.

Davidson, S.P., Kunpradid, T., Peerapornisal Y., Linh, N.T.M., Pathoumthong, B., Vongsambath, C. & P.A. Duc (2006) Biomonitoring of the lower Mekong River and selected tributaries. MRC Technical Paper No.13, Mekong River Commission, Vientiane. 100 pp.

MRC (2008) The Mekong River report card on aquatic ecological health (2004-2007). Mekong River Commission, Vientiane. 6 pp.

MRC (2008) Biomonitoring of the lower Mekong River and selected tributaries, 2004 – 2007, MRC Technical Paper No. 20, Mekong River Commission, Vientiane. 77 pp.

Vongsombath, C., Duc, P.A., Linh, N.T.M., Kunpradid, T., Davison, S.P., Peerapornpisal, Y., Khom, S. & M. Monyrak (2009) Report on the 2006 biomonitoring survey of the lower Mekong River and selected tributaries, MRC Technical Paper No. 22. Mekong River Commission, Vientiane. 124 pp.

Vongsombath, C., Duc, P.A., Linh, N.T.M., Kunpradid, T., Davison, S.P., Peerapornpisal, Y., Khom, S. & M. Monyrak (2009) Report on the 2007 biomonitoring survey of the lower Mekong River and selected tributaries, MRC Technical Paper No. 23. Mekong River Commission, Vientiane. 75 pp.

MRC (2009) The Mekong River report card on aquatic ecological health 2008. Mekong River Commission, Vientiane. 6 pp.

Giap, D.H., Kunpradid, T. Vongsombath, C., Loc, D.T.B. & P. Somany (2010) Report on the 2008 biomonitoring survey of the lower Mekong River and selected tributaries, MRC Technical Paper No. 27. Mekong River Commission, Vientiane. 69 pp.

Mekong River Commission

P.O.Box 6101, 184 Fa Ngoum Road, Unit 18, Ban Sithane Neua,
Sikhottabong District, Vientiane, Lao PDR

Telephone: (856) 21 263 263 Facsimile: (856) 21 263 264
E-mail: mrcs@mrcmekong.org
Website: www.mrcmekong.org