ENVIRONMENTAL SENSITIVITIES OF RIVERINE FISHES AND THE DEVELOPMENT OF A BIOASSESSMENT MODEL WITH WHICH TO EVALUATE WATER QUALITY

SAMPAN TONGNUNUI

A THESIS SUBMITTED IN PARTIAL FULFILLMENT OF THE
REQUIREMENTS FOR THE MASTER DEGREE OF SCIENCE
IN ENVIRONMENTAL SCIENCE
FACULTY OF SCIENCE
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The thesis of Sampan Tongnunui has been approved by the examining committee to be partial fulfillment of the requirements for the Master Degree of Science in Environmental Science of Burapha University

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Sampan Tongnunui

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CHAPTER 1
INTRODUCTION

Rivers provide important sources of water for a variety of domestic, recreational, agricultural and industrial services (Metcalfe-Smith, 1994; Sweeting, 1994; Mustow, 1999; Griffiths, Beamish, & Kongchaiya 2003), as well as habitat for many aquatic and terrestrial organisms. A potential consequence of water use is a change in its quantity and quality. In the extreme, this can be detrimental to the services provided by water and, importantly, to both aquatic and terrestrial ecosystems.

Historically, the integrity or general quality of water has been evaluated on the basis of acceptable levels of specific chemicals. This excludes factors associated with the destruction or alteration of ecological habitat such as sedimentation, discharge, flow and thermal addition. Drainage activities are also typically undetected by chemical monitoring programs that are usually conducted only during government working hours.

The concept of bioassessment integrates chemical, physical and ecological information in detecting and quantifying many types of environmental degradation and assigns an ecological integrity value. Its measurement is based on the health of organisms living in the habitat over long periods (Karr, 1991; Chapman, 1992; Mason, 2002). Biological methods for evaluating water quality began about 100 years ago when specific benthic invertebrates were associated with organic waste production (Forbes & Richardson, 1913; Richardson 1928). Water of good quality or minimally disturbed was associated with a variety of gill-breathing insects, for example, stoneflies (Plecoptera), mayflies (Ephemeroptera) and caddisflies (Trichoptera). Metabolic function in many of these taxa requires high ambient concentrations of dissolved oxygen. In contrast, some other benthic taxa such as in the family Tubificidae, Hirudineae (leeches) and Chironomidae thrive in or on organically-enriched sediment of rivers low in ambient oxygen. Strong relationships have been described between aquatic insects and environmental quality (Karr, 1981). The application of biological information to evaluate water quality integrates relevant knowledge for water management rather than the limited and, sometimes, misleading information provided by short term chemical measurement (Barbour, Gerritsen, Snyder, & Stribling, 1997).

Thailand has a large number of rivers with a rich diversity of freshwater fish (Smith, 1945; Nelson, 1994; Vidthayanon, Karnasuta, & Nabhitabhata, 1997; Monkolprasit, Sontirat, Vimollohakarn, & Songsirikul, 1997). In this study, fish have been chosen to evaluate water and habitat quality and ecological integrity because of their wide distribution, habitat preferences, ecological importance and their aesthetic value for many people. Fish can be collected and identified with relative easy to the level of genus or species. Trophic level (omnivores, carnivore and insectivores) can be assigned to most species from published information (Ward-Campbell, 2004; Ward-Campbell, Beamish, & Kongchaiya, 2005; Ward-Campbell & Beamish, 2005).

Karr (1981) used fish to assess ecological quality using a method he developed and called Index of Biotic Integrity (IBI). This approach embraced, specific environmental tolerances, composition of fish assemblages, presence of indicator species, representation by trophic level and overall structure. The present study monitored aspects of fish ecology in Thai streams consistent with the information applied in the Index of Biotic Integrity. Because of the scarcity of environmental information for Thai river fishes, a number of additional measurements were made to identify significant characteristics and individual sensitivities. The development of an Index of Biotic Integrity for Thailand will be useful for conservation purposes as well as for the management of surface water in Thailand's small rivers and streams.

Objective:

To develop a relatively simple bioassessment method to evaluate the ecosystem health of Thailand's small rivers and streams.

Hypothesis

1. The bioassessment approach can efficiently and accurately assess general water quality and environmental health for aquatic organisms.

2. Fish abundance, diversity and distribution are related to water quality.

3. Water quality is related to biogeomorphological and anthropogenic activities within the watershed.

Specific Environmental Benefit:

Conservation of the diverse and, in some cases unique fauna in the waterways of Thailand and, indeed, all of South East Asia will require quantification of ecosystem health, particularly habitat quality before meaningful management action is likely to be implemented. The chemical methods currently in use are recognized as inadequate because they measure water quality only for a short period of time and provide no information on the physical characteristics of the habitat. Biological methods integrate chemical and physical quality of the environment over long periods of time reflecting the life cycles of the organisms under study. The benefit of a simple bioassessment method is in its easy application, protection of water quality for human services and the conservation of aquatic biotic.

Scope in Research

1. To study numbers species of fish in small rivers

2. To study the relationship between species composition, fish abundances and water quality.

3. To modify an existing bioassessment model developed for temperate latitude to assess water quality of small rivers and stream in tropical Thailand.

CHAPTER 2
LITERATURE REVIEW

Relationship between Fish and Environmental Circumstances

Assessment and monitoring of water quality are important for conservation of aquatic life and human health. Fishes are an important biological component of the aquatic ecosystem and are represented by a large number of species. Nowhere is freshwater fish diversity better represented than in Thailand with about 570 species listed (Vidthayanon et al., 1997). Freshwater fish have evolved and adapted to virtually all environmental conditions and provide the basis for their application for bioassessment purposes. Important to this concept is the fact that not all species share the same range, optimum or lethal environmental characteristics (Hynes, 1970; Smith & Powell, 1971; Matthews & Robison, 1988; Rahel & Hubert, 1991; Horne & Goldman, 1994).

Diversity and distribution of fish species are influenced in part by environmental conditions, for example, dissolved oxygen, temperature, conductivity, pH, alkalinity, turbidity, velocity, discharges as well as nutrients (Wootton, 1990; Kamler, 1937) but equally by variations in tolerance among species. Streams display longitudinal changes in physical and chemical properties including width, depth, canopy, light, substrate, nutrients and oxygen. In concert with physiological variations in tolerance to environmental conditions, variations in morphology among species have contributed to multi species co-existence within habitats and efficient exploitation of resources (Ward-Campbell et al., 2005).

Dissolved Oxygen

Dissolved oxygen is an important chemical factor. Respiratory processes provide fish with oxygen which represents the fuel for all aerobic activities such as growth, swimming, feeding and reproduction. When the supply of oxygen is low, performance is impaired and, in the extreme, may lead to an individual's death

(Everhart & Young, 1981). Importantly, oxygen requirements differ among fish species. Further, some species have adapted to low oxygen through respiration organs or cutaneous respiration (Jobling, 1994). For instance, some temperate fishes such as *Salmo trutta*, *Phoxinus phoxinus* and *Nemachilus barbatulus* require high ambient concentrations of oxygen in excess of 9.8 mg/ l for optimum performance. In contrast, some other species such as *Rutilus rutilus* and *Acevina cevuna* perform quite well at oxygen concentrations of <6 mg/ l and stilling others such as *Carassius carassius* can survive for short periods under anaerobic conditions when the temperature is low (Blazka 1958; Nikolsky, 1963; Holopainen, Hyvarinen, & Piironen 1986; Van den Thillart & Van Waarde, 1985; Lloyd, 1992). Similarly, in tropical streams fishes occur in different habitats with respect to dissolved oxygen. Zakaria–Ismail and Sabariah (1995) reported that in the Malaysian peninsula, the Cyprinid *Hampala macrolepidota* was well adapted to a range of oxygen concentrations of <1 to approximately 8 mg/ l.

Ancillary respiratory organs are present in some fish species and can allow them to invade habitats unavailable to other species. Fish found in a blackwater peat swamp where dissolved oxygen was generally <2 mg/ l tended to have morphological modifications to their respiratory systems such as suprabranchial organs that assist in metabolism (Beamish, Beamish, & Lim, 2003). Often, Anabuntidae, Belontiidae and Channidae occurred in there areas because, fishes in these families have a suprabranchial organ as well as an auxiliary breathing. Also, described by Nelson (1994) *Clarius batrachus* which have suprabranchial organs and cutaneous respiration is burrowing fish and widely distributed. It can live in muddy habitat which is anaerobic.

Temperature

Temperature in tropical streams and rivers is a very significant factor for fish. Change in temperature of the surrounding water is associated with changes in the metabolic rate, feeding and digestion. Tropical and subtropical fish are more likely to be stenothermal than those of boreal and higher latitude (Nikolsky, 1963). Tropical fish do not seem able to survive temperature below 15 °C (Moyle & Joseph, 2000).

Temperature, both high and low can cause death (Everhart & Yong, 1981). Also, temperature can serve as a signal for some behaviors such as the timing of migration and spawning.

In tropical streams, fish distribution is also influenced by temperature. For example, Edds (1993) showed that *Neomacheilus rapecular*, *Euchiloglanis hodgarti*, *Schizothorax progasters* and *S. richardsoni* were found at low temperature in mountain habitat. While, *Barilus tileo, Danio devario, Esomus danricus, Babeo dero, Puntius guganio, P. ticto, Rasbora daniconius, Clupisoma garua and Glyptothorax telchitta* had been found in high temperature of Napal' Candaki River.

Turbidity

Turbidity or suspended particles in water can damage gill epithelia and hinder respiration. The occurrence of large concentration of the suspended particles may attach the gill's surface. Turbidity may also impair development of eggs, prey capture, visibility, swimming performance, and feeding. However, some groups of fish have adapted to high concentration of turbidity. For example, Rodriguez and Lewis (1997) found catfishes with their tactile organs and chemical sensors with which they can find food and refuge from predators were abundant in turbid water. It is worth noting that turbidity may also reduce the toxicity of potentially toxic materials such as organochlorine pesticides these substances can adsorb to the surfaces of suspended organic and non-toxic inorganic materials after which reducing their toxicity is reduced (Lloyd, 1992).

pH

Acid-base regulation is necessary for homeostasis (Claiborne, 1998). It maintains pH optimum in intrinsic; blood, intracellular and also environmental challenges. Internal buffering and transfer of acid-base molecules between fish and surrounding water are important processes for maintaining pH which occurs at the gills' surface (Heisler, 1993; Rankin, Henderson, & Brown, 1983). Blood and intracellular pH must be at an optimum to support pH sensitive enzyme activity (Van den Thillart & Van Waarde, 1991; Moyle & Joseph, 2000). The pH seriously affects to fish death associated with degradation of diversity, productivity and restricted distribution. Described by Jackson and Harvey (1993), Robison and Tonn (1989), Health (1995) normal pH range in aquatic had higher fish production and species numbers than acidic aquatic habitat. Jobling (1994) showed that fish fauna was depleted below pH 4. Johnson (1967) reported that the distribution pattern freshwater fish in Malaysia was restricted by pH. Only, seventeen species lived in this area of which pH was very low (pH 4.0 or less) such as *Rasbora caudimaculata, Rasbora einthovenii, Rasbora maculata, Osteochilus spilurus, Homaloptera tweedici,* ect. While, others had not been found. Studied by Trippel and Harvey (1987) the abundance of fish population had been reduced by low pH but survivors were increasing in their population.

Substratum characteristics

Type of substratum associates strongly with distribution of fish assemblages in aquatic ecosystems. Substratum can divide groupings of fish in streams and rivers where associated with substratum sizes. For example, benthic fish may have adhesive organs which can be used for attachment to substratum of cobbles and boulders. They often have a relatively long head, large mouth, deep body, wide pectoral and caudal fins (Watson & Balon, 1984a). Fins are used for maneuvering in pursuit of prey. Concordantly, Matin-Smith (1998); Beamish et al., (2003) showed that family balitoridae was the dominant group in streams with a substratum of boulders, rocks, cobbles, and also fast flowing water. Their pelvic fins are modified as adhesive suction discs and amplified of lateral fins, such as *Parhomaloptera microstoma, Protomyzon* sp, *Neohomalopter johorensis*. Family Siloridae, *Gryptothorax* cf. *major* modified also thoracic adhesive organs and *Garra borneensis* in family Cyprinidae amplified lateral fins. However, burrowing fish *Mastacembelus unicolor* and *Macrognathus maculatus* frequently habitats in a soft substrate or small substratum size (Bond, 1996; Rainboth, 1996).

Nutrients

Nutrients are almost always present in water and are essential for plant growth, the foundation of all higher productivities. In excessive concentrations, they can cause serious problems. An overabundance of nutrients may occur from effluents from farms, urban and industrial effluents. Depending on the amount and composition, these effluents can be harmful to fish (Steinman & Mulholland, 1996; Boonphakdee, Sawangwong, & Fujiwara, 1999).

Nitrogen is vital to growth of fish. It is used in the synthesis of amino acids and proteins. Deamination of proteins occurs in cells and result in the production of ammonia. Ammonia is toxic and eliminated via the gill epithelium and kidneys. Ammonia also occurs in the natural decomposition of organic matter (Wetzel, 1975). It occurs in both ionized and non-ionized form with the latter being the more toxic to fish. Ammonia is more toxic than either of the other forms of nitrogen, nitrite and nitrate. When ammonia concentration is increased, the rate of outward diffusion is slowed in some species leading to a toxic reaction (Fromm, 1970; Guerin-Ancey, 1976). Death occurs when relatively high concentrations of ambient ammonia (Colt & Tchobanogtous, 1976; Thurston & Russo, 1983). At sublethal concentrations, growth and reproduction may be impaired (Burrow, 1964; Smith & Piper, 1975).

Phosphorus is another essential element for growth of aquatic plant and animals and another key nutrient in streams. It is essential to cellular growth and reproduction of organisms such as phytoplankton (Horne & Goldman, 1994). Phosphorus is a major determinant in eutrophication and phytoplankton bloom (Gerdes & Kunst, 1998) as well as the key nutrient limiting productivity in streams (Reddy, Kadlec, Flaig, & Gale, 1999). In highly productive water, ambient oxygen tends to be low due to the demand by aerobic bacteria in decomposing dead organic matter as well as by other organisms in respiration (Giller & Malmqvist, 1998).

Trace elements usually occur in small concentrations in natural water and are essential for aquatic productivity. When they occur in abundance such as from industrial and mining effluents (Campbell & Parnrong, 2000), they may cause serious problems to resident fishes.

Conductivity

Conductivity is a function of the anion and cationic material in water representing the concentration of electrons (Cole, 1983). Although, some of these materials are essential to fish growth, whenever conductivity is high, it can affect fish communities and productivity (Almiron, Garcia, Menni, Protogino, & Solari, 2000). Geisler, Schmidt, and Sookvibul (1979) reported a low species richness and biomass in Muak Lek River of Central Thailand than in two other rivers which was associated with high ambient conductivity, the result of local agriculture activities.

Fish assemblages

Biological diversity and abundance of fish can be an indicator of integrity in stream and river ecosystems. Abundance and species richness are decreased by anthropogenic activities such as deforestation, agriculture activities and industrial discharge. Habitat alteration in streams and rivers commonly occur from these activities will impact to decreasing of fish abundance and species richness which can indicate a stream's health and environmental quality. For example, Greenfield and Bart (2005) reported that Elevenmile Creek in Florida had received kraft mill effluents and had lower species richness than other streams which were not similarly impacted. Decreases in benthic fish species as a result of mine discharges in Pennsylvania were described by Letternan & Mitsch (1978). Concordantly, Lee Kok Yuen (2001) showed that the Cyprinidae were not diverse in peat swamp forest with low pH water. In contrast, Zakaria-Ismail (1978) showed that streams and rivers of high dissolved oxygen (>4 mg/ l) in Ulu Endau in Malaysia had high abundance of fish that were free from physical deformities. Similarly, Lim (1987) reported that stenotopic species were well represented in streams where were not impacted from human activities and they required high oxygen concentration (>6 mg/ l).

Trophic Composition

The integrity of aquatic ecosystems may be interpreted using trophic composition. Usually trophic categories are assigned on the basis of the predominant dietary items as feeding patterns based on adult fish. The ecological process was changed by alteration in river quality. In the tropical streams, Ganasan and Hughes (1998) had used abundance of carnivore species to measure environmental or food supply degradations, carnivores were indicated as a declination of water quality which was disturbed from intensive human activities. Abundance of carnivores decreased in association with elevated urban. Thailand has a large number of insectivores in streams of pristine forests supported by an abundance and rich diversity of macro invertebrates. In contrast, perturbed streams have fewer insectivores (Tongnunui, personal observation). Typically, detritivores are present in habitats where there is the substratum contains much organic matter and fine particles and the water is low in pH and oxygen. Detritivores can be an indicator of water quality where habitats were probably high organic matters. Carnivores are also an important trophic group in stream ecosystems. In high abundance they can reduce biological diversity and abundance among native fish. This is especially true when the carnivores are exotic species (Lever, 1996; Lyons, Navarro-Perez, Cochran, Santana, & Guzman-Arroyo, 1995).

Pathogenesis

Fish are more susceptible to disease and exhibit a higher incidence of anomalies in water of poor quality. The incidence of diseases in fish is higher as are morphological anomalies and lesions. Fin erosions were much higher among fishes exposed to municipal waste water than in unexposed fishes (Cross, 1985). Skeletal deformities were more prevalent in fourhorn sculpins, *Myoxocephalus quadricornis*, in an area that received pulp and paper plant effluents that in a nearby unaffected area (Bengtsson, 1991). Slooff (1982) related the frequency of skeletal anomalies in bream (*Abramis brama*) from the Rhine River in Germany to concentrations of chemical substances. Studies by Mill, Starrett, and Bellrose (1966); Shotts, Gains, Martin, and Prestwood (1972) and Komada (1980) indicated that anomalies were few at non-polluted sites but high at sites receiving industrial effluent and sewage. Several others studies, Karr, Fausch, Angermier, Yant, and Schlosser (1986); Plumb (1994); Yoder and Rankin (1995) have found an inverse relationship between anomalies and degradation of water quality.

Non-indigenous fish

Rivers in which water chemistry or habitat have been altered by anthropogenic activities may provide a satisfactory environment for non indigenous species. Mostly of non indigenous fish were imported for aquaculture farm in fisheries activity and aquarium trade. They might rapid distributed through in the aquatic ecosystems where were effected by contamination of them. Hubbs (1961) reported that hybridization probably occurs as a result of stream degradation that prevents breeding fish from segregating along normal stream.

CHAPTER 3
MATERAILS AND METHODS

Small rivers were sampled within nine provinces; Chachoengsao, Nakhon Nayok, Prachin Buri, Chon Buri, Rayong, Chanthaburi and Trat to provided information on environmental sensitivities of river fishes in Eastern Thailand. A second series of sites sampled from Prachuap Khiri Khan and Petchaburi provinces provided information with which to examine for the general applicability of the bioassessment model for small rivers in Thailand. This information is considered in the discussion. The sites used to assess environmental sensitivities were located within five major rivers that discharge into the Gulf of Thailand (Figure 1). Bangpakong River is the largest with a watershed of 18,758 km^2 with its lower portion flowing through Chachoengsao and Chon Buri provinces prior to discharging into the Gulf of Thailand. The other rivers, Rayong, Prasae, Chanthaburi and Trat are smaller with watersheds of 2,300, 1,578, 1,755, and 1,970 of km^2, respectively. All watersheds in the study area drain land under intensive agriculture. The major crops include rubber, oil palm, sugar cane, pineapple, cassava, and rice. Animal husbandry is also intensive and includes fish such as *Oreochromis niloticus* and a hybrid catfish, (*Clarias macrocephalus* x *C. gariepinus*), shrimp, *Penaeus monodon*, and domestic pigs and chickens (Braaten & Flaherty, 2000; Bordalo, Nilsumranchit, & Chalermwat, 2001; Szuster & Flaherty, 2002). A number of cities and towns are located in the study area with an active array of industries (Graslund & Bengtsson, 2001; Cheevaporn & Menasveta, 2003). Commonly, waste is discharged directly into rivers throughout the study area.

The initial collection of fish was made from 95 sites with most (n=73) located in Chon Buri province with fewer sites in Rayong (n=13), Prachin Buri (n=1), Chachoengsao (n=1), Nakhon Nayok (n=2), Chanthaburi (n=3) and Trat (n=2) provinces, respectively. Sites were selected from road-accessible locations ranging from remote, heavily forested and sparsely inhabited to lightly settled areas where some subsistence to modest commercial agriculture occurred to more heavily farmed or urban areas. An effort was made to sample sites that displayed visual evidence of receiving varying amounts of organic effluent from sources such as pig and chicken farms, urban and industrial effluent as well as other sites for which there was little to no evidence of anthropogenic disturbance. Site selection also presented a few problems as some waterways were accessible only during short periods of the dry season and then only with difficulty. Some tributaries indicated on topographical maps could not be found and apparently no longer exist while others contained water only for portions of the year. Thus, for practical reasons, sites were selected as representative of landscapes and land use. Sites were not sampled closer than about 150 m from the nearest bridge.

A site was a length of stream relatively even in width, depth, water velocity and canopy. Within sites, these factors were visually estimated not to range beyond about 25% of the average except for depth and velocity along the margins. An effort was made also for evenness of substratum. Thus, for example, a stretch of river where the substratum was predominately of particles within a single Wentworth size class was considered to be relatively even with respect to this characteristic. In regions of rivers subjected to extremely high seasonal flow rates, the substratum was typically punctuated with boulders among which was cobble mixed with pockets of smaller particles. Stretches with much the same relative proportions of boulders, and other particle size categories were also considered to be relatively even in this characteristic. The search for relative evenness restricted the length of most sites to under 50 m. Physical constraints to sampling imposed by water velocity in concert with discharge also contributed to the length of stream that could be sampled.

Fish were captured with a Smith-Root, model 15 D backpack electro-fisher with variable output voltage (100-1100 volts), pulse width (1-120 Hz) and frequency (100 μs – 8 ms). The anode was fitted with a 28 cm diameter ring. Output voltage was varied inversely with water conductively and, for the sites in this study, was mostly between 200 and 600 volts in combination with a wave width of 60 Hz and frequencies of 1-4 ms. Settings were made, based on experience, to reduce damage to fish, particularly the initial impact.

Seine nets with about 3 mm mesh were installed across the upper and lower limits of a site and their groundlines weighted with rocks to reduce the probability of emigration from or immigration into the sample area. A site was electro-fished by moving in a zigzag pattern from one retaining net to the other, beginning downstream or upstream based on visibility, water depth and velocity. Usually four or five passes were made at a site. Relative capture efficiency between upstream and downstream direction of electro-fishing was compared in an earlier study (Beamish, Sa-adrit, & Cheevaporn, 2008) within several larger sites and not found to differ significantly (ANCOVA, $P<0.05$).

Fishing was conducted by three people, one to operate the electro-chocker and two to hand-net fish from the water. Except in areas of extremely fast flowing water almost all fish were captured by hand net rather than in either blocking seine. After each pass, fish were anaesthetized in methaine tricaine sulfonate (approximately 150 mg/ l), identified and examined for the presence of lesions, tumors, fin erosions, obvious skeletal deformities. Fishes that could confidently be identified were enumerated and, after recovery, released downstream from the site. When fishes could not be identified in the field they were killed by an overdose of anesthetic and preserved in 10% formalin for subsequent identification in the laboratory. Current systematics of Thai freshwater fishes is equivocal. For this report the classification system of Nelson (1994) was followed along with names given in the University of California, Catalog of Fishes (Eschmeyer, 2007). Fish were identified from a number of sources including: Smith (1945), Brittan (1954), Sontirat (1976), Dawson (1981), Robert (1982, 1986), Kottelat (1984, 1988, 1989, 1990, 1998, 2000), Karnasuta (1993), Ng & Kottelat (2000) and Nalbant (2002). A voucher collection was prepared and is maintained in the Institute of Marine Sciences at Burapha University, Bangsaen, Chon Buri (Catalogue number- BIMS: FF. 0001-002). Fish were preserved in 10% formalin for 10 days and then transferred to 80% ethanol for permanent storage.

On each sampling occasion, width (± 0.1 m), depth (±1 cm) and velocity (±1 cm/ s) were measured, each at least three times, and the mean used to estimate discharge (l/ s). Depth and velocity were the average of 3-5 measurements made at approximately equal intervals across a transverse transect located at about the mid length of a site. Velocity was measured with a propeller current meter at approximately mid depth which was recorded as the vertical average. Canopy was estimated visually and recorded as percentage with 100% representing complete cover. Regularly calibrated meters were used to measure temperature (±0.1 °C), conductivity (±5 μS/ cm), turbidity (NTU), pH (±0.1 unit) and dissolved oxygen (±0.1 mg/ l). In addition, a water sample was collected for the measurement of ammonia (mg NH_3N/ l) by the salicylate method, nitrate (mg NO_3N/ l) by the cadmium reduction method, total iron (mg Fe/ l) by the by the Ferro Ver method, alkalinity (as $CaCO_3$ mg/ l, pH 4.5) using the sulfuric acid titration method, silica (mg SiO_2/ l), using the heteropoly method and true color (mg/ l platinum as chloroplatinate ion; 1 color unit = 1 mg/ l chloroplatinate ion) (APHA, 1992). Elevation was measured with a Global Positioning Systems meter (GPS, ±10 m). Sites were sampled throughout the years, however, season was not included as a habitat variable. An earlier study by Beamish, Griffiths, Kongchaiya, Sa-adrit, & Sonchaeng (2005) indicated seasonal changes in fish abundance and assemblage similarity from several streams in Central Thailand to vary inversely with discharge which was included in this study as a habitat variable.

Substratum at each site was collected with a hand-held acrylic corer (5 cm inner diameter) to a depth of 10±3 cm. Particles on the surface larger than the diameter of the corer were removed before a sample was taken and included in the estimate. Samples were air dried and sieved to determine particle size distribution by weight. Six particle size categories were adopted from the Wentworth scale (Giller & Malmqvist, 1998), >150 mm (boulder to large cobble), 150-60.1 mm (large cobble to large pebble), 60-5.1 mm (large pebble to coarse gravel), 5-3.1 mm (medium to fine gravel), 3-0.51 mm (fine gravel to coarse sand) and <0.5 mm (medium sand to silt). The substratum at each site was coded into six categories based on mean particle size with 1 being the smallest and 6, the largest. The substratum at a few sites was solid or almost solid bedrock and coded as 7. In an earlier study (Beamish et al., 2008) an average of three substratum samples (range of 2-6 samples) was selected randomly within each of 40 sites. Variation was similar within each particle size category with an overall mean variation across all sizes (±SD) of 26±12% that is assumed for all measurements. A single sample was collected at all other sites.

Stepwise multiple linear regression analysis (MLR, SPSS 11.5) was applied to examine the relationships between species numbers and abundance and all habitat parameters (Steele & Torrie, 1980). Species, their abundances, and all habitat parameters, except for pH, were log (x+1) transformed to normalize the distribution of values and, in the case of habitat factors, to accommodate differences in scale. To avoid subjectivity all independent variables were included in the full model. The significance used to assess variable inclusion was $P<0.15$. For the final model, significance was accepted at $P<0.05$. In central Thailand, fish abundance in rivers reflected an aggregated distribution as between site variance increased linearly with mean population density (Beamish, Griffiths, Kongchaiya, Sa-adrit, & Sonchaeng, 2005). A log-transformation removed this correlation on the mean and suggested an overall standard deviation (SD) of 1.3 about the abundance estimate that is assumed for all species estimates in this study.

Canonical correspondence analysis (CCA, PC-ORD 3.2) was employed to identify important species-related environmental characteristics. Species numbers and abundances and environmental variables were centered and normalized within the program. In the canonical correspondence analysis, statistical significance of the relationship between a set of environmental factors and fish species was taken using a Monte Carlo permutation test with 1000 permutations. Statistical significance of all tests was accepted at $P<0.05$.

Total abundance of all fishes as well as individual species within a site were calculated by the maximum likelihood technique that also provided an estimate of capture efficiency for each electro-fishing pass (Carle & Straub, 1978). Numbers for many species were small and not amenable to this technique. When this occurred a conversion factor consisting of the estimated total abundance of all fish captured at a site divided by total number of all fish actually caught at the same site was applied to adjust the numbers of each species captured. Fish abundance was arithmetically adjusted to an area of 100 m^2.

Calculation of Index of Biotic Integrity (IBI)

The information collected from 95 streams sites was used to develop a bioassessment model on which to evaluate water quality in eastern rivers of Thailand. The model was based on the multifunctional ecological approach developed earlier by Karr (1981) for streams in Midwestern regions of U.S.A. His model assumed fish species and fish communities responded to habitat disturbances, particularly, degradation of water quality and was described by 12 characteristics of the fish community or assemblage that he referred to as metrics. Each metric targeted a different aspect of fish assemblage structure and total abundance recognizing differences in habitat preferences and tolerances among fishes (Fry, 1947). Karr's metrics included such characteristics as number of species or species richness, species and trophic composition, fish abundances and anomalies such as skeletal deformities, fin erosion and external lesions that Karr felt were induced by water quality.

Few fishes indigenous to the rivers in Midwestern U.S.A. are tolerant of water degraded by excessive organic material as indicated by low dissolved oxygen and high ammonia, a consequence of their aerobic metabolic requirements and inability to catabolise or excrete ammonia. In contrast, species numbers were high in water with only modest amounts of organic material and high concentration of dissolved oxygen.

Karr identified some species as indicators of water quality. For example, in the fast flowing regions of a stream he considered the presence of species in the family Etheostomidae, commonly called darters, to be indicative of water free or relatively free form excessive organic material. Where water flow was low as in pool habitats but comparatively high in ambient oxygen, number of sunfish species was expected to be high. Green sunfish, *Lepomis cyanellus*, was tolerated on low dissolved oxygen and high suspended solids siltation. It was expected to be high occurrence and abundance in habitats with low dissolved oxygen while other fish species were relatively absent. Species in family Catostomidae, commonly called suckers, were regarded as sensitive to physical and chemical degradation of water and were used as indicators of water acceptable quality.

Food resources are, of course, critical to the survival of fishes and just as water quality is important to fish it is also important to those on which they prey such as aquatic and terrestrial insects, molluscs, crustaceans and annelids. However, not all fishes share the same diets. Some species consume mainly detritus, others, algae or fish. Changes in the abundance of specific dietary items can be expected to impact on the abundance or even presence of those fishes within the relevant trophic category. This was represented also in Karr's metrics. For example, he found the proportion of omnivorous fishes within the community, species that consistently feed on a substantial proportion of plant and animal material, to increase as water declined in quality. This included the number of omnivorous species and their abundances which Karr used as a metric.

Invertivores or insectivores are those fishes that feed heavily on aquatic and terrestrial invertebrates, particularly insects. Insectivores were the dominant group of fish in both species numbers and abundances in those Midwestern streams that were of relatively good water quality and habitat characteristics. They declined in species numbers and abundant as habitat and water quality declined.

Those fishes whose diet consists mostly of large crustaceans such as crabs, shrimp and crayfish, annelids, small fish and molluscs were categorized as carnivores, Karr's study found that carnivore fish were relatively scarce in streams that received large amounts of organic matter.

Total fish abundance was also considered an important component of Karr's bioassessment model and was a metric. Total abundance was directly correlated with habitat integrity including water quality. In extremely disturbed streams, fish are unlikely to be present in easily detected numbers, while moderately disturbed sites can be expected to support more individuals but fewer than high quality of sites.

In undisturbed habitats species richness and abundance were comparatively high in Midwestern streams leaving few opportunities for invasion by non indigenous species. Only where habitat had been degraded by anthropogenic activities to the extent that species numbers and abundances were reduced were opportunities afforded non indigenous species. Karr's bioassessment model identified the presence of non indigenous fish with habitat alteration, particularly that by agriculture activities.

Fishes in Midwestern streams that received industrial wastes, particularly high concentrations of heavy metal often displayed abnormally high prevalence of tumours, external lesions, skeletal deformities and fin erosions. This association also recognized in earlier studies (Berra & Au, 1978) was used by Karr (1981) as a metric to identify streams of degraded quality, particularly from heavy metals and other industrial wastes.

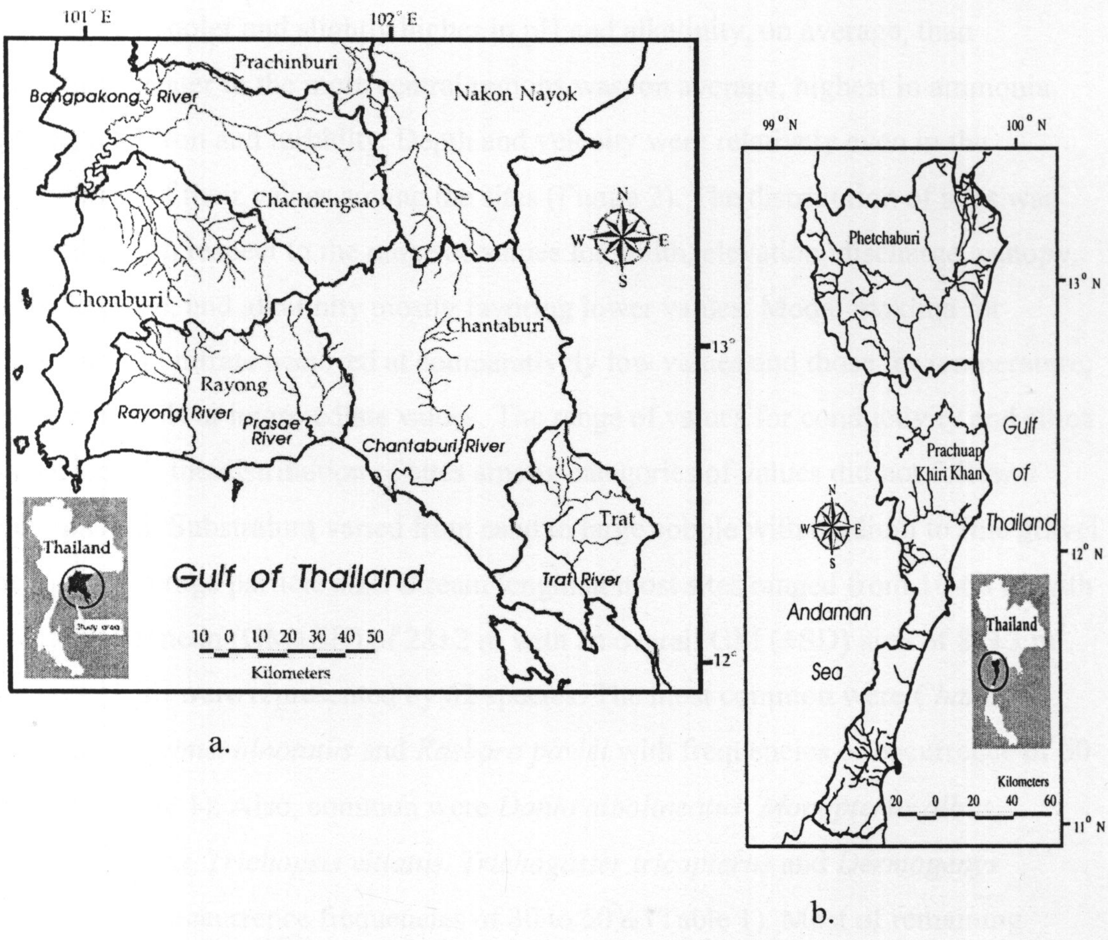


Figure 1 (a). Sampling sites were located in Chon Buri, Chachoengsao, Nakhon Nayok, Prachin Buri, Rayong, Chanthaburi and Trat provinces, (b). Assessment sites were located in Petchaburi and Prachuap Khiri Khan provinces in South-central Thailand.

CHAPTER 4
RESULTS

Water parameters varied among sites with those in the more eastern areas a few degrees cooler and slightly higher in pH and alkalinity, on average, than elsewhere. Water in the more central regions was, on average, highest in ammonia, nitrate, total iron and turbidity. Depth and velocity were relatively even in the distribution of their values among the sites (Figure 2). The distribution of sites was unimodal with respect to the range of values for width, elevation, discharge, canopy, turbidity, iron, and alkalinity mostly favoring lower values. Modal maxima for ammonia and nitrate occurred at comparatively low values and those for temperature, oxygen and pH at intermediate values. The range of values for conductivity and silica was wide but the distribution of sites among categories of values did not follow a clear pattern. Substratum varied from sand to large cobble with medium to fine gravel being the average particle size. Stream length at most sites ranged from 10-60 m with a geometric mean (GM±SD) of 28±2 m with an overall GM (±SD) area of 82±3 m^2.

Fish were represented by 62 species. The most common were *Channa gachua*, *Systomus binotatus* and *Rasbora paviei* with frequencies of occurrence of 60 to 75% (Table 1). Also, common were *Danio albolineatus, Monopterus albus*, *Channa striata, Trichopsis vittatus, Trichogaster tricopterus* and *Dermogenys pusillus* with occurrence frequencies of 30 to 50% (Table 1). Most of remaining species were found at ≤20% of the sites.

Species numbers were adjusted to an area of 100 m^2 from the overall GM using a calculated slope of 0.0018 (Beamish & Sa-ardrit, 2006) and varied from 0 to 21 (Figure 3). Most sites had 6-8 species and only 5 sites contained no fish. The relationship between species number and all habitat parameters were subjected to stepwise multiple linear regression analysis and found to be significantly related to two parameters, pH and dissolved oxygen:

$$\text{Log}(S+1) = 1.145 - 0.092(P) + 0.522\log(O+1)$$

where S, is species number, adjusted to an area of 100 m^2, P, pH and O, dissolved oxygen (mg/ l). Variables retained in the equation had significant t-values at P<0.05. The regression's F-value $_{2,85}$ is 13.25, P<0.05) and $R^2 = 0.25$. Thus, species numbers were negatively related with pH and positively associated with dissolved oxygen. For example, at pH 7, species numbers are predicted to increase from 6.3 to 9.0 species with an increase in oxygen from 4 to 8 mg/ l and at an oxygen concentration of 6 mg/ l to decrease from 9.8 to 6.1 with pH increase from 6 to 8.

Total fish abundance adjusted to a site area of 100 m^2 varied from 0 to 1425 with about half of the sites containing fewer than 100 fish/ 100 m^2 (Figure 3). Total fish abundance was related significantly with depth, discharge, substratum, oxygen, and ammonia and is described by the equation:

$$\text{Log}(N+1) = 0.650 + 0.468 \log(D+1) - 0.307 \log(d+1) + 0.938 \log(S+1) + 0.960 \log(O+1) - 0.213 \log(A+1)$$

where N is total fish abundance/ 100 m^2, D, depth, cm, d, discharge, l/ s, S, substratum coded value, O, oxygen, mg/ l, and A, ammonia, mg NH_3N/ l. The regression's F-value $_{5,84}$ is 6.8, P<0.05 and $R^2 = 0.3$. Thus, fish abundance is predicted to vary inversely with discharge and ammonia and directly with depth, substratum, and dissolved oxygen. The equation predicts with an increase in ammonia from 0.01 to 5 mg NH_3N/ l, fish abundance will decrease from 46.8 to 31.7 fish/ 100 m^2 when discharge is 200 l/ s, depth, 20 cm, dissolved oxygen, 4 mg/ l and the substratum is fine gravel to coarse sand with a coded value of 2.

In preparation for ordination analysis some adjustments were made to the data. No fish were captured at 5 sites. The efficiency of the electro-fisher over four passes was high at 90%, but still the possibility of not capturing all fish could not be excluded. Further, the statistical package available for CCA analysis requires that fish be present in order for a site to be included. Because of the importance of this information to the study's objective, a small number of individuals, 0.001 fish/ 100 m^2, of the most commonly encountered species, *R. paviei* was assigned to barren sites. Species and abundances were significantly correlated with eight environmental factors (P=0.001 along axes 1 and 2, Monte Carlo test with 1000 permutations). The first and second axes of the CCA analysis were both significant with axes 1 and 2 explaining 58 and 40% of the variability, respectively. The first axis illustrates a positive gradient of environmental factors; substratum (R^2=0.4), silica (R^2=0.5), oxygen (R^2=0.3) and width (R^2=0.1), respectively. Ammonia (R^2=0.04), pH (R^2=0.3), alkalinity (R^2=0.1) and conductivity (R^2=0.04) loaded positively on the second axis (Table 2). Environmental correlations were 0.88 and 0.86 for axis 1 and 2, respectively. Each significant environmental factor increased along a vector away from the origin with its length being a measure of magnitude (Figure 4).

Species were distributed clearly within four groups with respect to the significant habitat characteristics (Figure 4). The 29 species in group one were associated with mostly average habitat conditions for the sites sampled. Overall sites, GM±SD values for alkalinity, conductivity, silica, oxygen, ammonia and pH were 75±3 $CaCo_3$ mg/ l, 253±3 µS/ cm, 6.2±3.4 mg SiO_2/ l, 5.4±1.4 mg/ l, 1.7±7.6 mg NH_3N/ l and 7.1±0.7, respectively. Width and coded value for the substratum were 3±1.6 m and 3.1±1.4. Group 1 consisted of four of the generally most abundant species, *S. binotatus*, *R. paviei*, *C. gachua* and *D. pusillus*, respectively. The second group consisted of only seven species. Environmental conditions where species in group two were found indicate above average concentrations of oxygen and silica and below average levels of ammonia, alkalinity, conductivity and pH. Fish were not abundant at any of the sites in group two with no species having a mean abundance of >0.1 fish/ 100 m^2. Group three contained fourteen species with two, *T. vittatus* and *T. tricopterus*, being moderately abundant at 1.3±3.0 and.1.4±3.5 fish/ 100 m^2, respectively and widespread. The remaining species in this group were sparsely distributed being found at <41 sites with overall abundances of <1.0 fish/ 100 m^2. Ammonia and conductivity were exceptionally high at sites where group three fish were captured. Indeed, ammonia exceeded 500 mg NH_3N/ l at six sites and in three was 5000 mg NH_3N/ l or higher. At these latter sites, 5000, 6000 and 8000 mg NH_3N/ l (pH 7.5, 8.2 and 8.4), *T. vittatus* and *T. tricopterus* were present at approximately 12 and 3 individuals/ 100 m^2, respectively, despite ambient oxygen concentrations of only 2.7 mg/ l at the site with the highest ammonia. At each of the other two sites where ammonia was 6000 and 5000 mg NH_3N/ l one other species was present, *Esomus metallicus* at 5.6 / 100 m^2 and *C. striata* at 3.5 / 100 m^2, respectively. *O. niloticus* and *C. striata* along with *T. tricopterus* and *T. vittatus* were found at sites where ammonia ranged between 500 and 1700 mg NH_3N/ l (pH 7.6-7.8 and oxygen 4 to 6 mg/ l). Over half of the species in group three were found where ambient ammonia was >30 mg NH_3N/ l.

Habitat conditions were similar where fish in groups one and four were captured, except for ammonia which was lower for fish in the latter group. Species in group four were usually found at sites wider than the overall average (Figure 4). All the 12 species in this group displayed low abundances of <0.5 / 100 m^2 and were found at between 1 and 20 sites.

Table 1 Species captured at the sample sites, their frequencies of occurrence (Freq Occ, %) and GM abundances (N±SD/100 m^2). ID numbers were assigned as species coded in Figure 2.

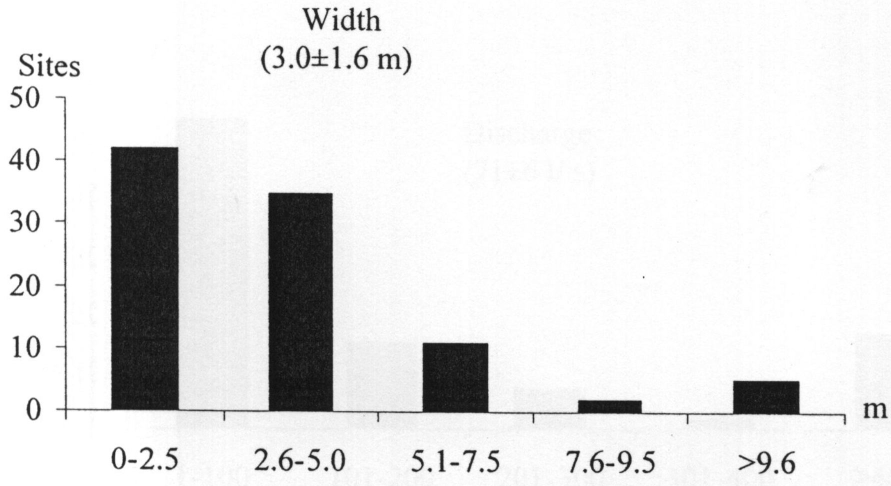
Species	ID	Freq Occ (%)	Abundance (±SD) (N/100 m^2)
Notopterus notopterus	1	1	<0.1
Barbodes gonionotus	2	4	<0.1
Danio albolineatus	3	30	1.5(5.3)
Cyclocheilichthys apogon	4	1	<0.1
Cyclocheilichthys armatus	5	2	<0.1
Cyclocheilichthys heteronema	6	1	<0.1
Esomus metallicus	7	16	0.6(3.0)
Hampala macrolepidota	8	7	<0.1
Labiobarbus leptocheilus	9	4	<0.1
Mystacoleucus marginatus	10	17	0.7(3.8)
Neolissochilus blanci	11	5	0.1(1.7)
Osteochilus hasselti	12	16	0.4(2.4)
Osteochilus lini	13	2	<0.1
Parachelia maculicauda	14	1	<0.1
Poropuntius deauratus	15	3	<0.1
Rasbora borapetensis	16	11	0.2(2.0)
Rasbora myersi	17	2	<0.1
Rasbora paviei	18	73	5.1(1.8)
Rasbora trilineata	19	2	<0.1
Systomus binotatus	20	65	5.7(5.4)
Systomus lateristriga	21	2	<0.1
Systomus orphoides	22	15	0.3(1.9)
Systomus partipentozona	23	6	<0.1

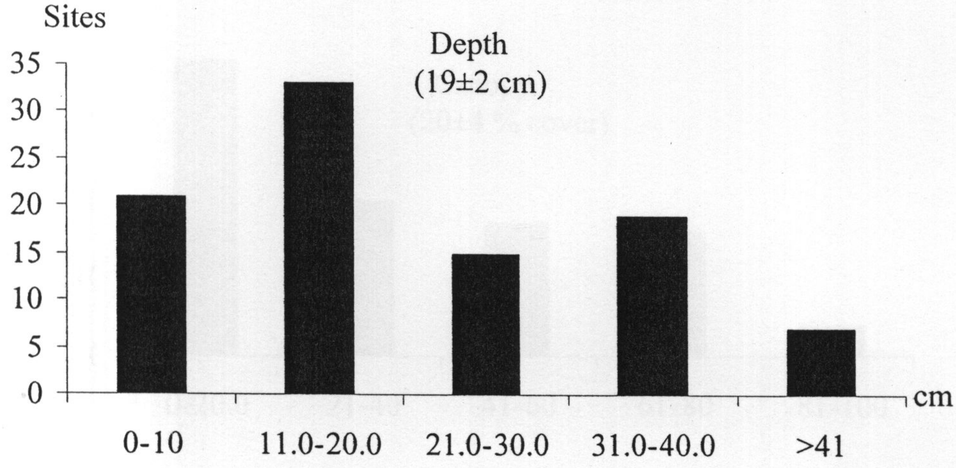
Acanthocobitis zonalternans	24	5	<0.1
Homaloptera smithi	25	4	<0.1
Nemacheilus masyae	26	5	<0.1
Nemacheilus platiceps	27	3	<0.1
Schistura kohchangensis	28	5	0.1(1.8)
Acanthopsis sp1.	29	4	<0.1
Acanthopsis sp2.	30	1	<0.1
Lepidocephalichthys berdmorei	31	1	<0.1
Lepidocephalichthys hasselti	32	22	0.5(2.3)
Pseudomystus siamensis	33	12	0.2(1.5)
Mystus mysticetus	34	5	0.1(1.8)
Hemibagrus nemurus	35	15	0.3(1.8)
Ompok bimaculatus	36	14	0.2(1.6)
Pterocryptis cochinchinensis	37	1	<0.1
Amblyceps mangois	38	16	0.2(1.7)
Clarias batrachus	39	14	0.2(1.4)
Dermogenys pusillus	40	48	2.2(4.3)
Xenenthodon cancilla	41	7	<0.1
Doryichthys boaja	42	1	<0.1
Doryichthys martensii	43	1	<0.1
Monopterus albus	44	37	0.5(2.0)
Macrognathus circumcintus	45	14	0.2(1.7)
Macrognathus siamensis	46	2	<0.1
Mastacembelus armatus	47	13	0.2(1.6)
Parambassis siamensis	48	20	0.5(2.4)
Nandus nebulosus	49	4	<0.1
Pristolepis fasciatus	50	2	<0.1
Oreochromis massambicus	51	1	<0.1
Oreochromis niloticus	52	10	0.4(2.6)
Oxyeleotris marmorata	53	7	<0.1
Rhinogobius sp.	54	10	0.2(1.3)

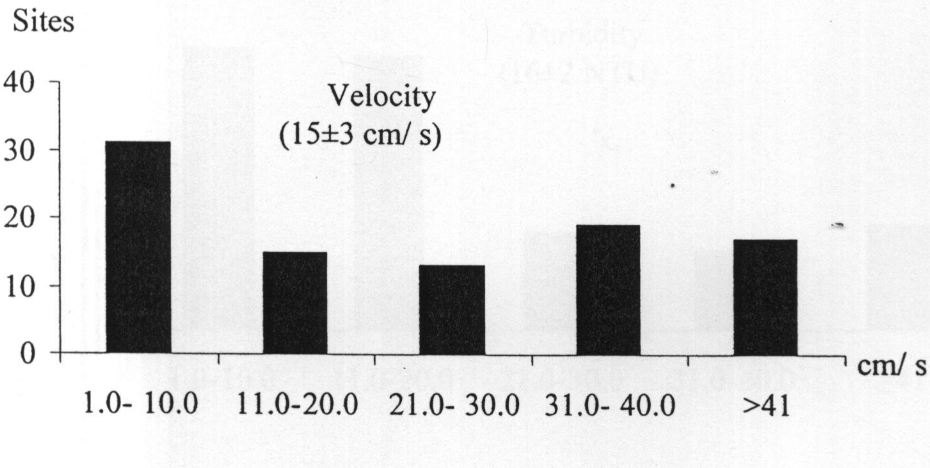
Anabas testudineus	55	5	0.1(1.7)
Betta prima	56	2	<0.1
Trichogaster tricopterus	57	45	1.4(3.5)
Trichopsis vittatus	58	43	1.3(3.0)
Channa gachua	59	61	2.3(3.7)
Channa lucius	60	5	<0.1
Channa striata	61	41	0.8(2.4)
Poecilia reticulata	62	2	<0.1

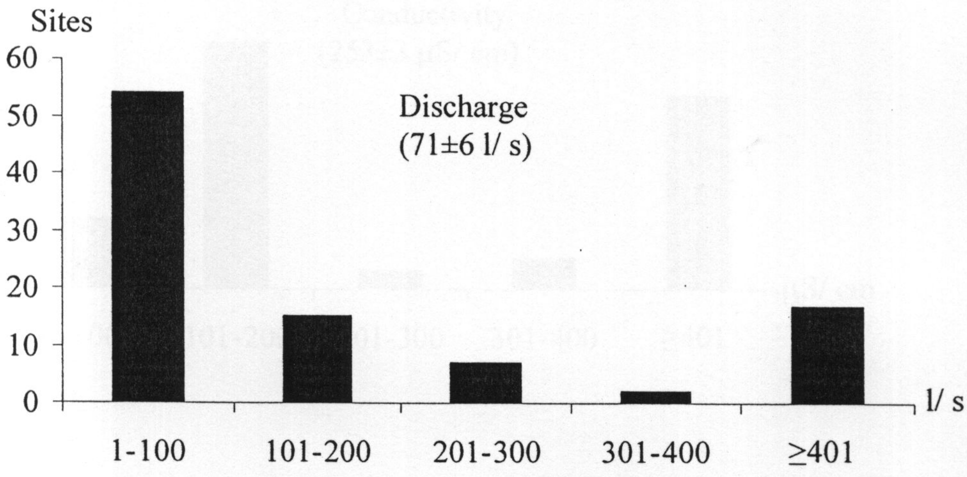
Table 2 Canonical coefficients for significant environmental variables at 95 stream sites.

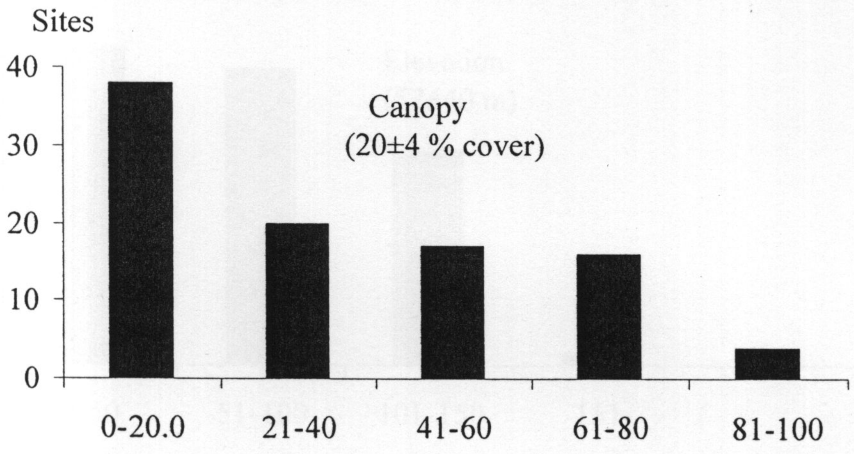
Variables	Axis 1	Axis 2
Width (m)	0.273	-0.531
Substratum (mm)	0.661	0.669
Conductivity (µS/ cm)	-0.763	0.189
pH	-0.723	0.547
Oxygen (mg/ l)	0.575	-0.224
Ammonia (mg NH_3N/ l)	-0.508	0.203
Silica (mg SiO_2/ l)	0.716	-0.062
Alkalinity (as $CaCO_3$ mg/ l)	-0.814	0.307

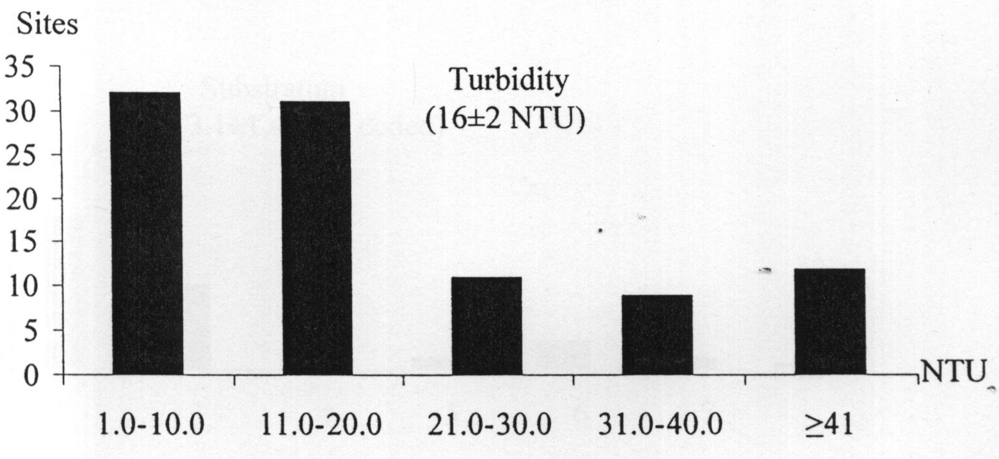

Width
(3.0±1.6 m)
Sites
50
40
30
20
10
0
m
0-2.5
2.6-5.0
5.1-7.5
7.6-9.5
>9.6

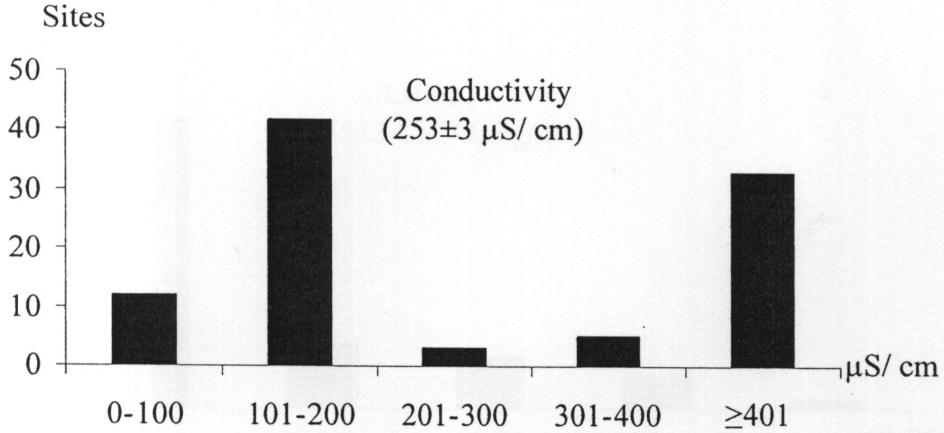

Sites
Depth
(19±2 cm)
35
30
25
20
15
10
5
0
cm
0-10
11.0-20.0
21.0-30.0
31.0-40.0
>41

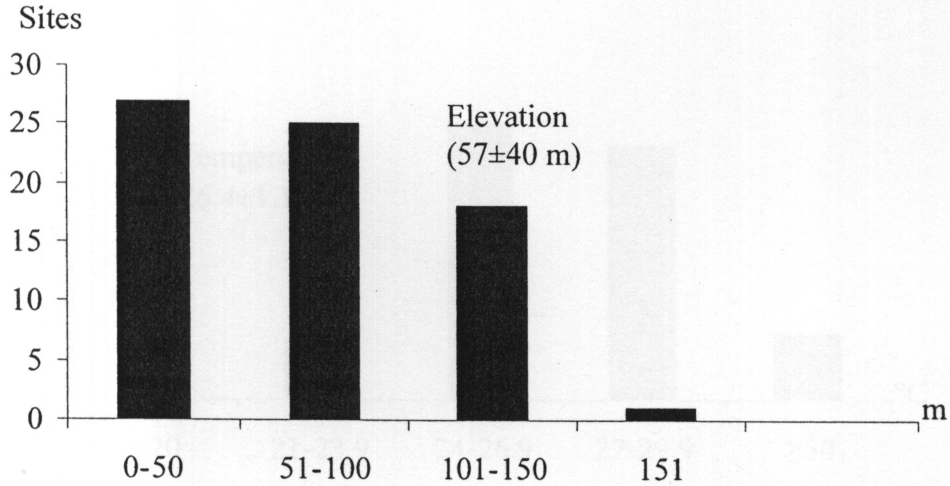

Sites
Velocity
(15±3 cm/ s)
40
30
20
10
0
cm/ s
1.0- 10.0
11.0-20.0
21.0- 30.0
31.0- 40.0
>41

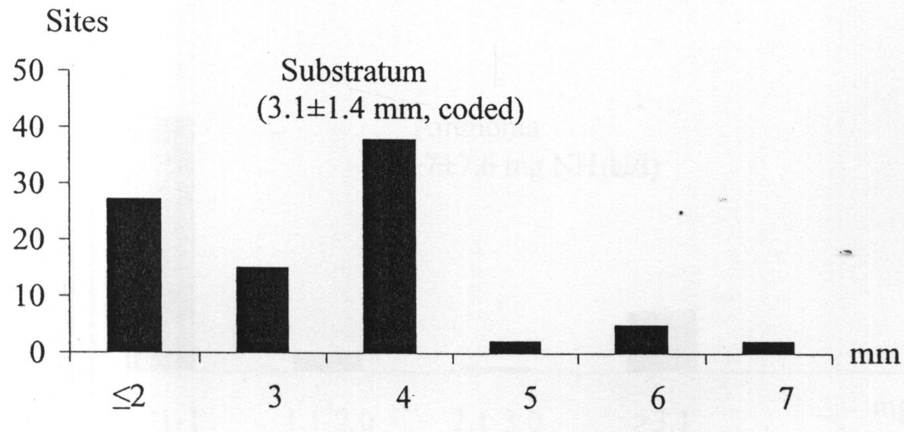

Sites
60
50
40
30
20
10
0
Discharge
(71±6 l/ s)
l/ s
1-100
101-200
201-300
301-400
≥401

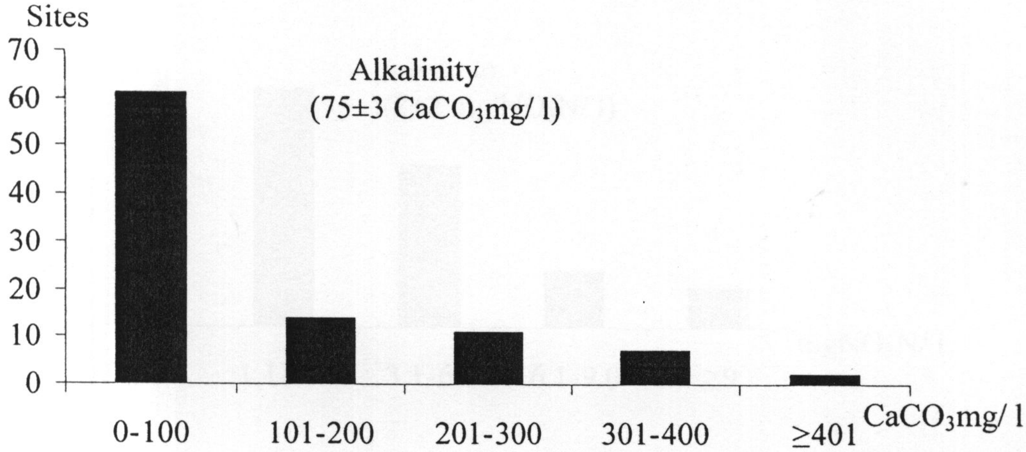

Sites
40
30
20
10
0
Canopy
(20±4 % cover)
0-20.0
21-40
41-60
61-80
81-100

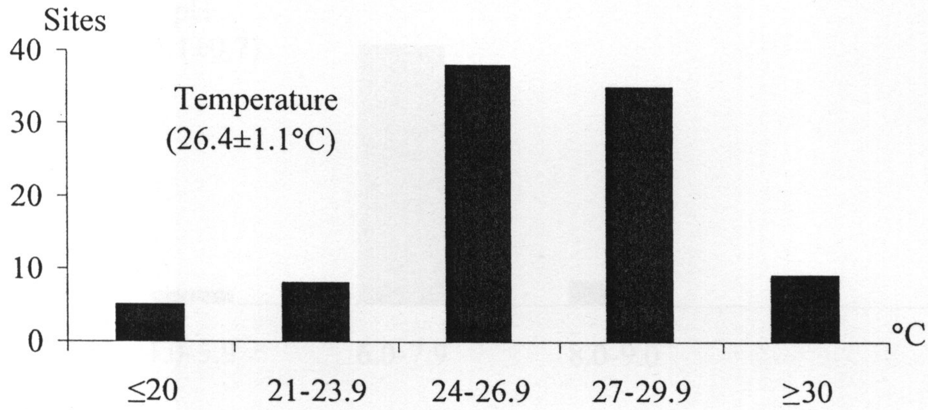

Sites
35
30
25
20
15
10
5
0
Turbidity
(16±2 NTU)
NTU
1.0-10.0
11.0-20.0
21.0-30.0
31.0-40.0
≥41

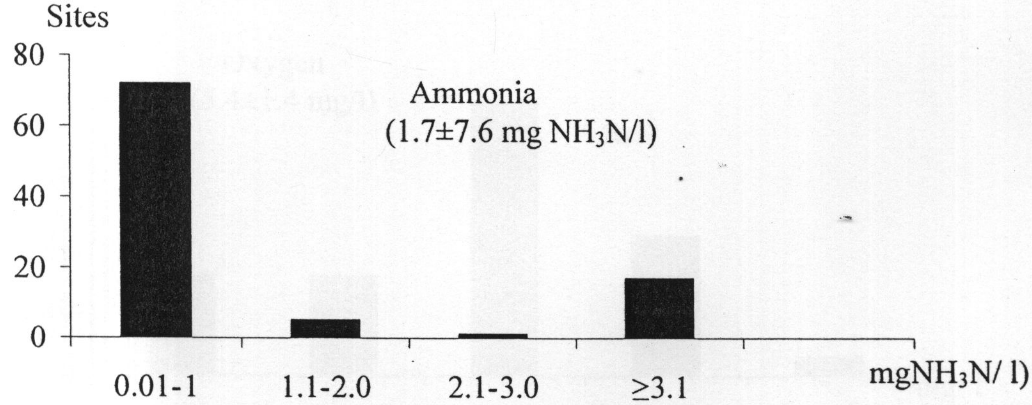

Sites
50
40
30
20
10
0
Conductivity
(253±3 µS/ cm)
µS/ cm
0-100
101-200
201-300
301-400
≥401

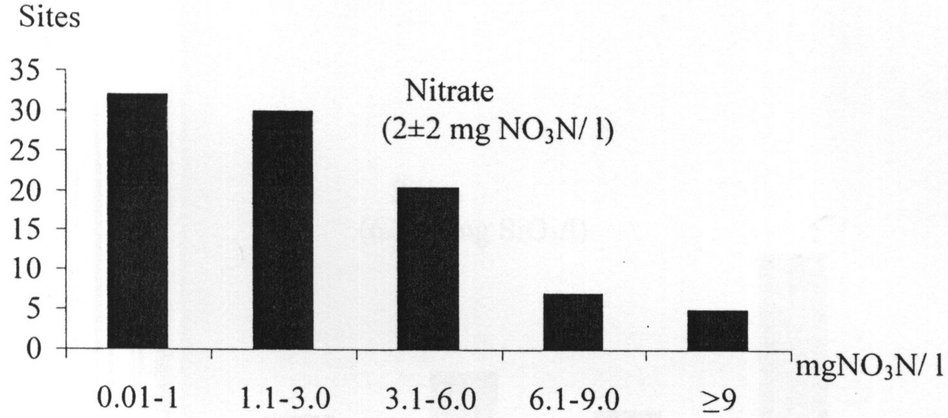

Sites
30
25
20
15
10
5
0
Elevation
(57±40 m)
m
0-50
51-100
101-150
151

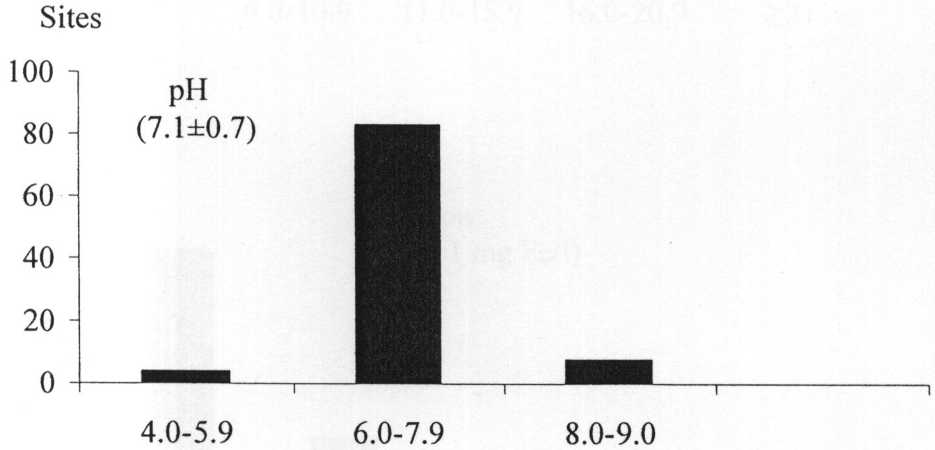

Sites
50
40
30
20
10
0
Substratum
(3.1±1.4 mm, coded)
mm
≤2
3
4
5
6
7

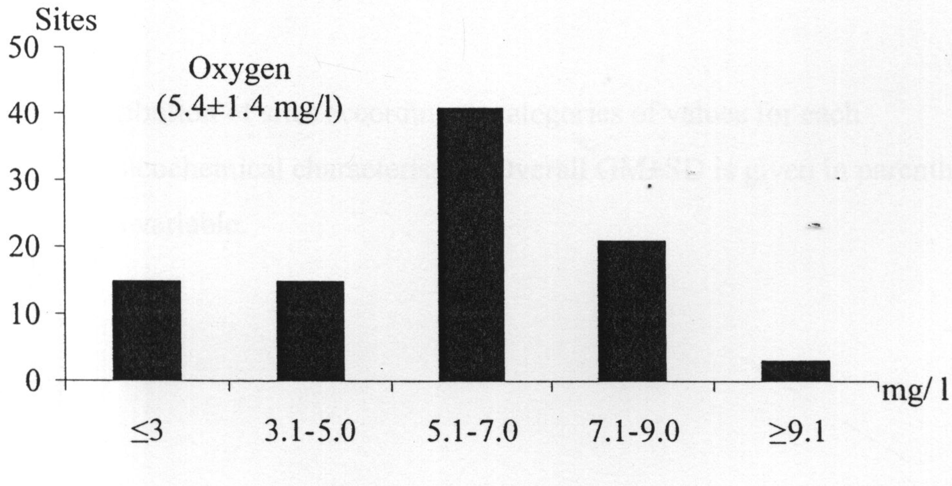

Sites
70
60
50
40
30
20
10
0
Alkalinity
(75±3 CaCO3mg/ l)
0-100
101-200
201-300
301-400
≥401
CaCO3mg/ l

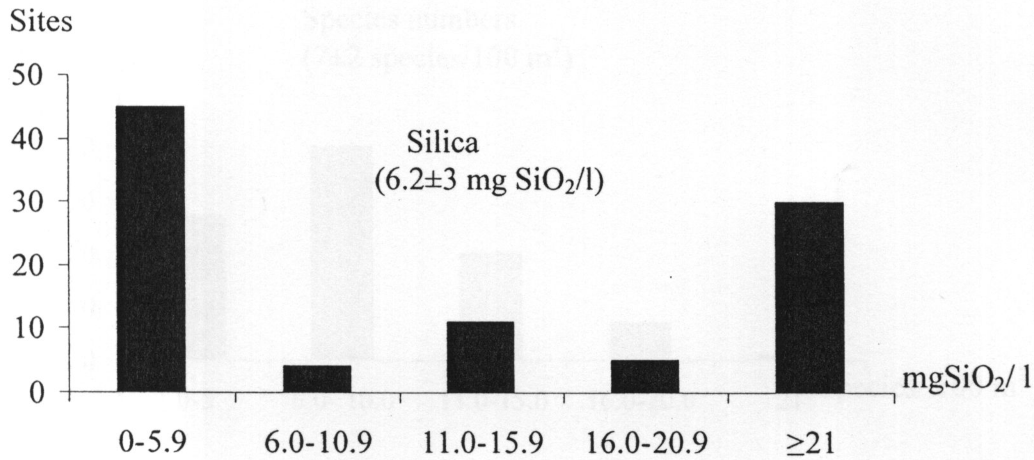

Sites
40
30
20
10
0
Temperature
(26.4±1.1°C)
≤20
21-23.9
24-26.9
27-29.9
≥30
°C


Sites
80
60
40
20
0
Ammonia
(1.7±7.6 mg NH3N/l)
0.01-1
1.1-2.0
2.1-3.0
≥3.1
mgNH3N/ l)


Sites
35
30
25
20
15
10
5
0
Nitrate
(2±2 mg NO3N/ l)
0.01-1
1.1-3.0
3.1-6.0
6.1-9.0
≥9
mgNO3N/ l


Sites
100
80
60
40
20
0
pH
(7.1±0.7)
4.0-5.9
6.0-7.9
8.0-9.0


Sites
50
40
30
20
10
0
Oxygen
(5.4±1.4 mg/l)
≤3
3.1-5.0
5.1-7.0
7.1-9.0
≥9.1
mg/ l



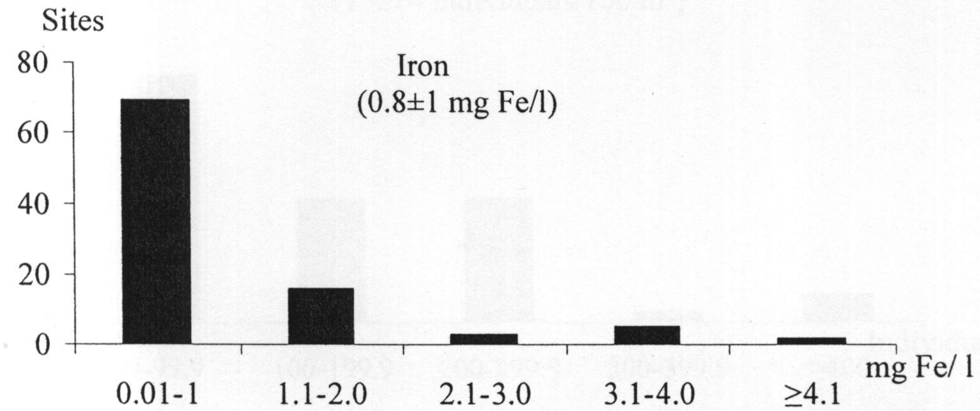


Figure 2 Distribution of sites according to categories of values for each physicochemical characteristics. Overall GM±SD is given in parenthesis for each variable.

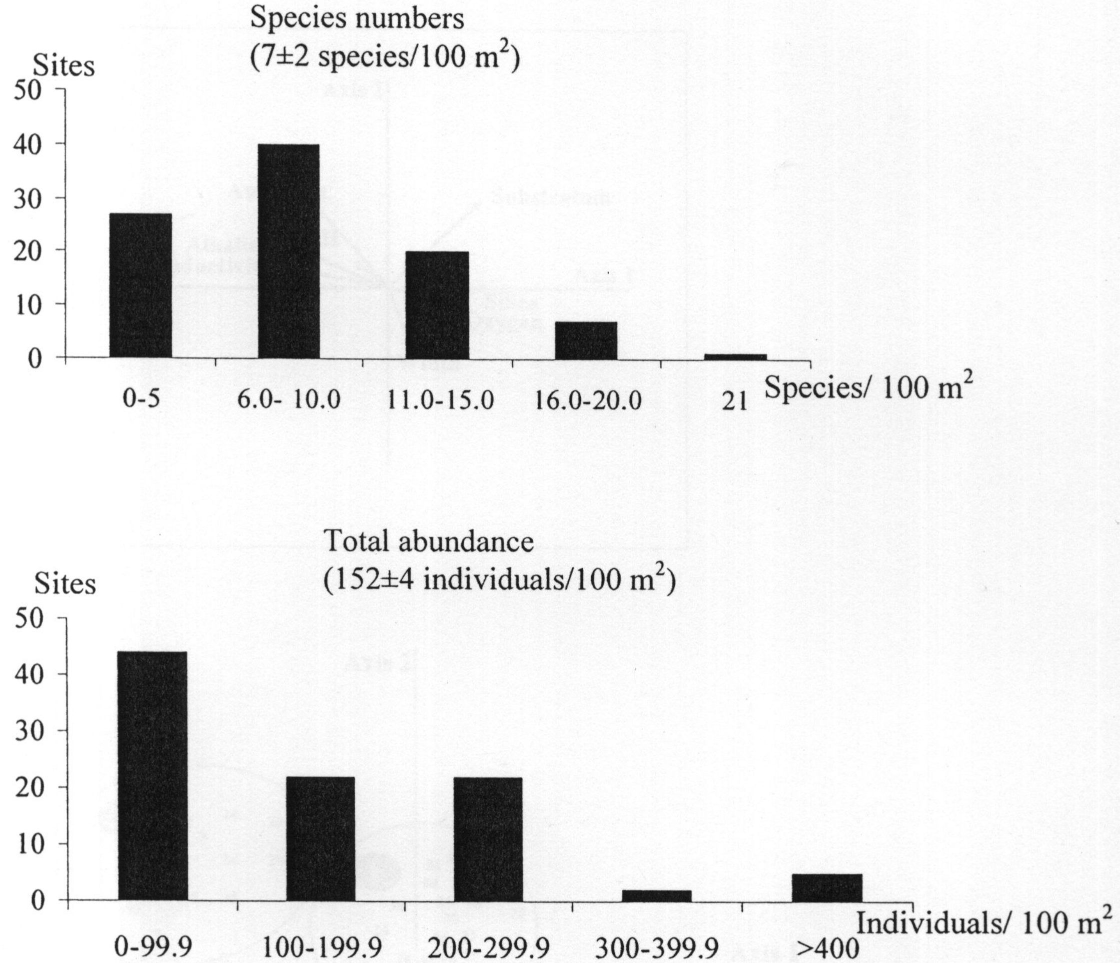


Figure 3 Distribution of sites according to categories of values in species numbers and total abundance adjusted to an area of 100 m^2. Overall GM±SD is given in parenthesis for each of species numbers and total abundance.

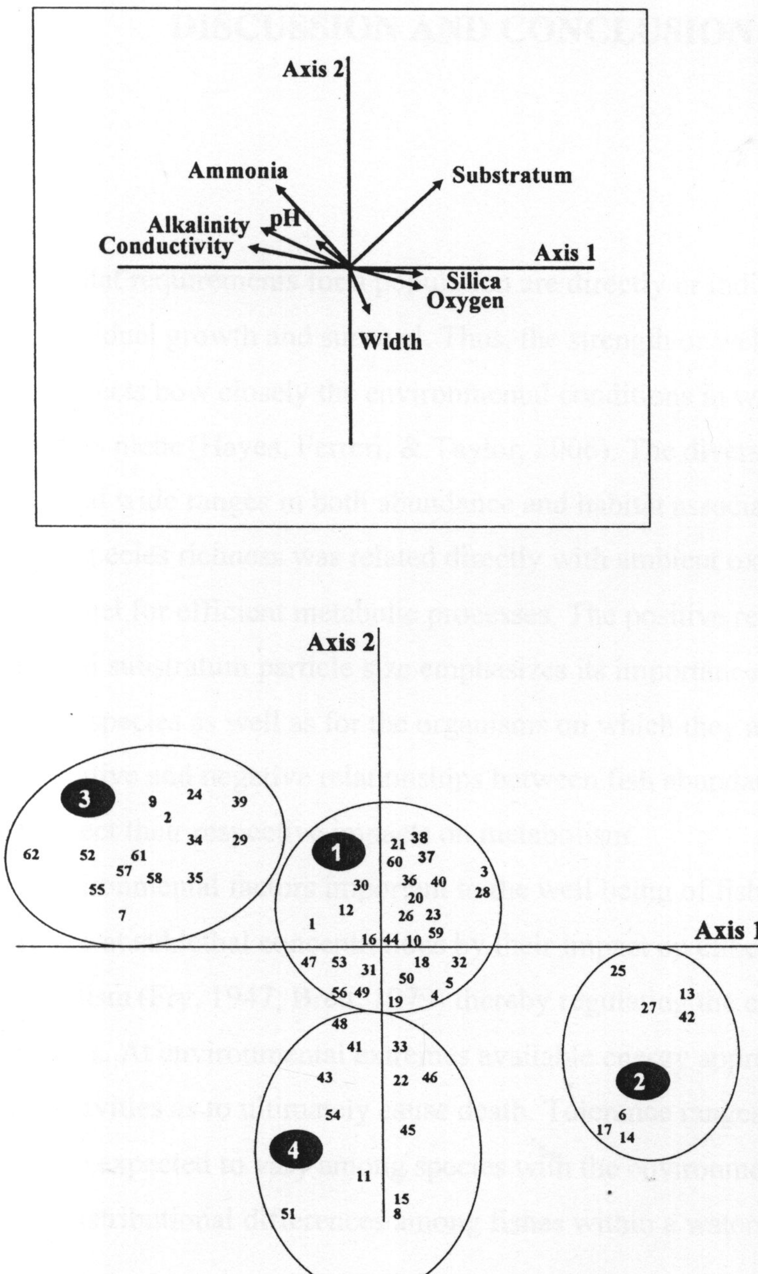


Figure 4 Distribution of fish species with respect to significant habitat variables. The upper panel illustrates the significant habitat variables on axis one and two and, for each, the vector length and direction. The lower panel locates species in relation to axis one and two.

CHAPTER 5
DISCUSSION AND CONCLUSION

Discussion

Habitat requirements for a population are directly or indirectly related to those for individual growth and survival. Thus, the strength or well being of a fish population reflects how closely the environmental conditions in which it lives approximate its niche (Hayes, Ferreri, & Taylor, 2006). The diverse species in this study displayed wide ranges in both abundance and habitat associations. Not surprisingly, species richness was related directly with ambient oxygen which provides the fuel for efficient metabolic processes. The positive relationship between abundance and substratum particle size emphasizes its importance in providing habitat for many fish species as well as for the organisms on which they and other species feed. The positive and negative relationships between fish abundance and oxygen and ammonia reflect their respective impacts on metabolism.

Environmental factors important to the well being of fish generally exercise their influence at sublethal concentrations by their impact on either or both active or basal metabolism (Fry, 1947; Brett, 1979) thereby regulating the energy available for all its activities. At environmental extremes available energy approaches zero, so restricting activities as to ultimately cause death. Tolerance ranges and performance optima can be expected to vary among species with the environmental factor and may account for distributional differences among fishes within a watershed or system of rivers.

The present study found eight environmental factors to be significantly related to species abundance and distribution. Substratum and ammonia were clearly the most important based on vector length. Conductivity, alkalinity, dissolved oxygen and silica followed and were of similar importance. Conductivity is positively related to alkalinity with the former reflecting all ions present while the latter, those contributing to buffering capacity. Stream width and pH were of least importance among the significant variables. Fishes have responded to variations in substratum through an assortment of adaptations that have allowed for the utilization and sharing of resources. Thus, morphologies have allowed some species to burrow in soft sediments providing protection from predators and, for other species, to live on hard substratum where water flows may be substantial (Roberts, 1986). Still other morphological variations ease habitat sharing by species through partitioning food resources (Wikramanayake, 1990; Ward-Campbell et al., 2005).

Ammonia is toxic to fish, particularly the undissociated form at high pH and low dissolved oxygen and hence can impose severe limitations on habitat. Ammonia toxicity as 96 h LC_{50} values have been reported for several freshwater and marine species (Thurston, Russo, & Philips, 1983a, b; Peng et al., 1998; Wang & Walsh, 2000) but for none does the concentration approach that at some sites where fish were found in the present study. Several mechanisms for ammonia tolerance have been described including its conversion to the less toxic urea or the amino acid, glutamine (Ip, Chew, & Randall, 2001) perhaps facilitated by an ammonia impermeable skin and reductions in protein catabolism (Lim, Anderson, Chew, & Ip, 2001). Conversion of ammonia to glutamine is employed also by *Oxyeleotris marmorata* (Jow, Chew, Lim, Anderson, & Ip, 1999), a species not associated with particularly high ammonia in the present study. Ammonia tolerance in some species including *Clarias batrachus* and *Anabas testudineus*, both found in the present study where ambient ammonia was comparatively high, is assisted through cutaneous and branchial excretion as well as by conversion to urea or non-essential free amino acids (Saha, Dutta, & Bhattacharjee, 2002; Tay et al., 2006). Presumably the accessory respiratory organ found in *A. testudineus* as well as in *T. vittatus* and *T. tricopterus*, the species found in this study where ammonia was exceptionally high, plays an important role in ammonia tolerance.

Oxygen's low solubility in water especially at high temperature, causes it to impose a hardship on many species. The need for oxygen varies widely among fish species (Beamish, 1964) as have the physiological adaptations associated with the extraction of ambient oxygen (Hughes, 1966; Palzenberger & Pohla, 1992). Other adaptations include accessory respiratory organs or suprabranchial chambers (Munshi & Hughes, 2005) and cutaneous respiration (Singh & Hughes, 1971; Munshi, Ghosh, Ojha, & Olson, 2005a, b).

Plants, especially algae are consumed partially or wholly by many fish species and by a large number of benthic invertebrates on which many Thai river fishes feed. Plant production is positively related to alkalinity, conductivity and pH at least within moderate concentrations. Diatoms, that are so abundant in streams and on which many organisms feed additionally require silica for the construction of their cell walls.

Each of the four groups of fish responded differently to the environmental factors identified as significant in the present study. Almost half of the 62 species are included in group one and occurred in habitats where the significant environmental factors were at approximately average values. Abundance in this group was far greater than the cumulative abundances in the other three groups. Cyprinids were dominant numerically as well as in species richness. Of the six most abundant species overall, all were from this group including four cyprinids, *D. albolineatus, S. binotatus, R. paviei* and *Mystacoleucus marginatus* followed by *D. pusillus* and *C. gachua*. Most of the other species were not represented by large numbers of individuals. It is interesting that included in this assemblage is *C. gachua* which with its suprabranchial organ (Musikasinthorn, 2003) is adapted for low ambient oxygen. Quite possibly the allure for this piscivorous species to group one is the abundance of other fishes.

Group two contained only seven species, mostly cyprinids, and all were present in low abundance with habitat associated with group two fishes were characterized by high ambient oxygen, silica and low ammonia, alkalinity, conductivity and pH. This implies that fish in this group may be sensitive to low oxygen and, also to high ammonia, conductivity, alkalinity and pH.

Group three contained 14 species and 25% of the total numerical abundance over all sites. Environmental conditions were represented by low dissolved oxygen and high ammonia concentration probably resulting from excessive organic productivity and decomposition. This group contained few cyprinids, *Barbodes gonionotus, E. metallicus, Labiobarbus leptocheilus*, all low in abundance. The anabantids, *T. tricopterus* and *T. vittatus*, with their relative independence from ambient oxygen through their lung-like labyrinth organs were the most abundant species in this group. *C. striata* and *A. testudineus*, both provided respiratory assistance through suprabranchial organs were also in this group although not in high abundance. *A. testudineus* and *C. batrachus* tolerate relatively high concentrations of ammonia and are likely residents of these habitats despite their low numbers. Others may be transients. In all probability branchial respiration in the scaleless silurids, *Mystus mysticetus* and *Hemibagrus nemurus,* the balitorid, *Acanthocobitis zonalternans* and cobitid, *Acanthopsis* sp. is at least partially subsidized by cutaneous respiration. It is interesting that two of the three non-indigenous species in this study, *O. nilotica* and *Poecilia reticulate* are in this group, indicating their capacity to tolerate unfavorable conditions that has undoubtedly contributed to their success as an invader species.

The fourth group contained 12 species and approximately 7% of the fish. Several are benthic and associated with wider than average locations where the substratum is rocky including *Rhinogobius* sp., *Macrognathus circumcintus, M. siamensis,* and *Pseudomystus siamensis.* The four cyprinids, *Systomus orphoides, Poropuntius deauratus, Neolissochilus blanci* and *Hampala macrolepidota* were not abundant but presumably favor, this habitat for the plentiful benthic invertebrates on which they feed (Saha et al., 2002). Presumably the piscivorous *Xenenthodon cancilla*, is attracted by potential prey. The non indigenous *Oreochromis mossambicus* was found in low abundance and is regarded as a transient.

In summary species numbers were positively and negatively related with ambient oxygen and pH while abundance varied inversely with river discharge and ammonia and directly with depth, substratum, oxygen and alkalinity. Fish species were related with eight habitat factors into four groups. Most of the 62 species were associated with a group in which the habitat factors were of overall average values. The group with the fewest species was associated with habitats of high oxygen and low ammonia and alkalinity. Fish in the other two groups were similiar in species numbers, however, they differed in habitat quality. In one, ammonia and alkalinity were high and oxygen, low with sites located near areas of domestic, industrial and agricultural activities and displaying evidence of abundant organic detritus. In the other, sites displayed minimal evidence of anthropogenic activities with ambient oxygen high and ammonia, low. These characteristics provide a basis from which to assess small river habitats.

Bioassessment methods

Bioassessment techniques were created to circumvent the shortcomings inherent in chemical indices in evaluating general water quality. In the present study, fish were selected for assessment of water quality for small rivers in Thailand as they associate with specific habitats where their abundances tend to be high relative to suboptimum habitats. Also, fishes encompass a number of trophic levels; invertivores, herbivores and carnivores and their abundances can be expected to vary directly with those of their prey.

Biota; such as aquatic insects are also useful indicators of water environmental quality; however, unlike fish they tend to be relatively difficult to identify below the Family level. Further, adult aquatic insects leave the water to mate during which time bioassessment is impaired. Other taxa such as diatoms or periphyton while acceptable indicators of environmental quality are, again, relatively difficult to identify to a suitable taxonomic level.

Development of IBI metrics for fish in small rivers in Thailand

The metrics used in the bioassessment model for fish in Thailand were patterned conceptually after those developed earlier by Karr (1981). In the present study, anomalies were not observed on fish at any of the sites, presumably reflecting the absence or low concentrations of heavy metals, often associated with anomalies (Berra & Au, 1975) and were not adopted as a metric.

Metrics developed in this study were based on the presence of fish in riffle habitats of smaller river in eastern. This model focused total species numbers, total fish abundance, species numbers of insectivores and carnivores, abundance of non indigenous. Fishes and abundance of all species within each of Balitoridae, Cyprinidae and a group of species selected as indicators. Indicator species were derived from the CCA analyses (Figure 4) and included seven species; *Cyclocheilichthys heteronema, Osteochilus lini*, *Parachelia maculicauda*, *Rasbora myersi*, *Homaloptera smithi, Nemacheilus platiceps* and *Doryichthys boaja*. These were members of group 2 in Figure 4 and were characterized by an association with high ambient oxygen, low ammonia, pH and alkalinity.

While chemical quality of water impacted by organics such as agricultural and domestic waste products is most accurately measured by a number of factors, dissolved oxygen and ammonia may serve as convenient surrogates. Not surprisingly, dissolved oxygen was significantly correlated with ammonia concentration at $P<0.05$ and $r = 0.45$, and is described by the equation;

$$\log (O + 1) = 0.838 - 0.073 \log (A + 1)$$

the regression's F-value $_{1, 93}$ is 19, $P<0.05$ and $R^2 = 0.2$. In view of the effort to sample approximately equal numbers of relatively undisturbed, moderately disturbed and severely disturbed sites throughout eastern Thailand, the average dissolved oxygen, 5.4 mg/ l, with a SD of 1.4, was assumed a reasonable approximation of the average range for this area. Thus, sites where dissolved oxygen ranged from 4 to 6.8 mg/l (5.4 ± 1.4 mg/ l) were considered moderately disturbed while those with oxygen above or below this range were taken to be undisturbed and extremely disturbed, respectively.

The results for each of the eight metrics for each site were placed into one of the three categories, undisturbed, moderately disturbed and severely disturbed to which was assigned a score of 5, 3 or 1, respectively. Metric ratings were then summed to yield a numerical score that was taken as a measure of site integrity.

Species numbers were higher where dissolved oxygen was also high. At sites with ambient oxygen concentrations between 4 and 6.8 mg/ l, and considered to be moderately disturbed, mean number of species adjusted to an area of 100 m^2 was 7±2. Sites with species numbers within this range were assigned a score of 3. Sites where species numbers were below 5 were considered to be severely disturbed and assigned a metric value of 1. When species numbers exceeded 9 species/ 100m^2 an integrity value of 5 was assigned.

Abundance of Cyprinidae fishes at moderately disturbed sites (4-6.8 mgO_2/ l) was 42±5 fish/ 100 m^2. Sites with Cyprinid abundances within this range were assigned a score of 3, above this range a score of 5 and below, a score of 1. Similarly, abundance of Balitoridae at moderately disturbed sites, based on ambient oxygen, was 1-2 fish/ 100 m^2. Sites with balitorid abundances within this range were assigned a score of 3, above this range a score of 5 and blow, a score of 1.

Number of indicator species, adjusted to an area of 100 m^2, at moderately disturbed sites based on ambient oxygen concentrations was 1 and greater than 1 in undisturbed sites. In severely disturbed sites these indicator species were absent. Scores of 5, 3 and 1 were assigned when numbers indicator species were >1, 1 and < 1, respectively.

Trophic categories of all species (Table 5) were classified in this study based on information from Rainboth (1996), Apitanakul & Wetchakul (2002), Lokitsathaporn, Pewnane, & Waipokha (2002), Pongsirijun, Boongarm, & Pongjanvakul (2002), Ward-Campbell (2004), Ward-Campbell & Beamish (2005), Ward-Campbell et al., (2005). Trophic level of fish was species differently feed in diet that relatively associated with natural foods supported to be fish survival in small rivers. Fish species existed in the streams based on relation with available preys in their habitats, thus, declining of available preys based on impacted from organic materials might be indirectly affected to fish diet in stream such as aquatic insect was declined in abundance lead to be declined fish species fed insects where affected from organic matters. The present study, species number of insectivores and carnivores were applied to indicate the water quality impacted by organic materials.

Species numbers of insectivores was 5.0±1.5 fish/ 100 m^2 at the 'standard' moderately disturbed sites (4- 6.8 mgO_2/l). Again, sites with numbers of insectivores within this range were assigned a score of 3, those with higher numbers, a score of 5 and those with fewer, a score of 1. Species numbers of carnivores was 2±2 fish/ 100 m^2 at moderately disturbed sites and were given a score of 3. Scores of 5 and 1 were assigned when numbers of carnivores were higher and lower, respectively.

Mean total fish abundance (all species, except non indigenous fishes) was 81±3 / 100 m^2 and sites within this range were scored a value of 3, while sites with a total fish abundance below 78 fish/ 100 m^2 was considered severely disturbed and assigned a score of 1. Sites with total abundances above 84 fish/ 100 m^2 were scored a 5. Abundance of non indigenous fish was taken to indicate severely disturbed conditions and sites were they were found were assigned a score of 1. The absence of non indigenous fish was considered positive evidence of habitat integrity and was scored a 5.

The cumulative score for the eight metrics was used to estimate water quality. IBI scores of 33-40 points were considered to indicate water of excellent quality. Scores of 25-32 and under 25 points were taken as indicators of good and poor water quality.

Bioassessment criteria in the present study was for rivers under 5 m in width. Among the sites studied, five of the 9 were wider than the overall average and ranged from 10-25 m. For these wider sites, mean species numbers were higher than at the more narrow sites with a mean of 13±1.4 species/ 100 m^2 (Table 3). Species of insectivores were higher at the wider sites with a mean of 8±2 species/ 100 m^2 in comparison to the 5±2 at the more narrow sites. Surprisingly, total fish abundance and abundance of Cyprinidae were lower in the wider sites, 61±4.0 and 36±5.0, respectively (Table 3). Values for the other metrics; species of carnivores and abundances of Balitoridae and indicator species were similar at sites of average and above average widths. Thus, while the number of sites sampled in wider rivers was small, it appears the bioassessment model proposed in this study may be equally applicable with only minor changes.

Classification of Thai Rivers

The IBI metrics and scoring criteria in Table 3 were used to assign general water quality or health (Table 4). Site scores of 33-40, 25-32 and17-24 were considered to indicate general water quality as being in excellent, good and poor health, respectively. The classification system developed in this study was applied also to 10 assessment sites located in Prachuap Khirikhan and Phetchaburi provinces in south-central Thailand (Figure 1, b). Fish and habitat parameters were sampled and measured as the same way of those in eastern Thailand. Fish were collected by electro-fishing in each site. Species of fish were counted and adjusted to an area of 100 m^2. Total fish abundance of all species as well as that of individual species were calculated by the maximum likelihood technique (Carle & Strub, 1978). Habitat parameters were measured as at all sites in eastern Thailand.

IBI scores indicated two sites were of excellent quality with scores of 33-40 points. Five sites were assigned as being of good water quality with scores of 25-32 points. Water at three sites was considered to be of poor quality with fewer than 24 cumulative points. On the basis of ambient oxygen concentrations water at four sites would be classified as being undisturbed and the overall quality as being of excellent quality. Five sites were considered moderately disturbed with water of good quality and one, severely disturbed and of poor water quality. The assigned quality of water based on dissolved oxygen was based on the mean ± 1SD (1 SD represented 68.3% of the variations among the 95 sites). Occasionally, values may exceed one standard deviation and this could be expected to bring the two assessment estimates closer in agreement. The advantage of the IBI is that the eight metrics are correlated with different ecological perspectives, each reflective of the impact of human use and summarized in Table 4. For example, few species are physiologically adapted to live were organic matter has seriously reduced ambient oxygen and elevated ammonia. The food resource base in rivers consisting to a large extent of benthic insectivores (invertivores), and attached algae will likewise vary with environmental quality. Fluctuations in food resources will dictate fish species and abundance. Thus, species number of insectivores (invertivores) and carnivores can be expected to relate directly with the general quality of water. Cumulative abundance of all species, Balitoridae species, Cyprinidae species and indicator species varied directly with general water quality.

All of the non indigenous species in the present study have morphological and, some, also physiological adaptations to low ambient oxygen. As well some of non indigenous species are able to tolerate high ammonia by reduction in the protein metabolism or direct excretion (Kobayashi et al., 2007). Hence successful habitat invasions by non indigenous species is more likely where competition with established populations of indigenous fishes is lessened such as where organic loads are high. For this reason non indigenous species were considered a strong indicator of general water quality and adopted as a metric.

The ecological approach to assess general water quality developed by Karr (1981) has been adopted widely throughout North America, Europe (Oberdorff & Hughes, 1992), Australia (Harris, 1995) and Southeast Asia (Ganasan & Hughes, 1998). In the present study eight of the metrics in Karr's model were considered conceptually relevant also for water quality evaluation of Thai rivers. Interestingly, species numbers in headwater temperate streams, 10- 15/ 100 m^2, appears to be comparable (Matthews, 1998) to that for rivers of similar size in eastern Thailand. In wider rivers 20-25 species was common in temperate regions (Karr, 1981; Matthews, 1998) compared with about 15 in eastern Thailand. This is especially interesting in view of the much higher number of species in Thailand than in the geographically larger USA where Matthews and Karr made their estimates.

Insectivores and carnivores are relatively abundant in temperate streams in which water is of good quality, as are their prey (Mathew, 1998). Conversely insectivores, carnivores and their prey are all less abundant where water quality is degraded (Karr, 1981, Oberdoff & Porcher, 1994; Belpaire et al., 2000; Price & Birge, 2005). In tropical waters a large proportion of the riverine fish species feed intensively on invertebrates (Ward-Cambell, 2004; Ward-Cambell & Beamish, 2005; Ward-Campbell et al., 2005) whose abundance also varies directly with overall water quality. Thus, when general water quality was good, species diversity of insectivores and carnivores was much higher than when of poor quality.

Several species were found in water judged to be of poor water quality, however, no single indigenous species was consistently present. This was in contrast to the findings of Karr in the Arkansas River in Midwestern U.S.A. where green sunfish, *Lepomis cyanellus* and *Lepomis humilis* were considered to be representative of poor water quality (Bramblett & Fausch, 1991). In Thailand, the abundance of the non indigenous *O. niloticus* and *Poecilia reticulata* was taken as ecological surrogate of Karr's green sunfish. In the present study species in the family Balitoridae were taken as indicators of water of good to excellent overall quality and the ecological surrogate of species in the family as Etheostomidae (darters) in Karr's model. Species in the family Cyprinidae and their cumulative abundances were greater than those for any other family and both varied directly with overall water quality in accord with earlier observations in American rivers by Schrader (1989), Bramblett & Fausch (1991). Total fish abundance was declined as water quality degraded accord with the earlier observations by Karr (1981; Ganasan & Hughes, 1998). Seven indicator species were declined in abundant as water quality was high organic matters observed by in this study.

The general observational pattern of agreement between the earlier findings by Karr (1981) and for other ecological regions throughout the world including Thailand in the present study supports the usefulness of this bioassessment approach for evaluating water quality (Karr, Fausch, Angermier, Yant, & Schlosser, 1986; Angermeier & Karr, 1986; Fausch & Schrader, 1987; Steedman, 1998). My recommendation based on my study is that this model should be considered for adoption in Thailand.

Conclusion

In the present study is the first quantitative study to relate environmental quality of rivers in eastern Thailand to the distribution and abundance of fishes. In this study found eight habitat parameters to be significantly related to species abundance and distribution. Substratum and ammonia were distinctly the most important based on vector length. Conductivity, alkalinity, dissolved oxygen and silica followed and were of similar importance. Conductivity is positively related to alkalinity with the former reflecting all ions present while the latter, those contributing to buffering capacity. Width of stream and pH were of least importance among the significant variables. Fishes have responded to variations in substratum through an assortment of adaptations that have allowed for the utilization and sharing of resources. Hence, morphologies have allowed some species to burrow in soft sediments providing protection from predators and, for other species, to live on hard substratum where water flows may be substantial. Still other morphological variations ease habitat sharing by species through partitioning food resources.

The quantitative environmental associations found for fishes in Thai rivers provided the basis for the development of a bioassessment model with which to evaluate general water quality. Bioassessment techniques were created to circumvent (overcome) the shortcomings inherent in chemical indices in evaluating general water quality. In the present study, fish were selected for assessment of water quality for small rivers in Thailand as they associate with specific habitats where their abundances tend to be high relative to suboptimum habitats. Also, fishes encompass a number of trophic levels; invertivores, herbivores and carnivores and their abundances can be expected to vary directly with those of their preys. The first step in actual model construction was derived from characteristics of fish communities including species composition, trophic levels and fish condition resulting in eight factors or metrics. Metrics or factors included species richness, abundance of Cyprinidae, Balitoridae, and indicator species; species number of insectivores and carnivores; total fish abundance and number of non indigenous species. These metrics should be suitable also to conservation, in particular in providing restoration guidelines for impaired habitats.

Table 3 Integrity of Index of Biotic and scoring criteria for metrics. Moderately disturbed sites were those where ambient oxygen was within the overall geometric mean ± SD, 4.0-6.8 mg/ l. Minimally disturbed were those sites where oxygen concentration was above 6.8 mg/l and severely disturbed were those sites with oxygen below 4.0 mg/ l.

Metrics/categories	Minimally disturbed	Moderately disturbed	Severely disturbed
Score	5	3 (n=51)	1
(O_2, mg/ l)	>6.8	4.0-6.8	<4.0
Taxonomic richness			
Species richness (numbers of species)	>9	5-9	<5
Indicator fish			
Abundance of Cyprinidae (fish/ 100 m^2)	>47	37-47	<37
Abundance of Balitoridae (fish/ 100 m^2)	>2	1-2	0
Indicator species (number of species/ 100 m^2)	>1	1	0
Trophic categories			
Species number of insectivore (fish/ 100 m^2)	>7	3-7	<3
Species number of carnivore (fish/ 100 m^2)	>4	1-4	0
Total all fish abundance and fish condition			
Total fish abundance (fish/ 100 m^2)	>110	104-110	<104
Non indigenous fish (all species)	absent		present

Table 4 Index score interpretation is described for environmental health is based on habitat characteristics of Thai Rivers.

Overall IBI Score	Integrity Class	Rating Characteristics
33-40	Excellent	Very good water and habitat quality; includes, high dissolved oxygen was >7 mg/ l, ammonia was very low as <0.01 $mgNH_3N$/ l of which present in very low organic materials. The surrounding landscape shows limited deforestation, agriculture, aquaculture and no industry exercising. Negative influence on the environment, improper road construction not evident; urban development was not impacting negatively on the environment.
25-32	Good	Good water quality where dissolved oxygen was 5.7±1.3 mg/ l and ammonia concentration was 0.3±3.3 mg NH_3N/ l. A little present of agriculture activity, urban and industrial effluent, stream habitats were a little disturbed by road construction and deforestation.
<25	Poor	Water quality is large impacted by agriculture activity, urban discharge, road construction, waste water effluent. For example, dissolved oxygen was probably decreased as <3.4 mg/ l, while organic materials were lead to be high amounts contributed by concentration of ammonia was increased as 1.5±6.0 mg NH_3N/ l). Relative few species include decreased species richness and non-indigenous fish (all species) sometimes common, especially *O. niloticus* was likely to be present as well as high individual abundant.

Table 5 Species of fish in the Eastern Rivers Thailand at 95 sites were classified the trophic categories. ID numbers were assigned as species coded in Figure 4.

Species	ID	Trophic categories of fish
Notopterus notopterus	1	C
Barbodes gonionotus	2	Om
Danio albolineatus	3	I
Cyclocheilichthys apogon	4	I
Cyclocheilichthys armatus	5	I
Cyclocheilichthys heteronema	6	I
Esomus metallicus	7	I
Hampala macrolepidota	8	C
Labiobarbus leptocheilus	9	Om
Mystacoleucus marginatus	10	I
Neolissochilus blanci	11	H
Osteochilus hasselti	12	H, D
Osteochilus lini	13	H, D
Parachelia maculicauda	14	I
Poropuntius deauratus	15	I
Rasbora borapetensis	16	C
Rasbora myersi	17	I
Rasbora paviei	18	I
Rasbora trilineata	19	I
Systomus binotatus	20	I
Systomus lateristriga	21	I
Systomus orphoides	22	I, D
Systomus partipentozona	23	Om
Acanthocobitis zonalternans	24	I
Homaloptera smithi	25	I

Nemacheilus masyae	26	I
Nemacheilus platiceps	27	I
Schistura kohchangensis	28	I
Acanthopsis sp1.	29	I
Acanthopsis sp2.	30	I
Lepidocephalichthys berdmorei	31	Om
Lepidocephalichthys hasselti	32	Om
Pseudomystus siamensis	33	I
Mystus mysticetus	34	C
Hemibagrus nemurus	35	I
Ompok bimaculatus	36	C
Pterocryptis cochinchinensis	37	-
Amblyceps mangois	38	I
Clarias batrachus	39	C
Dermogenys pusillus	40	I
Xenenthodon cancilla	41	C
Doryichthys boaja	42	I
Doryichthys martensii	43	I
Monopterus albus	44	I
Macrognathus circumcintus	45	I
Macrognathus siamensis	46	I
Mastacembelus armatus	47	I
Parambassis siamensis	48	I
Nandus nebulosus	49	I
Pristolepis fasciatus	50	C
Oreochromis massambicus	51	C
Oreochromis niloticus	52	C
Oxyeleotris marmorata	53	C
Rhinogobius sp.	54	C
Anabas testudineus	55	C
Betta prima	56	I
Trichogaster tricopterus	57	I

Trichopsis vittatus	58	I
Channa gachua	59	C
Channa lucius	60	C
Channa striata	61	C
Poecilia reticulata	62	I

Where; H=herbivore, Om=omnivore, C=carnivore, I=insectivore, and D=detritivore. *Pterocryptis cochinchinensis* is not reported in trophic categories and not calculated.

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APPENDIX

ENVIRONMENTAL SENSITIVITIES OF FISHES FROM SMALL RIVERS IN EASTERN THAILAND

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Keyword: abundance, frequency of occurrence, habitat, water quality, species richness.

Synopsis

Total abundance of fish and number of species captured by electrofishing at 95 sites in small rivers varied significantly with physicochemical factors. Species numbers were negatively and positively related with pH and dissolved oxygen, respectively. Abundance varied inversely with discharge and ambient ammonia and, directly with depth, substratum, ambient oxygen and alkalinity. Fish were represented by 62 species, the most common being *Rasbora paviei*, *Systomus binotatus* and *Channa gachua*. Canonical correspondence analysis related species and their abundances with eight environmental variables into four groups. The most species-rich group was associated with approximately average values for the significant variables. The group with the fewest species was associated with high oxygen and low ammonia and alkalinity. The other two groups had similar numbers of species, one being associated with high ammonia and alkalinity and low oxygen. Species in the other group were found at locations where rivers were relatively wide with comparatively high oxygen and low ammonia concentrations.

INTRODUCTION

Ambient abiotic factors characterize habitat quality and, collectively, the suitability for fish species (Brett, 1979). Several important environmental factors have been identified for some river and lake-dwelling fishes, particularly in temperate regions of the world (Jackson, Peres-Neto, & Olden, 2001; Yamazaki, Haramato, & Fukasawa, 2006) and represent an important step in the identification of both water quality and critical habitat. By comparison these relationships are less well understood in tropical regions, particularly South East Asia.

An appreciation of the physical and chemical requirements of aquatic biota is embraced within an enthusiastic array of bioassessment models developed largely to overcome shortcomings inherent in traditional chemical indices of water quality (Karr, 1981, 1991; Angermeier & Karr, 1986). Rivers in Southeast Asia have been subjected to extensive anthropogenic alterations. Today, it is unlikely any river in this region can be considered as pristine (Dudgeon, 2000). A common view is that the numbers and diversity of river fishes are declining and that habitat degradation is a major cause (Beamish & Sa-ardrit, 2006; Beamish, Sa-ardrit, & Tongnunui, 2006). While this view is likely correct few studies have quantified changes in habitat. The Eastern rivers system in Thailand (Vidthayanon, Karnasuta, & Nabhitabhata, 1997) provide important sources of water for a variety of domestic, agricultural and industrial services (Boonphakdee, Sawangwong, & Fujiwara, 1999; Szuster & Flaherty, 2002) and, as a consequence, have almost certainly suffered changes in quality (Cheevaporn, Jacinto, & San Diego-McGlone, 1995; Mangmeechai, Chaiwatpongsakorn, Marhaba, Wattanachaira, & Pavasant, 2004). The present study sought to quantify those significant environmental factors important to the habitat of common river fishes of this region with the ultimate view of developing a bioassessment model specifically for Thai fishes.

Materials and methods

Small rivers representing first to third order tributaries, ≤25 m in width, of larger rivers were sampled within seven provinces; Chachoengsao, Nakon Nayok, Prachinburi, Chon Buri, Rayong Chanthaburi and Trat. Sites were located within five major rivers that discharge into the Gulf of Thailand (Figure 1). Bangpakong river is the largest with a watershed of 18,758 km^2 with its lower portion flowing through Chachoengsao and Chon Buri provinces prior to discharging into the Gulf of Thailand. The other rivers, Rayong, Prasae, Chanthaburi and Trat are smaller with watersheds of 2,300, 1,578, 1,755, and 1,970 of km^2, respectively. All watersheds in the study area drain land under intensive agriculture. The major crops include rubber, oil palm, sugar can, pineapple, cassava, and rice. Animal husbandry is also intensive and includes fish such as *Oreochromis niloticus* and a hybrid catfish, (*Clarias macrocephalus* x *C. gariepinus*), shrimp, *Penaeus monodon*, and domestic pigs and chickens (Braaten & Flaherty, 2000; Bordalo, Nilsumranchit, & Chalermwat, 2001; Szuster & Flaherty, 2002). A number of cities and towns are located in the study area with an active array of industries (Graslund & Bengtsson, 2001; Cheevaporn & Menasveta, 2003). Commonly, waste is discharged directly into rivers throughout the study area.

Fish were collected from 95 sites with most (n=73) located in Chon Buri province with fewer sites in Rayong (n=13), Prachinburi (n=1), Chachoengsao (n=1), Nakhon Nayok (n=2), Chanthaburi (n=3) and Trat (n=2) provinces, respectively. Sites were selected from road-accessible locations ranging from remote, heavily forested and sparsely inhabited to lightly settled areas where some subsistence to modest commercial agriculture occurred to more heavily farmed or urban areas. Site selection presented a few problems as some waterways were accessible only during short periods of the dry season and then only with difficulty. Some tributaries indicated on topographical maps could not be found and apparently no longer exist while others contained water only for portions of the year. Thus, for practical reasons, sites were selected as representative of landscapes and land use. Sites were not sampled closer than about 150 m from the nearest bridge.

A site was a length of stream relatively even in width, depth, water velocity and canopy. Within sites, these factors were visually estimated not to range beyond about 25% of the average except for depth and velocity along the margins. An effort was made also for evenness of substratum. Thus, for example, a stretch of river where the substratum was predominately of particles within a single Wentworth size class was considered to be relatively even with respect to this characteristic. In regions of rivers subjected to extremely high seasonal flow rates, the substratum was typically punctuated with boulders among which was cobble mixed with pockets of smaller particles. Stretches with much the same relative proportions of boulders, and other particle size categories were also considered to be relatively even in this characteristic. The search for relative evenness restricted the length of most sites to under 50 m. Physical constraints to sampling imposed by water velocity in concert with discharge also contributed to the length of stream that could be sampled.

Fish were captured with a Smith-Root, model 15 D backpack electro-fisher with variable output voltage (100-1100 volts), pulse width (1-120 Hz) and frequency (100 μs – 8 ms). The anode was fitted with a 28 cm diameter ring. Output voltage was varied inversely with water conductively and, for the sites in this study, was mostly between 200 and 600 volts in combination with a wave width of 60 Hz and frequencies of 1-4 ms. Settings were made, based on experience, to reduce damage to fish, particularly the initial impact.

Seine nets with about 3 mm mesh were installed across the upper and lower limits of a site and their groundlines weighted with rocks to reduce the probability of emigration from or immigration into the sample area. A site was electro-fished by moving in a zigzag pattern from one retaining net to the other, beginning downstream or upstream based on visibility, water depth and velocity. Usually four or five passes were made at a site. Relative capture efficiency between upstream and downstream direction of electro-fishing was compared in an earlier study (Beamish, Sa-adrit, & Cheevaporn, 2008) within several larger sites and not found to differ significantly (ANCOVA, $P<0.05$).

Fishing was conducted by three people, one to operate the electro-chocker and two to hand-net fish from the water. Except in areas of extremely fast flowing water almost all fish were captured by hand net rather than in either blocking seine. After each pass, fish were anaesthetized in methaine tricaine sulfonate (approximately 150 mg/ l), and those fishes that could confidently be identified were enumerated and, after recovery, released downstream from the site. When fishes could not be identified in the field they were killed by an overdose of anesthetic and preserved in 10% formalin for subsequent identification in the laboratory. Current systematics of Thai freshwater fishes is equivocal. For this report the classification system of Nelson

(1994) was followed along with names given in the University of California, Catalog of Fishes (Eschmeyer, 2007). Fish were identified from a number of sources including: Smith (1945), Brittan (1954), Sontirat (1976), Dawson (1981), Robert (1982, 1986, 1989), Kottelat (1984, 1988, 1989, 1990, 1998, 2000), Karnasuta (1993), Ng & Kottelat (2000) and Nalbant (2002). A voucher collection was prepared and is maintained in the Institute of Marine Sciences at Burapha University, Bangsaen, Chon Buri (Catalogue number- BIMS: FF. 0001-002). Fish were preserved in 10% formalin for 10 days and then transferred to 80% ethanol for permanent storage.

On each sampling occasion, width (± 0.1 m), depth (±1 cm) and velocity (±1 cm/ s) were measured, each at least three times, and the mean used to estimate discharge (l/ s). Depth and velocity were the average of 3-5 measurements made at approximately equal intervals across a transverse transect located at about the mid length of a site. Velocity was measured with a propeller current meter at approximately mid depth which was recorded as the vertical average. Canopy was estimated visually and recorded as percentage with 100% representing complete cover. Regularly calibrated meters were used to measure temperature (±0.1°C), conductivity (±5 µS/ cm), turbidity (NTU), pH (±0.1 unit) and dissolved oxygen (±0.1 mg/ l). In addition, a water sample was collected for the measurement of ammonia (mg NH_3N/ l) by the salicylate method, nitrate (mg NO_3N/ l) by the cadmium reduction method, total iron (mg Fe/ l) by the by the Ferro Ver method, alkalinity (as $CaCO_3$ mg/ l, pH 4.5) using the sulfuric acid titration method, silica (mg SiO_2/ l), using the heteropoly method and true color (mg/ l platinum as chloroplatinate ion; 1 color unit = 1 mg/ l chloroplatinate ion) (APHA 1992). Elevation was measured with a Global Positioning Systems meter (GPS, ±10 m). Sites were sampled throughout the year, however, season was not included as a habitat variable. An earlier study by Beamish, Griffiths, Kongchaiya, Sa-ardrit, & Sonchaeng (2005) indicated seasonal changes in fish abundance and assemblage similarity from several streams in Central Thailand to vary inversely with discharge which was included in this study as a habitat variable.

Substratum at each site was collected with a hand-held acrylic corer (5 cm inner diameter) to a depth of 10±3 cm. Particles on the surface larger than the diameter of the corer were removed before a sample was taken and included in the estimate. Samples were air dried and sieved to determine particle size distribution by weight. Six particle size categories were adopted from the Wentworth scale (Giller & Malmqvist, 1998), >150 mm (boulder to large cobble), 150-60.1 mm (large cobble to large pebble), 60-5.1 mm (large pebble to coarse gravel), 5-3.1 mm (medium to fine gravel), 3-0.51 mm (fine gravel to coarse sand) and <0.5 mm (medium sand to silt). The substratum at each site was coded into six categories based on mean particle size with 1 being the smallest and 6, the largest. The substratum at a few sites was solid or almost solid bedrock and coded as 7. In an earlier study (Beamish, Sa-ardrit, & Chevaporn, 2008) an average of three substratum samples (range of 2-6 samples) was selected randomly within each of 40 sites. Variation was similar within each particle size category with an overall mean variation across all sizes (±SD) of 26±12% that is assumed for all measurements. A single sample was collected at all other sites.

Stepwise multiple linear regression analysis (MLR, SPSS 11.5) was applied to examine the relationships between species numbers and abundance and all habitat parameters (Steele & Torrie, 1980). Species, their abundances, and all habitat parameters, except for pH, were log (x+1) transformed to normalize the distribution of values and, in the case of habitat factors, to accommodate differences in scale. To avoid subjectivity all independent variables were included in the full model. The significance used to assess variable inclusion was $P<0.15$. For the final model, significance was accepted at $P<0.05$. In central Thailand, fish abundance in rivers reflected an aggregated distribution as between site variance increased linearly with mean population density (Beamish et al., 2005). A log-transformation removed this correlation on the mean and suggested an overall standard deviation (SD) of 1.3 about the abundance estimate that is assumed for all species estimates in this study.

Canonical correspondence analysis (CCA, PC-ORD 3.2) was employed to identify important species-related environmental characteristics. Species numbers and abundances and environmental variables were centered and normalized within the program. In the canonical correspondence analysis, statistical significance of the relationship between a set of environmental factors and fish species was taken using a Monte Carlo permutation test with 1000 permutations. Statistical significance of all tests was accepted at $P<0.05$.

Total abundance of all fishes as well as individual species within a site were calculated by the maximum likelihood technique that also provided an estimate of capture efficiency for each electro-fishing pass (Carle & Strub, 1978). Numbers for many species were small and not amenable to this technique. When this occurred a conversion factor consisting of the estimated total abundance of all fish captured at a site divided by total number of all fish actually caught at the same site was applied to adjust the numbers of each species captured. Fish abundance was arithmetically adjusted to an area of 100 m^2.

Figure 1 Sampling sites were located in Chon Buri, Chachoengsao, Nakon Nayok, Prachinburi, Rayong, Chanthaburi and Trat provinces in Thailand.

RESULTS

Water parameters varied among sites with those in the more eastern areas a few degrees cooler and slightly higher in pH and alkalinity, on average, than elsewhere. Water in the more central regions was, on average, highest in ammonia, nitrate, total iron and turbidity. Depth and velocity were relatively even in the distribution of their values among the sites (Figure 2). The distribution of sites was unimodal with respect to the range of values for width, elevation, discharge, canopy, turbidity, iron, and alkalinity mostly favoring lower values. Modal maxima for ammonia and nitrate occurred at comparatively low values and those for temperature, oxygen and pH at intermediate values. The range of values for conductivity and silica was wide but the distribution of sites among categories of values did not follow a clear pattern. Substratum varied from sand to large cobble with medium to fine gravel being the average particle size. Stream length at most sites ranged from 10-60 m with a geometric mean (GM±SD) of 28±2 m with an overall GM (±SD) area of 82±3 m^2.

Fish were represented by 62 species. The most common were *Channa gachua*, *Systomus binotatus* and *Rasbora paviei* with frequencies of occurrence of 60 to 75% (Table 1). Also, common were *Danio albolineatus, Monopterus albus*, *Channa striata*, *Trichopsis vittatus, Trichogaster tricopterus* and *Dermogenys pusillus* with occurrence frequencies of 30 to 50% (Table 1). Most of remaining species were found at ≤20% of the sites.

Species numbers were adjusted to an area of 100 m^2 from the overall GM using a calculated slope of 0.0018 (Beamish & Sa-ardrit, 2006) and varied from 0 to 21 (Figure 3). Most sites had 6-8 species and only 5 sites contained no fish. The relationship between species number and all habitat parameters were subjected to stepwise multiple linear regression analysis and found to be significantly related to two parameters, pH and dissolved oxygen:

$$\text{Log}(S+1) = 1.145 - 0.092\,(P) + 0.522\,\log(O+1)$$

where S, is species number, adjusted to an area of 100 m^2, P, pH and O, dissolved oxygen (mg/ l). Variables retained in the equation had significant t-values at $P<0.05$. The regression's F-value $_{2,85}$ is 13.25, $P<0.05$) and $R^2 = 0.25$. Thus, species numbers were negatively related with pH and positively associated with dissolved oxygen. For example, at pH 7, species numbers are predicted to increase from 6.3 to 9.0 species with an increase in oxygen from 4 to 8 mg/ l and at an oxygen concentration of 6 mg/ l to decrease from 9.8 to 6.1 with pH increase from 6 to 8.

Total fish abundance adjusted to a site area of 100 m^2 varied from 0 to 1425 with about half of the sites containing fewer than 100 fish/ 100 m^2 (Figure 3). Total fish abundance was related significantly with depth, discharge, substratum, oxygen, and ammonia and is described by the equation:

$$\text{Log}(N+1) = 0.650 + 0.468 \log(D+1) - 0.307 \log(d+1) + 0.938 \log(S+1) + 0.960 \log(O+1) - 0.213 \log(A+1)$$

where N is total fish abundance/ 100 m^2, D, depth, cm, d, discharge, l/ s, S, substratum coded value, O, oxygen, mg/ l, and A, ammonia, mgNH_3N/ l. The regression's F-value $_{5, 84}$ is 6.8, $P<0.05$) and $R^2 = 0.3$. Thus, fish abundance is predicted to vary inversely with discharge and ammonia and directly with depth, substratum, and dissolved oxygen. The equation predicts with an increase in ammonia from 0.01 to 5 mgNH_3N/ l, fish abundance will decrease from 46.8 to 31.7 / 100 m^2 when discharge is 200 l/ s, depth, 20 cm, dissolved oxygen, 4 mg/ l and the substratum is fine gravel to coarse sand with a coded value of 2.

In preparation for ordination analysis some adjustments were made to the data. No fish were captured at 5 sites. The efficiency of the electro-fisher over four passes was high at 90%, but still the possibility of not capturing all fish could not be excluded. Further, the statistical package available for CCA analysis requires that fish be present in order for a site to be included. Because of the importance of this information to the study's objective, a small number of individuals, 0.001 /100 m^2, of the most commonly encountered species, *R. paviei* was assigned to barren sites. Species and abundances were significantly correlated with eight environmental factors (P=0.001 along axes 1 and 2, Monte Carlo test with 1000 permutations). The first and second axes of the CCA analysis were both significant with axes 1 and 2 explaining 58 and 40% of the variability, respectively. The first axis illustrates a positive gradient of environmental factors; substratum (R^2=0.4), silica (R^2=0.5), oxygen (R^2=0.3) and width (R^2=0.1), respectively. Ammonia (R^2=0.04), pH (R^2=0.3), alkalinity (R^2=0.1) and conductivity (R^2=0.04) loaded positively on the second axis (Table 2). Environmental correlations were 0.88 and 0.86 for axis 1 and 2, respectively. Each significant environmental factor increased along a vector away from the origin with its length being a measure of magnitude (Figure 4).

Species were distributed clearly within four groups with respect to the significant habitat characteristics (Figure 4). The 29 species in group one were associated with mostly average habitat conditions for the sites sampled. Overall sites, GM±SD values for alkalinity, conductivity, silica, oxygen, ammonia and pH were 75±3 $CaCo_3$mg/ l, 253±3 μS/ cm, 6.2±3.4 mg SiO_2/ l, 5.4±1.4 mg/ l, 1.7±7.6 mgNH_3N/ l and 7.1±0.7, respectively. Width and coded value for the substratum were 3±1.6 m and 3.1±1.4. Group 1 consisted of four of the generally most abundant species, *S. binotatus*, *R. paviei*, *C. gachua* and *D. pusillus,* respectively. The second group consisted of only seven species. Environmental conditions where species in group two were found indicate above average concentrations of oxygen and silica and below average levels of ammonia, alkalinity, conductivity and pH. Fish were not abundant at any of the sites in group two with no species having a mean abundance of >0.1 /100 m^2. Group three contained fourteen species with two, *T. vittatus* and *T. tricopterus,* being moderately abundant at 1.3±3.0 and 1.4±3.5 /100 m^2, respectively and widespread. The remaining species in this group were sparsely distributed being found at <41 sites with overall abundances of <1.0 /100 m^2. Ammonia and conductivity were exceptionally high at sites where group three fish were captured. Indeed, ammonia exceeded 500 mgNH_3N/ l at six sites and in three was 5000 mgNH_3N/ l or higher. At these latter sites, 5000, 6000 and 8000 mg NH_3N/ l (pH 7.5, 8.2 and 8.4), *T. vittatus* and *T. tricopterus* were present at approximately 12 and 3 individuals/ 100 m^2, respectively, despite ambient oxygen concentrations of only 2.7 mg/ l at the site with the highest ammonia. At each of the other two sites where ammonia was 6000 and 5000 mgNH_3N/ l one other species was present, *Esomus metallicus* at 5.6 / 100 m^2 and *C. striata* at 3.5 / 100 m^2, respectively. *O. niloticus* and *C. striata* along with *T. tricopterus* and *T. vittatus* were found at sites where ammonia ranged between 500 and 1700 mgNH_3N/ l (pH 7.6-7.8 and oxygen 4 to 6 mg/ l). Over half of the species in group three were found where ambient ammonia was >30 mg NH_3N/ l.

Habitat conditions were similiar where fish in groups one and four were captured, except for ammonia which was lower for fish in the latter group. Species in group four were usually found at sites wider than the overall average (Figure 4). All the 12 species in this group displayed low abundances of <0.5 / 100 m^2 and were found at between 1 and 20 sites.

Table 1 Species captured at the sample sites, their frequencies of occurrence (Freq Occ, %) and GM abundances (N±SD/ 100 m^2). Numbers under ID designate species in Figure 2.

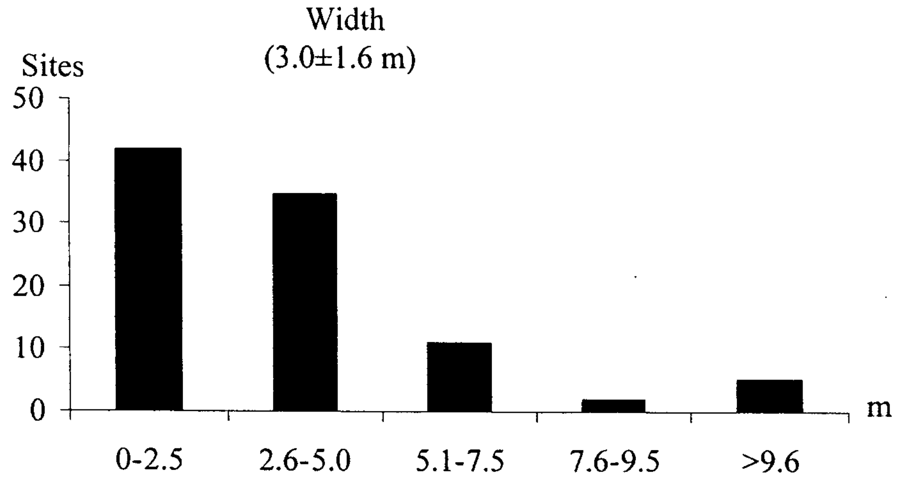
Species	ID	Freq Occ (%)	Abundance (±SD) (N/ 100 m^2)	Species	ID	Freq Occ (%)	Abundance (±SD) (N/ 100 m^2)
Notopterus notopterus	1	1	<0.1	*Neolissochilus blanci*	11	5	0.1(1.7)
Barbodes gonionotus	2	4	<0.1	*Osteochilus hasselti*	12	16	0.4(2.4)
Danio albolineatus	3	30	1.5(5.3)	*Osteochilus lini*	13	2	<0.1
Cyclocheilichthys apogon	4	1	<0.1	*Parachelia maculicauda*	14	1	<0.1
Cyclocheilichthys armatus	5	2	<0.1	*Poropuntius deauratus*	15	3	<0.1
Cyclocheilichthys heteronema	6	1	<0.1	*Rasbora borapetensis*	16	11	0.2(2.0)
Esomus metallicus	7	16	0.6(3.0)	*Rasbora myersi*	17	2	<0.1
Hampala macrolepidota	8	7	<0.1	*Rasbora paviei*	18	73	5.1(1.8)
Labiobarbus leptocheilus	9	4	<0.1	*Rasbora trilineata*	19	2	<0.1
Mystacoleucus marginatus	10	17	0.7(3.8)	*Systomus binotatus*	20	65	5.7(5.4)

Systomus lateristriga	21	2	<0.1	*Ompok bimaculatus*	36	14	0.2(1.6)
Systomus orphoides	22	15	0.3(1.9)	*Pterocryptis cochinchinensis*	37	1	<0.1
Systomus partipentozona	23	6	<0.1	*Amblyceps mangois*	38	16	0.2(1.7)
Acanthocobitis zonalternans	24	5	<0.1	*Clarias batrachus*	39	14	0.2(1.4)
Homaloptera smithi	25	4	<0.1	*Dermogenys pusillus*	40	48	2.2(4.3)
Nemacheilus masyae	26	5	<0.1	*Xenenthodon cancilla*	41	7	<0.1
Nemacheilus platiceps	27	3	<0.1	*Doryichthys boaja*	42	1	<0.1
Schistura kohchangensis	28	5	0.1(1.8)	*Doryichthys martensii*	43	1	<0.1
Acanthopsis sp1.	29	4	<0.1	*Monopterus albus*	44	37	0.5(2.0)
Acanthopsis sp2.	30	1	<0.1	*Macrognathus circumcintus*	45	14	0.2(1.7)
Lepidocephalichthys berdmorei	31	1	<0.1	*Macrognathus siamensis*	46	2	<0.1
Lepidocephalichthys hasselti	32	22	0.5(2.3)	*Mastacembelus armatus*	47	13	0.2(1.6)
Pseudomystus siamensis	33	12	0.2(1.5)	*Parambassis siamensis*	48	20	0.5(2.4)
Mystus mysticetus	34	5	0.1(1.8)	*Nandus nebulosus*	49	4	<0.1
Hemibagrus nemurus	35	15	0.3(1.8)	*Pristolepis fasciatus*	50	2	<0.1

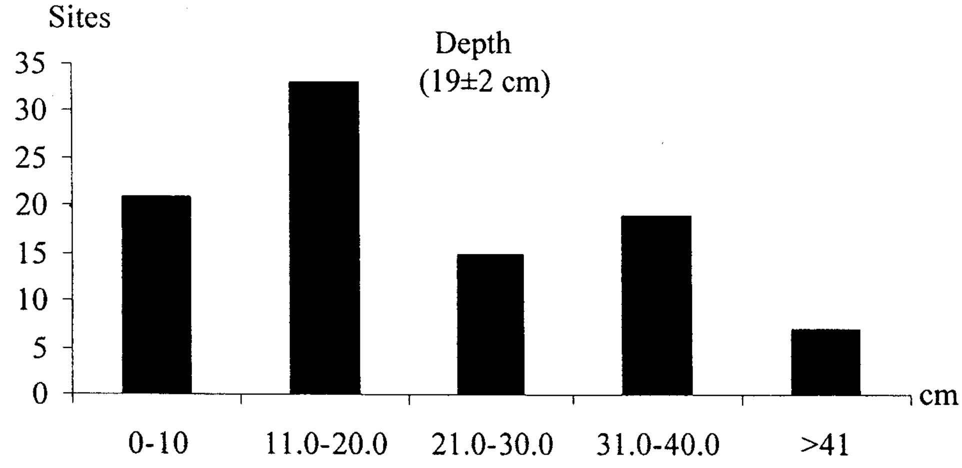
Oreochromis massambicus	51	1	<0.1	*Trichogaster tricopterus*	57	45	1.4(3.5)
Oreochromis niloticus	52	10	0.4(2.6)	*Trichopsis vittatus*	58	43	1.3(3.0)
Oxyeleotris marmorata	53	7	<0.1	*Channa gachua*	59	61	2.3(3.7)
Rhinogobius sp.	54	10	0.2(1.3)	*Channa lucius*	60	5	<0.1
Anabas testudineus	55	5	0.1(1.7)	*Channa striata*	61	41	0.8(2.4)
Betta prima	56	2	<0.1	*Poecilia reticulata*	62	2	<0.1

Table 2 Canonical coefficients for significant environmental variables at 95 stream sites.

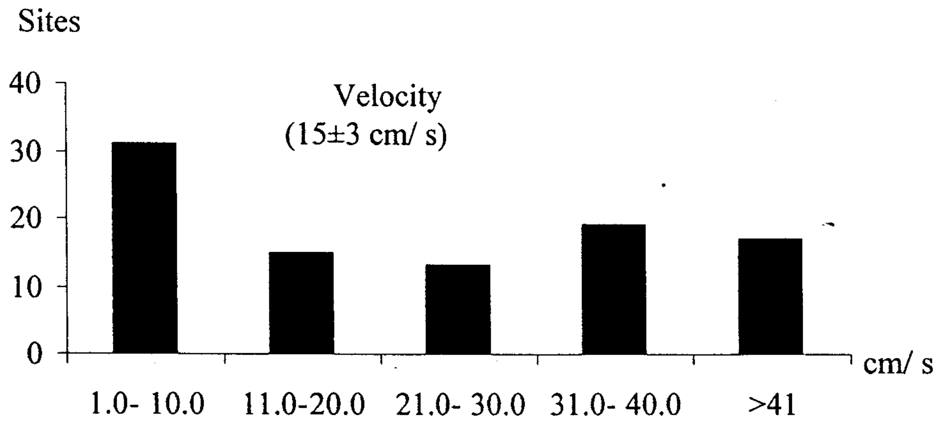
Variables	Axis 1	Axis 2
Width (m)	0.273	-0.531
Substratum (mm)	0.661	0.669
Conductivity (μS/ cm)	-0.763	0.189
pH	-0.723	0.547
Oxygen (mg/ l)	0.575	-0.224
Ammonia (mgNH_3N/ l)	-0.508	0.203
Silica (mg SiO_2/ l)	0.716	-0.062
Alkalinity (as $CaCO_3$ mg/ l)	-0.814	0.307



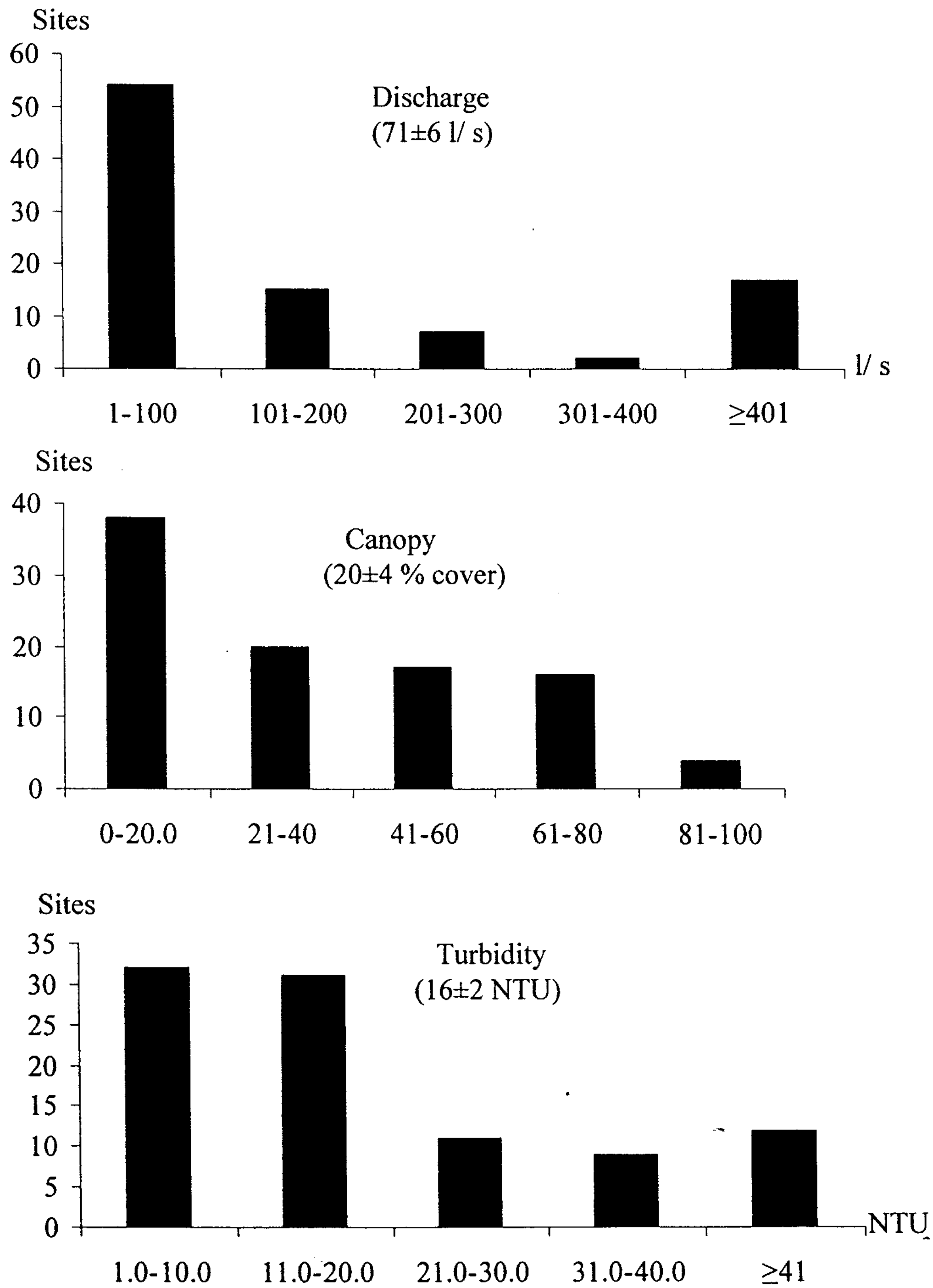
Width
(3.0±1.6 m)
Sites
50
40
30
20
10
0
m
0-2.5
2.6-5.0
5.1-7.5
7.6-9.5
>9.6

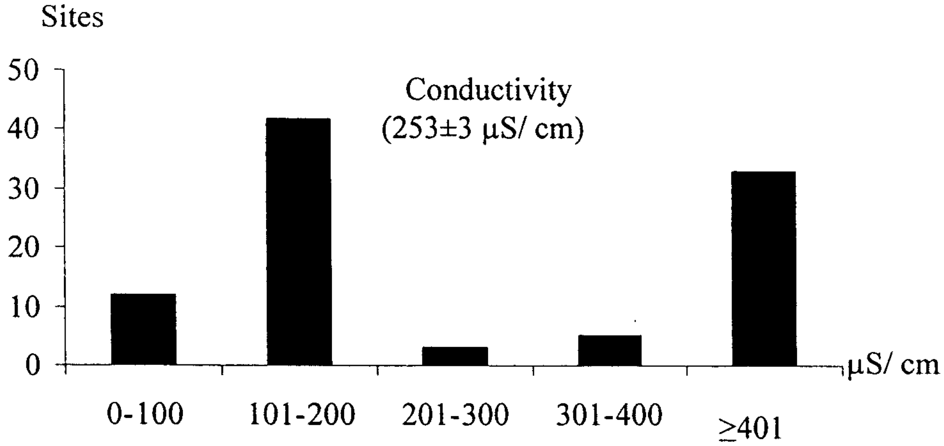


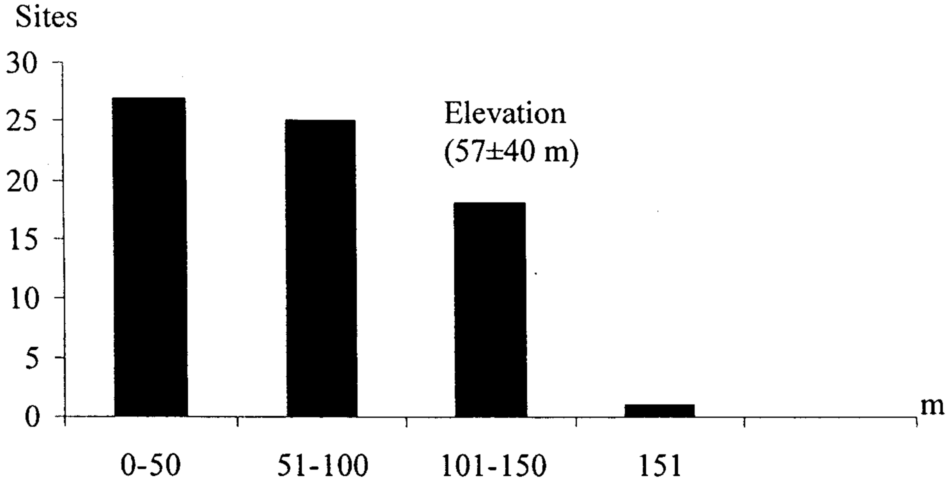
Sites
Depth
(19±2 cm)
35
30
25
20
15
10
5
0
cm
0-10
11.0-20.0
21.0-30.0
31.0-40.0
>41

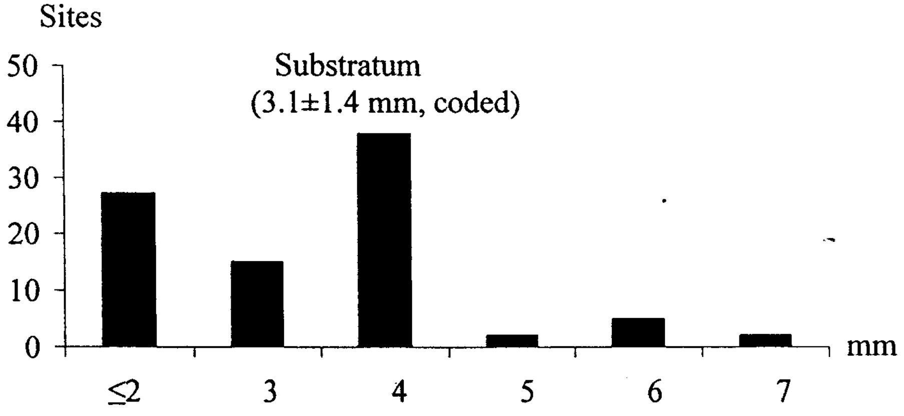


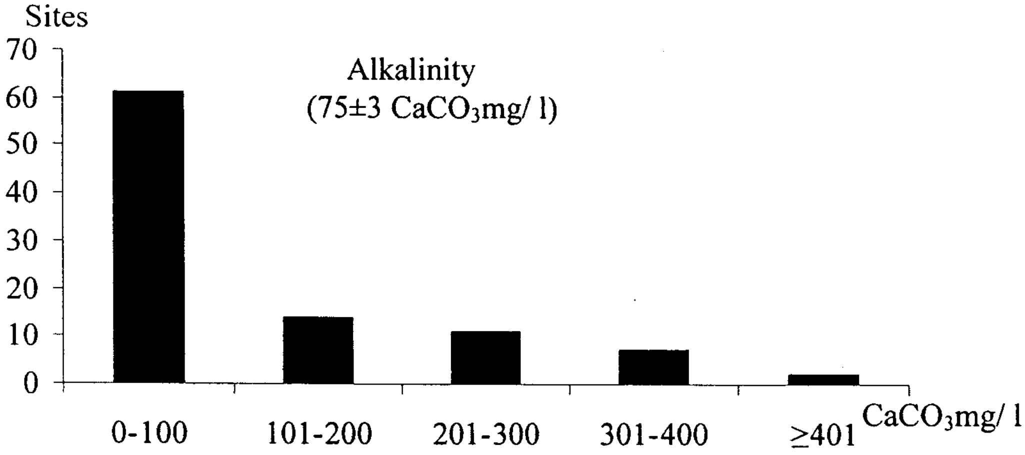
Sites
Velocity
(15±3 cm/ s)
40
30
20
10
0
cm/ s
1.0- 10.0
11.0-20.0
21.0- 30.0
31.0- 40.0
>41

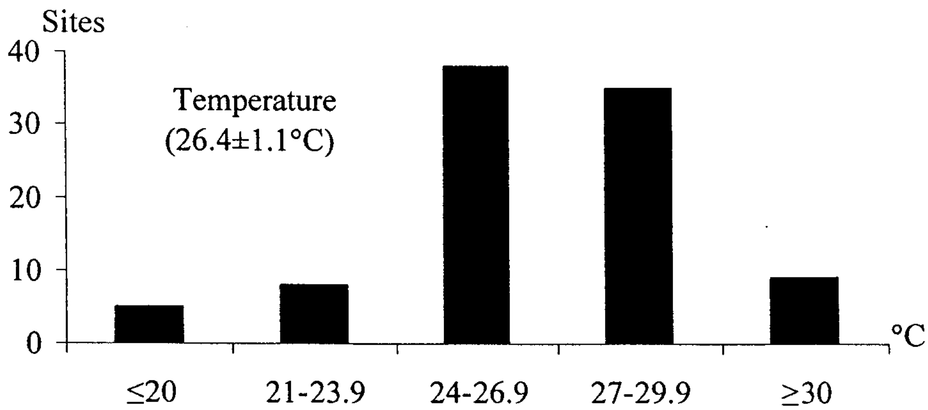

Sites
60
50
40
30
20
10
0
Discharge
(71±6 l/ s)
l/ s
1-100
101-200
201-300
301-400
≥401
Sites
40
30
20
10
0
Canopy
(20±4 % cover)
0-20.0
21-40
41-60
61-80
81-100
Sites
35
30
25
20
15
10
5
0
Turbidity
(16±2 NTU)
NTU
1.0-10.0
11.0-20.0
21.0-30.0
31.0-40.0
≥41

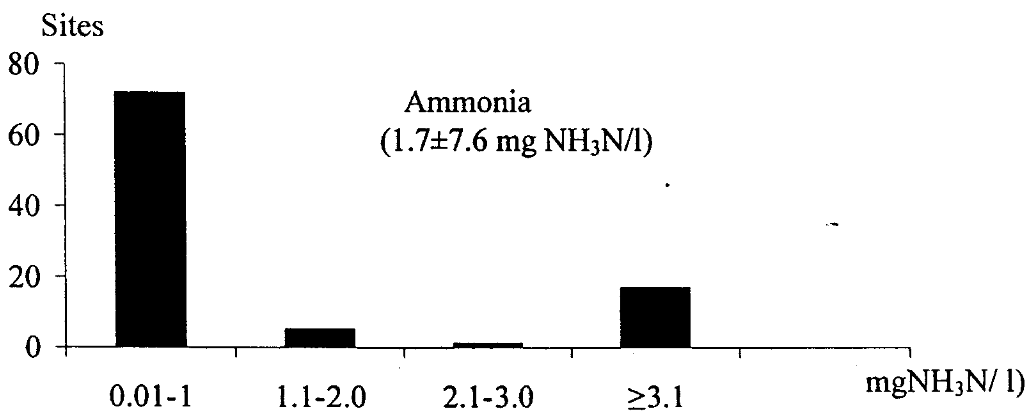

Sites
50
40
30
20
10
0
Conductivity
(253±3 μS/ cm)
μS/ cm
0-100
101-200
201-300
301-400
≥401

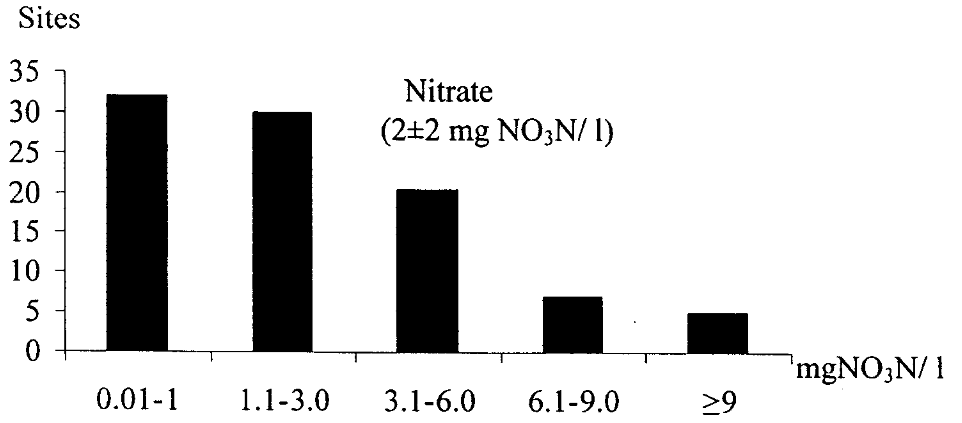

Sites
30
25
20
15
10
5
0
Elevation
(57±40 m)
m
0-50
51-100
101-150
151

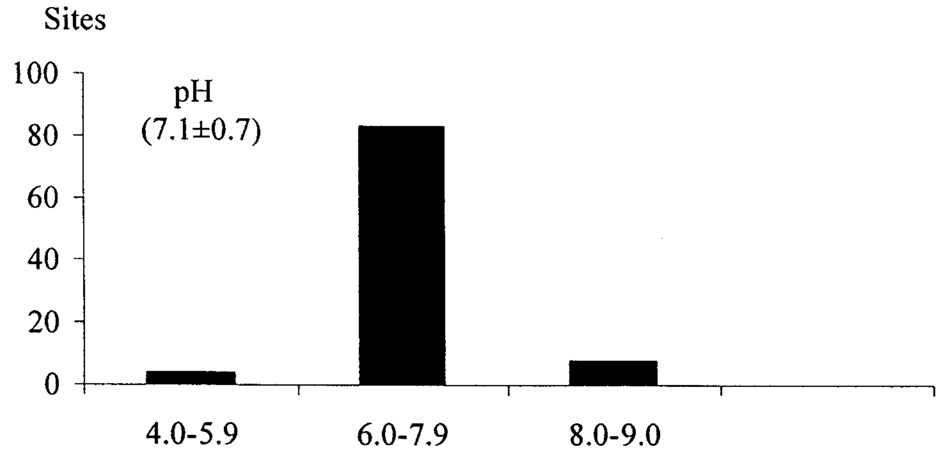

Sites
50
40
30
20
10
0
Substratum
(3.1±1.4 mm, coded)
mm
≤2
3
4
5
6
7

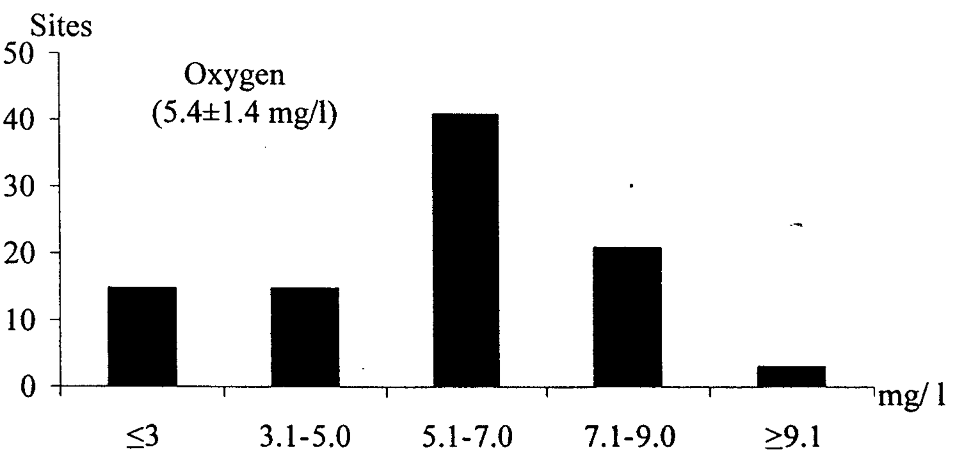

Sites
70
60
50
40
30
20
10
0
Alkalinity
(75±3 CaCO3mg/ l)
0-100
101-200
201-300
301-400
≥401
CaCO3mg/ l


Sites
40
30
20
10
0
Temperature
(26.4±1.1°C)
≤20
21-23.9
24-26.9
27-29.9
≥30
°C


Sites
80
60
40
20
0
Ammonia
(1.7±7.6 mg NH3N/l)
0.01-1
1.1-2.0
2.1-3.0
≥3.1
mgNH3N/ l)


Sites
35
30
25
20
15
10
5
0
Nitrate
(2±2 mg NO3N/ l)
0.01-1
1.1-3.0
3.1-6.0
6.1-9.0
≥9
mgNO3N/ l


Sites
100
80
60
40
20
0
pH
(7.1±0.7)
4.0-5.9
6.0-7.9
8.0-9.0


Sites
50
40
30
20
10
0
Oxygen
(5.4±1.4 mg/l)
≤3
3.1-5.0
5.1-7.0
7.1-9.0
≥9.1
mg/ l

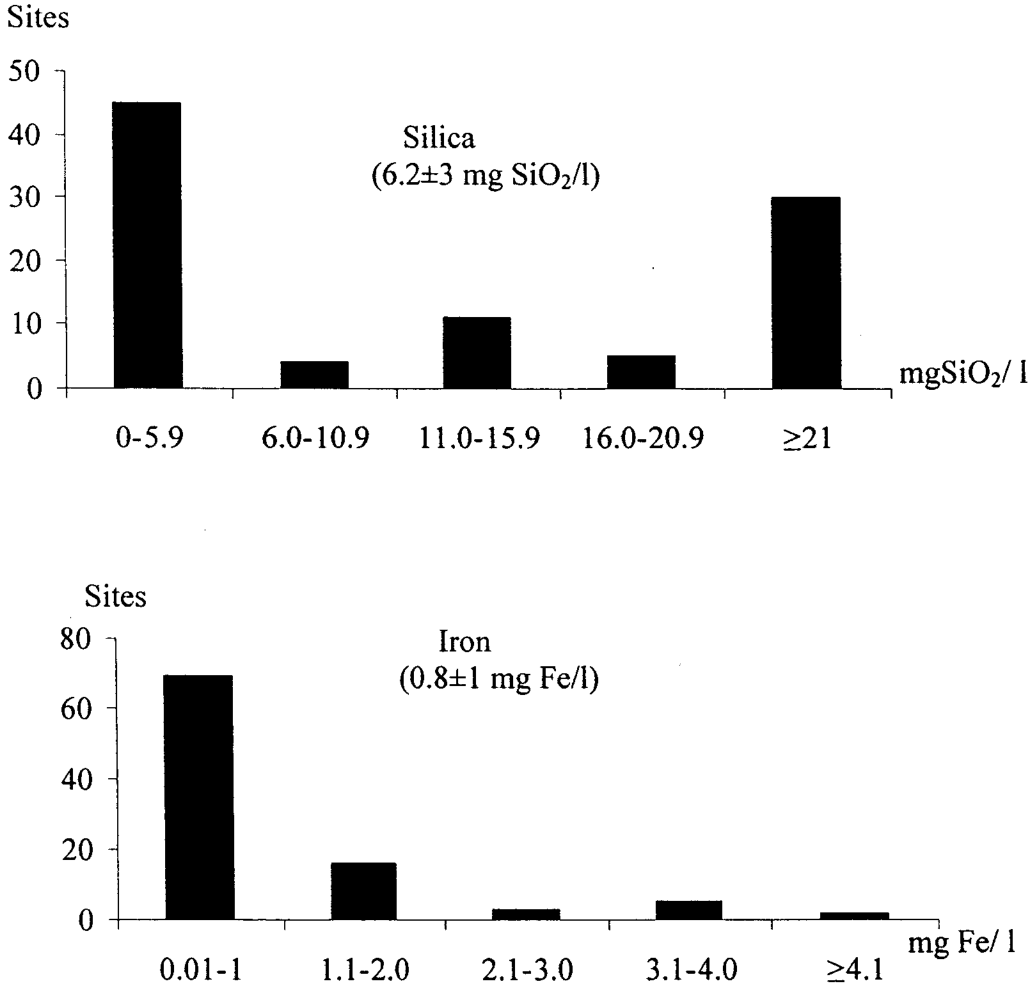
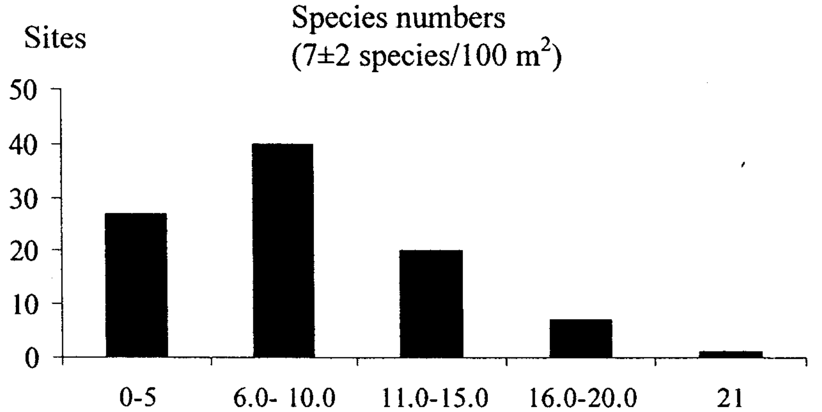


Figure 2 Distribution of sites according to categories of values for each physicochemical characteristics. Overall GM±SD is given in parenthesis for each variable.



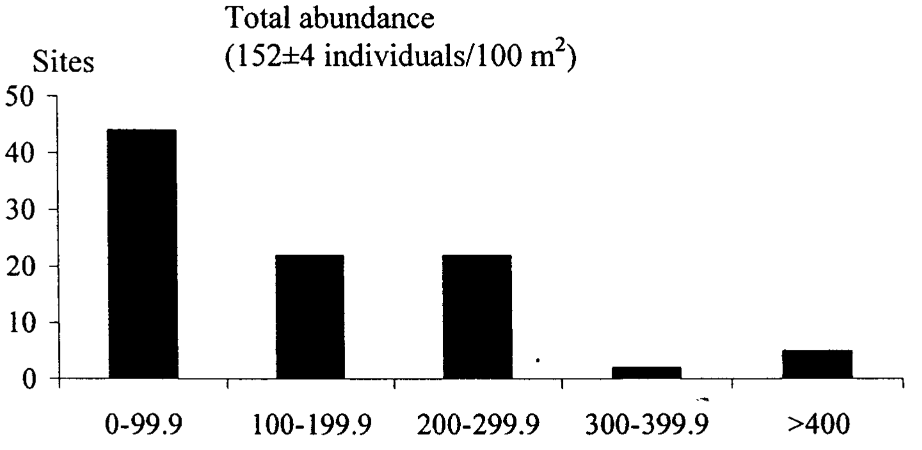


Figure 3 Distribution of sites according to categories of values in species numbers and total abundance adjusted to an area of 100 m^2. Overall GM±SD is given in parenthesis for each of species numbers and total abundance.

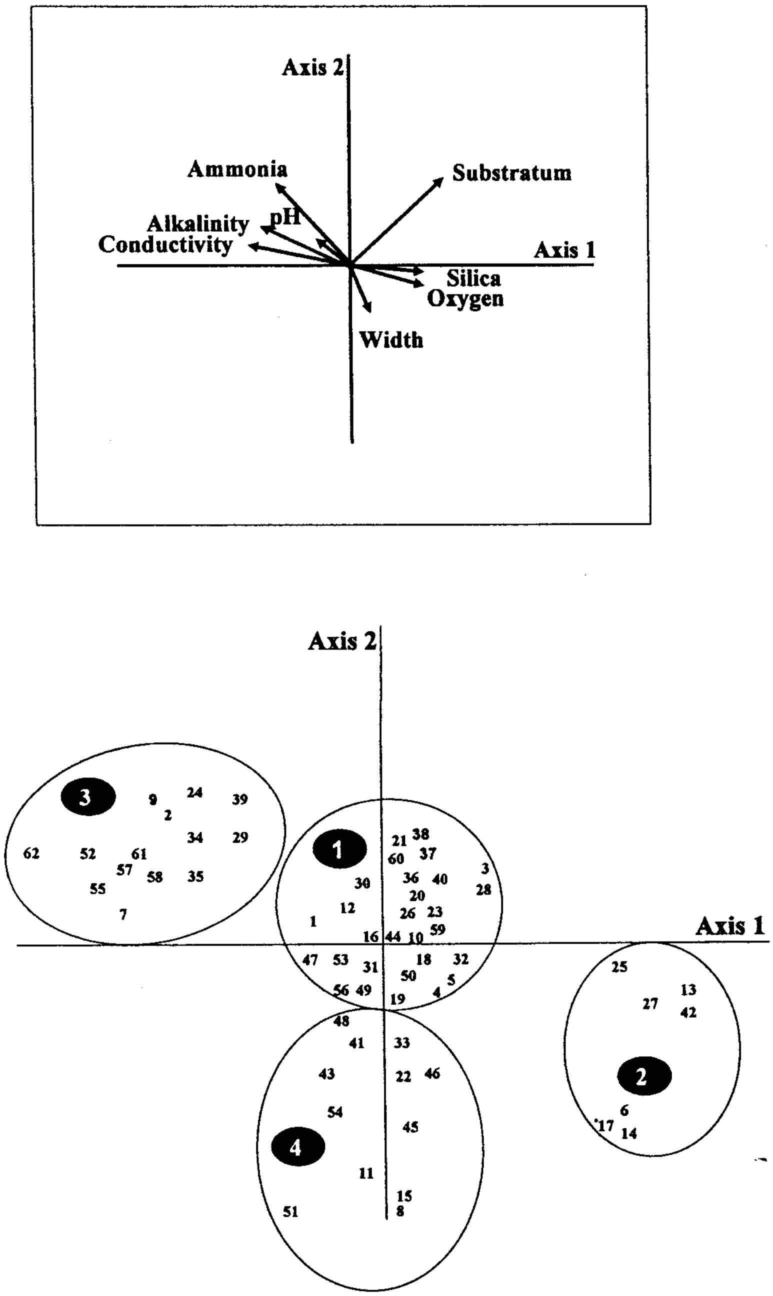


Figure 4 Distribution of fish species with respect to significant habitat variables. The upper panel illustrates the significant habitat variables on axis one and two and, for each, the vector length and direction. The lower panel locates species in relation to axis one and two.

DISCUSSION

Habitat requirements for a population are directly or indirectly related to those for individual growth and survival. Thus, the strength or well being of a fish population reflects how closely the environmental conditions in which it lives approximate its niche (Hayes, Ferreri, & Taylor, 2006). The diverse species in this study displayed wide ranges in both abundance and habitat associations. Not surprisingly, species richness was related directly with ambient oxygen which provides the fuel for efficient metabolic processes. The positive relationship between abundance and substratum particle size emphasizes its importance in providing habitat for many fish species as well as for the organisms on which they and other species feed. The positive and negative relationships between fish abundance and oxygen and ammonia reflect their respective impacts on metabolism.

Environmental factors important to the well being of fish generally exercise their influence at sublethal concentrations by their impact on either or both active or basal metabolism (Fry, 1947; Brett, 1979) thereby regulating the energy available for all its activities. At environmental extremes available energy approaches zero, so restricting activities as to ultimately cause death. Tolerance ranges and performance optima can be expected to vary among species with the environmental factor and may account for distributional differences among fishes within a watershed or system of rivers.

The present study found eight environmental factors to be significantly related to species abundance and distribution. Substratum and ammonia were clearly the most important based on vector length. Conductivity, alkalinity, dissolved oxygen and silica followed and were of similar importance. Conductivity is positively related to alkalinity with the former reflecting all ions present while the latter, those contributing to buffering capacity. Stream width and pH were of least importance among the significant variables. Fishes have responded to variations in substratum through an assortment of adaptations that have allowed for the utilization and sharing of resources. Thus, morphologies have allowed some species to burrow in soft sediments providing protection from predators and, for other species, to live on hard substratum where water flows may be substantial (Roberts, 1986). Still other morphological variations ease habitat sharing by species through partitioning food resources (Wikramanayake, 1990; Ward-Campbell, Beamish, & Kongchaiya, 2005).

Ammonia is toxic to fish, particularly the undissociated form at high pH and low dissolved oxygen and hence can impose severe limitations on habitat. Ammonia toxicity as 96 h LC_{50} values have been reported for several freshwater and marine species (Thurston, Russo, & Phillips, 1983a, b; Peng et al., 1998; Wang & Walsh, 2000) but for none does the concentration approach that at some sites where fish were found in the present study. Several mechanisms for ammonia tolerance have been described including its conversion to the less toxic urea or the amino acid, glutamine (Ip, Chew, & Randall, 2001) perhaps facilitated by an ammonia impermeable skin and reductions in protein catabolism (Lim, Anderson, Chew, & Ip, 2001). Conversion of ammonia to glutamine is employed also by *Oxyeleotris marmorata* (Jow, Chew, Lim, Anderson, & Ip, 1999), a species not associated with particularly high ammonia in the present study. Ammonia tolerance in some species including *Clarias batrachus* and *Anabas testudineus*, both found in the present study where ambient ammonia was comparatively high, is assisted through cutaneous and branchial excretion as well as by conversion to urea or non-essential free amino acids (Saha, Dutta, & Bhattacharjee, 2002; Tay et al., 2006). Presumably the accessory respiratory organ found in *A. testudineus* as well as in *T. vittatus* and *T. tricopterus*, the species found in this study where ammonia was exceptionally high, plays an important role in ammonia tolerance.

Oxygen's low solubility in water especially at high temperatures, causes it to impose a hardship on many species. The need for oxygen varies widely among fish species (Beamish, 1964) as have the physiological adaptations associated with the extraction of ambient oxygen (Hughes, 1966; Palzenberger & Pohla, 1992). Other adaptations include accessory respiratory organs or suprabranchial chambers (Munshi & Hughes, 2005) and cutaneous respiration (Singh & Hughes, 1971; Munshi, Ghosh, Ojha, & Olson, 2005a, b).

Plants, especially algae are consumed partially or wholly by many fish species and by a large number of benthic invertebrates on which many Thai river fishes feed. Plant production is positively related to alkalinity, conductivity and pH at least within moderate concentrations. Diatoms, that are so abundant in streams and on which many organisms feed additionally require silica for the construction of their cell walls.

Each of the four groups of fish responded differently to the environmental factors identified as significant in the present study. Almost half of the 62 species are included in group one and occurred in habitats where the significant environmental factors were at approximately average values. Abundance in this group was far greater than the cumulative abundances in the other three groups. Cyprinids were dominant numerically as well as in species richness. Of the six most abundant species overall, all were from this group including four cyprinids, *D. albolineatus, S. binotatus, R. paviei* and *Mystacoleucus marginatus* followed by *D. pusillus* and *C. gachua*. Most of the other species were not represented by large numbers of individuals. It is interesting that included in this assemblage is *C. gachua* which with its suprabranchial organ (Musikasinthorn, 2003) is adapted for low ambient oxygen. Quite possibly the allure for this piscivorous species to group one is the abundance of other fishes.

Group two contained only seven species, mostly cyprinids, and all were present in low abundance with habitat associated with group two fishes were characterized by high ambient oxygen, silica and low ammonia, alkalinity, conductivity and pH. This implies that fish in this group may be sensitive to low oxygen and, also to high ammonia, conductivity, alkalinity and pH.

Group three contained 14 species and 25% of the total numerical abundance over all sites. Environmental conditions were represented by low dissolved oxygen and high ammonia concentration probably resulting from excessive organic productivity and decomposition. This group contained few cyprinids, *Barbodes gonionotus, E. metallicus*, *Labiobarbus leptocheilus*, all low in abundance. The anabantids, *T. tricopterus* and *T. vittatus*, with their relative independence from ambient oxygen through their lung-like labyrinth organs were the most abundant species in this group. *C. striata* and *A. testudineus*, both provided respiratory assistance through suprabranchial organs were also in this group although not in high abundance. *A. testudineus* and *C. batrachus* tolerate relatively high concentrations of ammonia and are likely residents of these habitats despite their low numbers. Others may be transients. In all probability branchial respiration in the scaleless silurids, *Mystus mysticetus* and *Hemibagrus nemurus,* the balitorid, *Acanthocobitis zonalternans* and cobitid, *Acanthopsis* sp. is at least partially subsidized by cutaneous respiration. It is interesting that two of the three non-indigenous species in this study, *O. nilotica* and *Poecilia reticulate* are in this group, indicating their capacity to tolerate unfavorable conditions that has undoubtedly contributed to their success as an invader species.

The fourth group contained 12 species and approximately 7% of the fish. Several are benthic and associated with wider than average locations where the substratum is rocky including *Rhinogobius* sp., *Macrognathus circumcintus, M. siamensis,* and *Pseudomystus siamensis.* The four cyprinids, *Systomus orphoides, Poropuntius deauratus, Neolissochilus blanci* and *Hampala macrolepidota* were not abundant but presumably favor, this habitat for the plentiful benthic invertebrates on which they feed (Saha et al., 2002). Presumably the piscivorous *Xenenthodon cancilla*, is attracted by potential prey. The non indigenous *Oreochromis mossambicus* was found in low abundance and is regarded as a transient.

In summary species numbers were positively and negatively related with ambient oxygen and pH while abundance varied inversely with river discharge and ammonia and directly with depth, substratum, oxygen and alkalinity. Fish species were related with eight habitat factors into four groups. Most of the 62 species were associated with a group in which the habitat factors were of overall average values. The group with the fewest species was associated with habitats of high oxygen and low ammonia and alkalinity. Fish in the other two groups were similiar in species numbers, however, they differed in habitat quality. In one, ammonia and alkalinity were high and oxygen, low with sites located near areas of domestic, industrial and agricultural activities and displaying evidence of abundant organic detritus. In the other, sites displayed minimal evidence of anthropogenic activities with ambient oxygen high and ammonia, low. These characteristics provide a basis from which to assess small river habitats.

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