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Ecological condition, biodiversity and major environmental challenges in a tropical river network in the Bago District in South-central Myanmar: First insights to the unknown

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ABSTRACT

Freshwater ecosystems in the Indo-Burma biodiversity hotspot face immediate threats through habitat loss and species extinction. Systems to monitor ecological status and trends in biodiversity are therefore crucially needed. Myanmar is part of Indo-Burma but with no past experience of biomonitoring in freshwaters. In this study, we aimed to assess the ecological and biodiversity status of a lowland river network in south-central Myanmar by identifying and quantifying pressures using macroinvertebrates as bioindicators. Novel data on water quality (nutrients, sediments and metals), hydromorphology (Morphological Quality Index; MQI), habitat quality (Litter-Siltation Index; LSI), land use, and macroinvertebrates were collected from 25 river sites. The dominant pressures on rivers were urban land use, inputs of untreated sewage, in-stream and riparian garbage littering, run-off from agricultural fields and plantations, as well as physical habitat degradation. Water chemistry data indicated inputs of sediments and nutrients to degraded streams, but no obvious metal pollution. The LSI and MQI indices indicated high perturbation in agricultural and urban areas, respectively. Ecological status was assessed using a first version of a modified Average Score per Taxon index (ASPT), while biodiversity was assessed by family richness within the orders Ephemeroptera, Plecoptera, Trichoptera, Coleoptera and Odonata (EPTCO), which was tested against the pressure gradient by principal component regressions. ASPT had high diagnostic capabilities ($R^2 = 0.68$, $p < 0.001$) and showed that the index can be used to evaluate ecological water quality in this region. Biodiversity, expressed as family richness, also declined along the gradient ($R^2 = 0.59$, $p = 0.041$), giving support to the fact that current land-use practices in this area are unsustainable.

1. Introduction

Southeast Asia is known for an exceptionally high rate of biodiversity and endemism but is also one of the most biologically threatened regions worldwide (Brooks et al., 2002; Hughes, 2017; Myers et al., 2000). Freshwater aquatic communities remains relatively understudied in parts of the region (e.g. Allen et al., 2012) despite living in areas that are especially exposed to human degradation (Sala et al., 2000; Vorosmarty et al., 2010; Young et al., 2016). In this study, we explore threats to freshwaters and management options in a river network in Myanmar, a part of the Indo-Burma biodiversity hotspot (Myers et al., 2000) with no history of biomonitoring, and where the ecological and biodiversity status of surface waters is largely unknown.

Myanmar currently lacks systems for evaluating ecological status of its surface waters, though there have been attempts to adopt Integrated Water Resources Management (IWRM) for this purpose through a number of recent governmental initiatives, including the Myanmar National Water Policy (NWP) and the Myanmar National Water Framework Directive (NWRC, 2014), inspired by the EU Water Framework Directive (EU WFD; European Community, 2000). Ecological status evaluations, per the EU WFD, are primarily based on bioindicators (biological quality elements; BQE), such as fish, algae, macrophytes and

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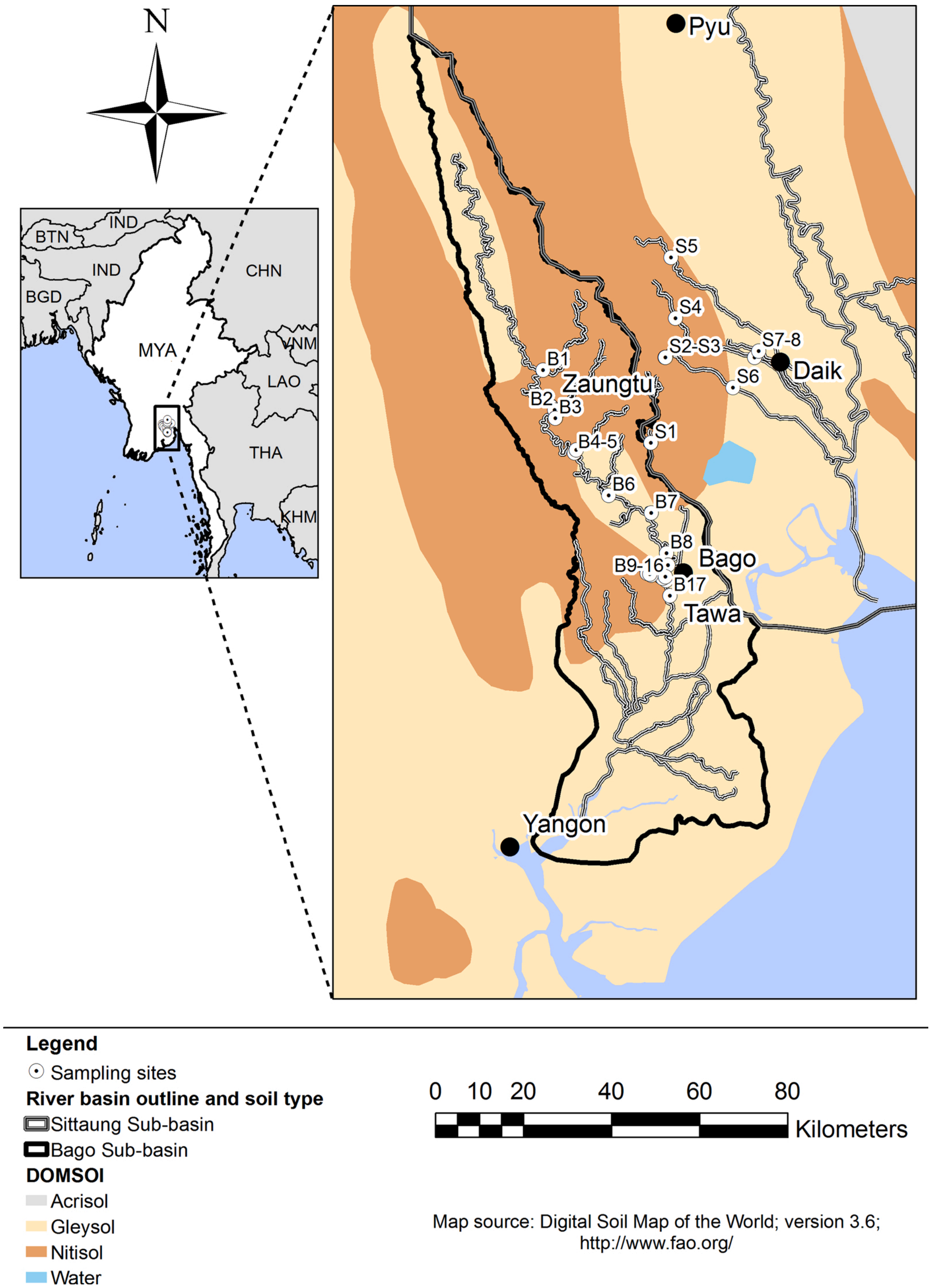

Fig. 1. Map showing the location of sampling sites and dominating soil types.

macroinvertebrates, which are supported by physical and chemical parameters. The use of these parameters is therefore desirable in the management strategies for Myanmar, but with little previous experience in biomonitoring, it is necessary to develop the appropriate tools. Recent attempts were therefore made to test the applicability of bioindicators in Myanmar, including riverine macroinvertebrates, lake algae and lake macrophytes (Ballot et al., 2018, 2020; Ko et al., 2020). Apart from some biomonitoring initiated by the Mekong River Commission in the lower Mekong River and its tributaries, including Cambodia, Lao PDR, Thailand and Vietnam, there is to our knowledge no biomonitoring programs for rivers in Indo-Burma.

Macroinvertebrates have a good track record as reliable bioindicators of anthropogenic degradation of surface waters worldwide (Cairns and Pratt, 1993; Wright et al., 2000; Morse et al., 2007; Damanik-Ambarita et al., 2016; Mangadze et al., 2019), and are used more frequently than any other groups of organisms for assessing the water quality of lotic systems (Carter et al., 2017). A widely applied system to evaluate ecological water quality by macroinvertebrates is the Biological Monitoring Working Party (BMWP). It was originally designed for use in the United Kingdom (Hawkes, 1998) but has later been applied worldwide, with and without modifications (e.g. Armitage et al., 1983; Dickens and Graham, 2002; Mustow, 2002; Rios-Touma et al., 2014). Its main advantage is that it can be introduced to new areas, even when there is limited taxonomic knowledge, and enable the detection of

Table 1
Variables collected in the various sampling campaigns, measuring device and analysis methods.

Variables	Sampling campaign	Measuring device	Analysis method
Biological			
Macroinvertebrates	1	Kicknet/handnet	Stereo microscope (Leica MZ205)
Hydromorphological			
Morphological quality index (MQI)	1		Visual assessment
Spatial			
Land use	1	ArcGIS	Remote sensing
Habitat			
Substrate-litter index	1		Visual assessment
Inorganic litter	1		Visual assessment
Physical and chemical (units)			
Water temperature (°C)	1 & 2	HQ40D multi meter[1]	
Specific conductivity (µS/cm)	1 & 2	HQ40D multi meter[1]	
pH	1 & 2	HQ40D multi meter[1]	
Dissolved oxygen (mg O2/L & % saturation)	1 & 2	HQ40D multi meter[1]	
Total nitrogen (µg TN/L)	1 & 2	Sequential flow analyzer[2]	NS 4743:1997
Total phosphorus (µg TP/L)	1 & 2	Sequential flow analyzer[2]	NS-EN ISO 6878
Orthophosphate (µg PO4-P/L)	1	Sequential flow analyzer[2]	NS-EN ISO 6878
Alkalinity (mmol/l)	1 & 2	Man-Tech Robot[3]	NS-EN ISO 9963−1
Ions (Na, K, Ca, Mg, Cl, SO4, NO3)	1 & 2	Ion chromatograph[4]	NS-EN ISO 14911:1999 & NS-EN ISO 10304−1:2009
Heavy metals (Cr, Fe, As, Cd, Zn, Ni and Pb)	1 & 2	ICP mass spectrometry[5]	NS-EN ISO 17294−1:2007
Total suspended solids (mg/L)	2		NS-4764:1980
Hg (total and methylated)	3	Atomic Fluorescence Detector[6]	Braaten et al. (2014)[8]
Average flow velocity (m/s)	1	Electromagnetic flow meter[7]	
Bacteriological			
Escherichia coli (*E. coli*)	4		9222 D and 9222 G (APHA et al. 2012)[9]

[1] HACH, Loveland, USA
[2] Skalar, Breda, Netherlands
[3] Man-Tech Co., Canada
[4] Dionex, ThermoFisher Scientific, USA
[5] PerkinElmer, New York, USA
[6] Brooks Rand Labs MERX, Seattle, USA
[7] OTT MF Pro, Loveland, USA
[8] Braaten, H. F. V., H. A. de Wit, E. Fjeld, S. Rognerud, E. Lydersen & T. Larssen, 2014. Environmental factors influencing mercury speciation in Subarctic and Boreal lakes. Sci Total Environ 476:336−345
[9] APHA, AWWA & WEF, 2012. Standard Methods for the examination of water and wastewater. 22 ed. edn. American Public Health Association, Washington, USA

perturbations of organic pollution and general degradation of the environment (Paisley et al., 2014; Turley et al., 2014). For Myanmar, like other parts of Southeast Asia, this is particularly relevant as multiple pressures including prominent sewage pollution are common (Hartmann et al., 2010; Li et al., 2010; Park et al., 2018). Biodiversity is a major determinant of ecosystem productivity, stability, and nutrient cycling (Tilman et al., 2014), where high diversity is generally favorable to ecosystem processes (Loreau et al., 2001). Species richness is a fundamental measure of community diversity (Gotelli and Colwell, 2001), and species or taxon richness are widely used to describe the integrity of river ecosystems, such as in the orders Ephemeroptera, Plecoptera, Trichoptera, Coleoptera and Odonata (Boonsoong et al., 2009; Raburu et al., 2009; Baptista et al., 2013; Huang et al., 2015). Such metrics could therefore be suitable tools for evaluation of the ecological and biodiversity status of rivers in Myanmar.

The overall aim of the present study was to investigate ecological and biodiversity status in response to environmental quality in parts of a lowland tropical river network in Myanmar using macroinvertebrates as a bioindicator. We took a range of physical and chemical measurements and sampled the macroinvertebrate communities at a large number of sites with some sampling repeated in time. We aimed to elucidate the main pressures in the river network to provide a baseline for coming monitoring efforts that can contribute towards a sustainable future for Myanmar. Our hypotheses were that 1) as there is no heavy industry in this area, land-use such as deforestation and point source pollution from urban areas would be the main contributors to environmental degradation, in part because both stressors may impact water oxygen levels from already low baselines, and consequently impact ecological and biodiversity status, and 2) methods and approaches used in the EU WFD, and elsewhere, to assess ecological status of rivers can be applied in Myanmar and are able to differentiate sites along degradation gradients.

2. Methods

2.1. Study area

The focal point of our study was the Bago District that covers approx. 3000 km^2, has a population around 2 million, and is representative in terms of land-use for a large proportion of lowland Myanmar. We studied a river network consisting of 25 lowland locations (< 75 m a.s.l.) in the lower part of the Sittaung river basin, which is comprised of the Sittaung and Bago Sub-basins located in the south central of Myanmar (Fig. 1; station coordinates are given in Supplementary material, Table 1). The climate in this region is tropical monsoon with distinct wet and dry seasons. The dry season lasts from December to April and the wet season from May to October. December and January are the coldest months with mean temperatures ranging from 24 °C to 31 °C (Haruyama and Hlaing, 2013). For the period 1990–2009 the average annual precipitation was 3185 mm and 2746 mm at Bago City and Zaungtu, respectively (Shrestha and Htut, 2016). According to the classifications by the world reference base for soil resources (WRB, 2015), the two dominant soil types in this area are eutric gleysols (faosoil GE37−2/3a; with clear signs of groundwater influence), and dystric nitisols (faosoil Nd55−2/3b; red soils with a clayey horizon).

There are several pressures acting on the river network in this area with large forested areas being converted to agricultural fields (rice, corn, peanut, sesame, chili, pigeon pea and vegetables), rubber plantations, roads and other infrastructure at an increasing rate (Eriksen et al., 2017). This leads to potentially increased erosion and diffuse pollution loads that will influence the aquatic environment negatively through excess sedimentation, high levels of nutrients and xenochemicals. Furthermore, sewage and other wastewater enter the river network in urbanized areas, with the major input of untreated sewage coming from the Bago City area. There is also extensive garbage littering in riverside locations as river corridors are used for disposal of garbage and only inadequate refuse systems are in place. Dams for hydroelectric power and diversion of water for irrigation, human consumption and industrial purposes are found in most parts of the river network. There is little heavy industry in this area but sand mining from the riverbed is frequent and there is also illegal gold mining.

Table 2
Variables used to quantify the degradation of stream sites, expected response and score categories.

Scale and variable	Expected response to degradation	Categories
Catchment		
1. Prop. area forested	–	0–1
2. Prop. area agriculture or plantations	+	0–1
3. Prop. area settlements/ urbanized	+	0–1
Reach		
4. Hydromorphology - MQI index	+	0–150
5. Prop. area villages/urban 500 m	+	0–1
Site		
6. Percent inorganic litter	+	0–100
7a. SLI - sedimentation/sludge layer/FPOM[a]	+	No (3), low (2), medium (1), high (0)
7b. SLI - organic litter (allochthonous material)[a]	–	No (0), low (1), medium (2), high (3)
8. Total nitrogen (μg TN/L)	+	
9. Total phosphorus (μg TP/L)	+	
10. Visible sewage input	+	Yes (1) or no

[a] The substrate litter index (SLI) was calculated as the total score of variables 7a and 7b, where a high score corresponds to high habitat quality.

2.2. Selection of sampling sites

Selection of sampling sites within the river network was as representative as possible and covered a gradient of degradation. We included a wide range of river sizes, sampling depths and water current velocities. The stream sediments were dominated by sand and silt. We used elements of the EU WFD criteria for the delineation of water bodies based on characteristics relating to the chemical, physico-geological and morphological attributes (Eriksen et al., 2017). There was almost no available assessment data for this purpose as there is very limited environmental monitoring in the Bago District. However, by applying geological soil type and land use maps, together with selected water chemical data measured as part of a previous Japanese funded project (JICA, 2014), a delineation of 59 water bodies in the lower Sittaung basin was possible; with 35 lying in the Bago Sub-basin (Eriksen et al., 2017) and 24 in the Sittaung Sub-basin (unpublished material). Sampling campaigns were subsequently initiated to document degradation in the river network. In a few cases there were more than one sampling location per water body (in five of the water bodies). Sampling of additional locations were done to estimate within water body variability. Sampling campaigns were subsequently initiated to document degradation in the river network. The relatively small Bago Sub-basin was our pilot focus area, and only here did we attempt to cover the whole catchment. Sites from Sittaung Sub-basin were located only in the lower part of the basin and belonged to the Bago District.

2.3. Sampling strategy

Novel data on water chemistry, hydromorphology, habitat quality, land use and macroinvertebrates were collected during the period 2016–2018 (Table 1). Four sampling campaigns were undertaken. Sampling campaign 1 focused on an integrated approach by investigating all the data types from 30 samples and 25 sites. The sampling took place in the dry season (February – March) when all streams were accessible, and only in areas without saltwater intrusion. Spot measurements of water chemistry, hydromorphology and habitat quality were taken at the same time as the collection of macroinvertebrates, whereas land use information was gathered from remote sensing data following the collection. Sampling campaign 2 focused entirely on water chemistry and served the purpose of continuous monitoring, by monthly to bimonthly collection in the study period, also covering areas with saltwater intrusion. Sampling campaign 3 was a screening for mercury, which we suspected could be a problem resulting from illegal gold mining. Sampling campaign 3 was conducted on one occasion in 2016 at 13 stream sites, covering most parts of the Bago Sub-basin (B1 to Yangon; Fig. 1). In campaign 4 water samples were taken from two sites for the enumeration of *Escherichia coli* (*E. coli*) to detect fecal pollution.

The degradation of stream sites was quantified at the scale of catchment, reach and site by applying ten degradation variables: 1) % of catchment forested, 2) % of catchment converted to agriculture or plantations, 3) % of catchment converted to settlements and urbanized areas, 4) hydromorphological degradation of stream reach (Morphological Quality Index; MQI-index), 5) % of area urbanized, or with settlements, measured within 500 m of sampling site, 6) % inorganic litter on the stream bed, 7) sedimentation/sludge layer and quantity of organic litter on the stream bed (Substrate Litter Index; SLI), 8) total nitrogen, 9) total phosphorus, and 10) visible sewage inputs at the site during collection (Table 2). Non-impacted sites (reference sites) were designated based on no or low impacts observed during surveys (substrate, riparian vegetation and flow modifications), as well as low likelihood of any human activity based on remote sensing (satellite images). The reference sites were distributed across both sub-basins and reflected various altitudes (34–72 m), river widths (1.5–68 m), water current velocities (0.01 – 0.53 m/s) and geological conditions (1.6–18.8 mg Ca/L). As we avoided the lower parts of the river networks (slow flowing and deep river sections; sometimes with saltwater intrusion), the degradation gradient was only to little extent confounded by a natural upstream to downstream gradient.

2.4. Water chemistry sampling

During sampling campaign 1, we measured dissolved oxygen (DO; spot measurement in daytime), pH, alkalinity, calcium (Ca), sodium (Na), potassium (K), magnesium (Mg), chloride (Cl), sulfate (SO4), total nitrogen (totN), nitrate (NO_3), total phosphorus (totP), orthophosphate (PO_4-P), copper (Cu), chromium (Cr), iron (Fe), arsenic (As), cadmium (Cd), zinc (Zn), nickel (Ni), and lead (Pb). During sampling campaign 2 suspended sediments, pH, alkalinity, Ca, conductivity, Cu, Zn, As, Cd, Zn, Ni, Pb, totN and totP were measured. Total mercury (totHg; unfiltered and filtered) and methylmercury (MeHg; unfiltered and filtered) were measured in sampling campaign 3. The water samples in campaigns 1 and 3 were analyzed in Norway because there was a lack of necessary infrastructure in Myanmar. Samples from campaign 2 were analyzed in Myanmar, although nutrients and metals were not always successfully analyzed (technical issues). Samples were conserved by adding acids immediately following collection. Water samples for heavy metals were filtered prior to analysis (0.45 μm mesh; Merck Millex-HA) in order to estimate their dissolved forms. The conservation of nutrient and metal water samples was done by applying 1% sulphuric acid (4 M H_2SO_4) and 1% nitric acid (7 M HNO_3), respectively. We did not have the facilities to conduct analysis of biochemical oxygen demand (BOD) in Myanmar, and for bacterial analysis, we experienced challenges related to traveling distances and storage of samples following collection. Therefore, although these two parameters may be useful to quantify organic pollution, they could not be used in any of the sampling campaigns on a routine basis.

For most metals, the thresholds for effects on aquatic life were evaluated based on criteria from the United States Environmental Protection Agency (U. S. EPA), although with some modifications for copper because of uncertainty in the criteria (U. S. EPA, 1986, 2007). Levels for acute and chronic effects were set respectively as: Pb (65 and 2.5 μg/L), Cu (dependent on physicochemical water properties and thresholds for aquatic life are variable; we set a tentative threshold of 2 μg/L to consider this substance harmful), Cr (dependent on physicochemical water properties, lowest threshold (soft water) at 16 and 11 μg/L), Cd (1.8 and 0.8 μg/L), Ni (470 and 52 μg/L), Zn (120 μg/L for both), As (340 and 150 μg/L), Fe (not given, 1000 μg/L), and totHg (1400 and 770 ng/L).

2.5. Assessment of in-stream habitat characteristics

Habitat data were collected by visual assessment in connection with macroinvertebrates sampling, typically covering an area of 50 - 200 m^2, depending on river size. This was done by the same person (T. E. Eriksen) throughout the study period to limit variability. Water current velocities (0.6x depth from the surface) were measured using a handheld electromagnetic flow meter from multiple locations within the study site area. Substrate grain sizes were categorized, following Wentworth (1922): silt and clay (< 0.063 mm diameter), sand (0.064−2 mm), small and medium pebbles (2.1−16 mm), coarse pebbles (16.1–64), cobbles (64.1−256 mm), and boulder (> 256 mm). Substrate size was logarithmically transformed to Krumbein scale, *phi* units (φ), and a score was calculated as the average of values based on the relative substrate composition. The following *phi* units were adopted for these calculations: silt and clay = 8.89; sand = 2.97, small and medium pebbles = -3.24; coarse pebbles = -5.24; coarse gravel = -7; cobble = -8.5; boulder = -10. Positive *phi* scores are therefore associated with fine sized substrate particles and negative numbers with coarser particles. The proportion of substrate covered by inorganic litter (garbage and other foreign objects) was estimated visually in shallow streams and by foot tactility and hand net in deep or turbid waters. Cover was estimated as percent of the wetted stream bed at the site. The substrate-litter index (SLI index) was estimated as substrate covered by allochthonous material (coarse particulate organic matter, CPOM) and the cover of silt/-sludge. The CPOM cover densities were assigned to levels nil, low, medium and high, corresponding to approximately 0 %, 1–20 %, > 20–40 % and > 40 % cover. The categories were based on the cover observed in reference conditions and scored 0–3, respectively. The siltation/sludge layer was scored likewise, based on 1) the existence of a sludge layer, and 2) the clogging frequency of a 250 μm kick net during macroinvertebrate sampling (< 1, 1, 2 or ≥ 3 subsamples for the net to fully clog; see macroinvertebrate sampling and identification). Hence, the SLI index had a maximum score of 6. Any pipes or ditches with obvious sewage inputs to the stream were scored 1 and no score was given otherwise.

2.6. Assessing hydromorphological features

Hydromorphological degradation was assessed using a modified version of the morphological quality index (MQI index; Rinaldi et al., 2013). We omitted the use of historical information on flow alterations and historical maps as this was not accessible to us. The hydromorphological features were visually assessed in the field along 200–1000 m stream reaches depending on channel width and the surveyor's ability to get an uninterrupted view of the river channel and its surroundings. All sites were assessed by the same surveyor (N. Friberg). Features surveyed included loss of continuity of sediment and wood, presence of an active floodplain, naturalness of bed sediments, number and types of artificial structures among others. A total number of 25 features were assessed resulting in a score ranging from 0 (pristine) to 150 (most degraded).

2.7. Identification of catchment land use

At the catchment scale, degradation of each sampling site was estimated based on the proportion of the catchment that was 1) forested, 2) cultivated (agriculture/plantations), and 3) converted to villages/urban development. Furthermore, the proportion of urban development/houses in an area 500 m upstream the sampling sites was estimated from orthophotos (Google Earth; Landsat/Copernicus). This was done by investigating a 500-m reach, at a 180 degrees angle from the sampling point, facing upstream. Assessments of land use were undertaken by remote sensing in ArcGIS (version 10.1) by the application of orthophotos and open source landcover maps (https://www.arcgis.com; Myanmar's Land Cover Change from 2002 to 2016; retrieved 20.2.2018). Geological composition of soils was determined from a 1: 5,000,000 soil map (http://www.fao.org/; Digital Soil Map of the World; version 3.6; retrieved 20.1.2018). A digital elevation model (DEM) was provided by the Directorate of Water Resources and Improvement of River Systems of Myanmar and used for the delineation of catchments.

2.8. Macroinvertebrate sampling and identification

Macroinvertebrates were sampled in the dry season by kick sampling, using a standardized, semi-quantitative approach commonly used in Europe for routine monitoring (Friberg et al., 2006). Nine subsamples each covering 1 m were collected from each site and later pooled to a single sample. The kicking movement was maintained for 20 s for each 1 m subsample. In total, each sample covered approximately 2.25 m^2 of the substratum and the sampling time was a total of 3 min covering all habitats with sampling time in each habitat reflecting their occurrence (Friberg et al., 2006). The sampling net dimensions were according to the CEN standard, 25 × 25 cm and mesh size 250 μm, which is recommended for surveys requiring complete taxa lists including rare taxa for conservation evaluations (NS-EN ISO 10870, 2012). All macroinvertebrate samples were collected and analyzed by the same person (T. E. Eriksen). The sample was immediately preserved by adding 99 % ethanol to the collected material. All the collected material was brought back to the laboratory. If the size of the specimen allowed, the specimens were identified to the level of family (most groups) or subclass/class (Oligochaeta and Polychaeta), using stereoscopic microscope (Leica M205C). The following taxonomic literature were applied: Dudgeon (1999) and Sangpradub and Boonsoong (2006).

2.9. Data treatment and index calculation

Macroinvertebrate data were evaluated by means of family richness within the orders Ephemeroptera, Plecoptera, Trichoptera, Odonata and Coleoptera (EPTCO) and the biotic index Average Score Per Taxon (ASPT; Armitage et al., 1983). ASPT is calculated as the average score of Biological Monitoring Working Party (BMWP) indicators in a sample and is based on presence/absence at the family levels, except for Oligochaeta (order level). In this system, the bivalve family Sphaeriidae is assigned with the score 3 whereas the related family Corbiculidae has no score. Because minute specimen of these two families were difficult to distinguish by morphology, Sphaeriidae/Corbiculidae were combined and assigned the score 3 in this study for the purpose of ASPT. This is comparable to Mustow (2002) who also scored Corbiculidae by 3 in his study of Thailand rivers. Richness of EPTCO taxa are much used for biomonitoring purposes of rivers worldwide because they are common to most surface waters and show an overall strong response to changes in the environment (Boonsoong et al., 2009; Raburu et al., 2009; Baptista et al., 2013; Huang et al., 2015).

Statistical analyses were performed in R (R Core Team, 2017), version 3.4.0. A principal component analysis (PCA) was performed on relevant catchment, physical and chemical variables, using the built-in function *prcomp*. The data were centered and scaled for the analysis. A principal component regression (PCR) was subsequently applied to test the biological response of ASPT and EPTCO to the principal components.

3. Results

3.1. Geological differences in water chemistry

Local geology (shown in Fig. 1) was found to have a clear effect on

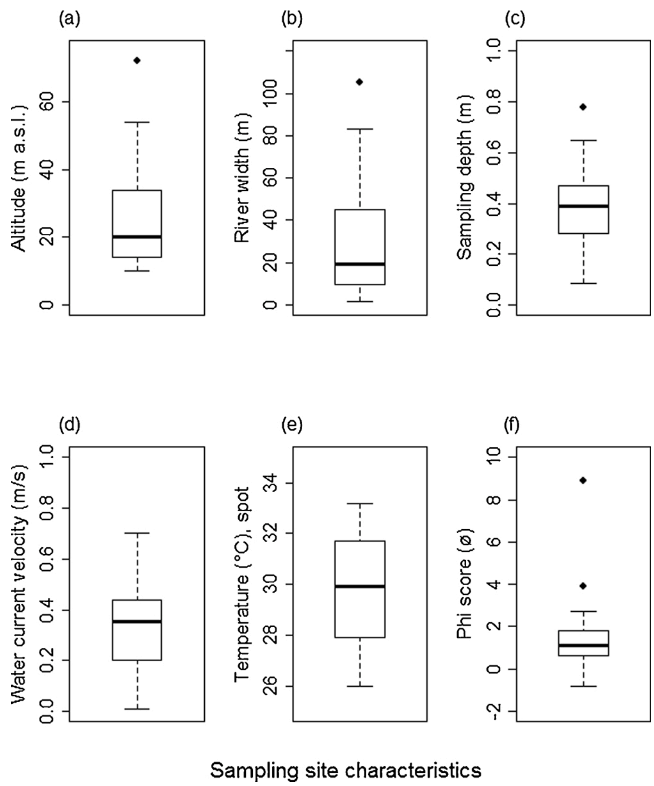

Fig. 2. Characteristics of the sampling sites for Sampling campaign I. The distribution of data for are shown for (a) altitude, (b) wetted river width, (c) sampling depth, (d) water current velocity (measured at 0.6 x depth from the surface), (e) water temperature (spot measurement at the time of sampling), and (f) substrate grain size in *phi* units (φ). Within each boxplot, the thick, horizontal lines represent the median, the top and bottom of the boxes represent the 75th and 25th percentiles, the dashed error bars extend to the most extreme data point which is no more than 1.5 times the interquartile range from the box, and separate points represent outliers.

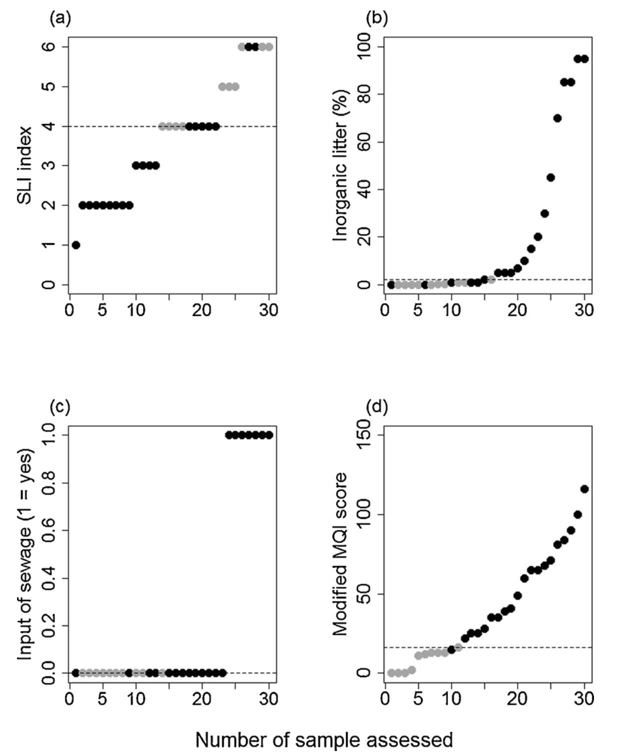

Fig. 3. The distribution of data for a) the siltation and organic litter index, b) inorganic litter index, c) visible sewage inputs to streams during collection, and d) MQI (modified) scores assessing hydromorphological degradation. The MQI scores range from 0 (pristine) to 150 (extremely impacted) based on European criteria. References sites are denoted by grey dots and the horizontal lines are drawn at the lowest quality measurements from reference stations. Data in each plot are ordered by ascending numbers.

water chemistry with water samples from gleysol soils having lower levels of calcium (< 4 mg/L) and alkalinity (< 1 mmol/L) compared to nitisol soils (calcium > 8 mg/L and alkalinity > 1.5 mmol/L; Supplementary material Fig. 1). Sites with transitional soil type (gleysol/nitisol) had intermediate levels for these parameters (calcium 4–8 mg/L and alkalinity 1–1.5 mmol/L). Conductivity and pH also followed this trend with lower levels in areas dominated by gleysol soils. Levels of 20−40 μg/L totP were measured from reference and low impact sites located in the nitisol soils without any known settlements or perturbations, whereas < 20 μg/L totP were recorded from equivalent gleysol sites (Supplementary material Fig. 2), hence indicating that the nitisol soils (clayish) have higher background levels of totP. Total nitrogen was generally in the range 200−400 μg N/L and no clear difference between soil types was observed in our data.

3.2. Water chemistry along the degradation gradient

The amounts of suspended sediments and water turbidity were highest in areas with intensified land-use and increased notably in the Bago River from Bago City (Supplementary material Fig. 3). Levels of totP and totN were highest in areas having a high proportion of agriculture and plantations in the catchment. In gleysol stream sites receiving sewage inputs, levels of 20−40 μg totP /L were measured, showing that totP levels were above expected background levels for gleysol soils, although not for nitisols. This difference was further supported by the detection of *E. coli* in the gleysol site, but not in the nitisol site despite having similar totP levels. Oxygen concentrations measured during daytime were in the range 11.3 mg O_2/L (155 %) - 6.1 mg O_2/L (77 % saturation). Based on filtered samples, the detected levels of heavy metals were below the following concentrations: Pb 0.5 μg/L; Cu 1.3 μg/L; Cr 1.3 μg/L; Cd 0.03 μg/L; Ni 2.5 μg/L; Zn 14 μg/L; As 1.7 μg/L, Fe < 1000 μg/L, totHg < 2.8 ng/L; MeHg 0.11 ng/L (Supplementary material Figs. 4,5). Hence, based on the water quality criteria by the U. S. EPA, and data from reference localities, there seem to be limited heavy metal pollution in the study area (Fig. 2).

3.3. Catchment land-use, habitat quality and hydromorphological conditions

The upper parts of the network were generally undisturbed by human activities with degradation increasing downstream. The general trend downwards through the network was for forested areas to be converted into cultivated areas (agriculture and plantations), human settlements and urban development (Supplementary material Fig. 6). Habitat quality at the site scale showed that some sites were degraded by higher siltation/sludge layer and less natural organic litter located on the substratum (SLI index; Fig. 3a). At some degraded sites, a layer of fine particles covered coarser particles and skewed the *phi* score in the direction of finer sediments. Moreover, many sites had high levels of in-stream and riparian garbage littering and inputs of untreated sewage (Fig. 3b-c). The water temperature was higher at sites where the riparian vegetation had been completely removed, and sites near reservoirs had lower water temperature because of inputs from the deeper parts of reservoirs during releases in the dry season. Hydromorphological conditions ranged from pristine rivers segments, with no evidence of modifications, to severely degraded reaches (Fig. 3d). However, most sites assessed showed relatively little degradation, especially in the upper parts of the river network. The main impacts on the

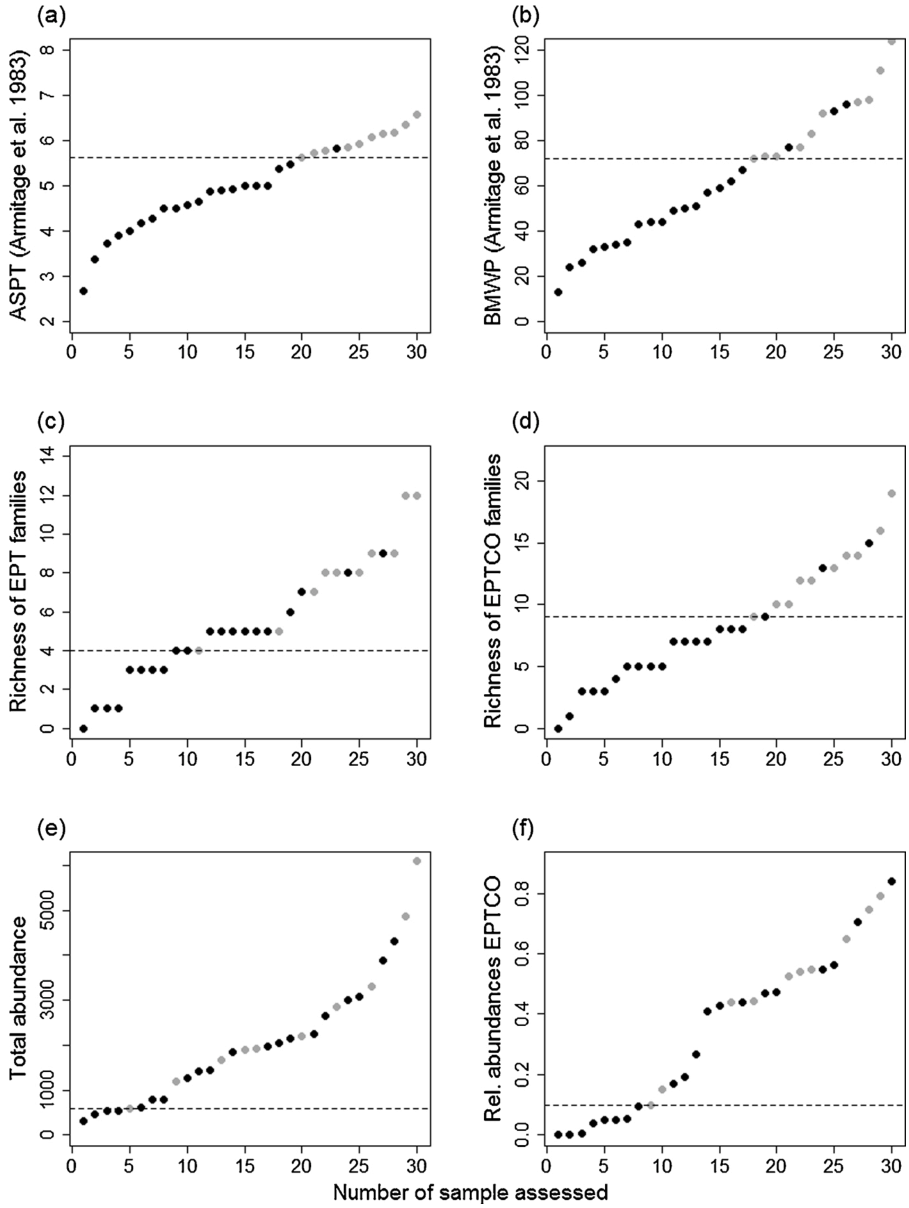

Fig. 4. The recorded metric values for from Sampling campaign I. References sites are denoted by grey dots and the horizontal lines are drawn at the lowest measurements from reference stations.

hydromorphology were the presence of dams upstream, embankments and extensive loss of riparian vegetation. In and around Bago City, most river reaches have been physically modified and the most impacted sites were found here.

3.4. Macroinvertebrate communities

We recorded a total of 60 families, mostly within the orders Ephemeroptera, Trichoptera, Coleoptera, Odonata, Diptera and Gastropoda (Appendix). Only one plecopteran taxon was recorded. The ASPT index ranged from 2.2 to 6.6, the BMWP 13–124, the family richness of EPT, i.e., Ephemeroptera (E), Plecoptera (P) and Trichoptera (T) 0–12, and the composite family richness of EPTCO, i.e. EPT plus Coleoptera (C) and Odonata (O), 0−19. The non-degraded sites (references sites) had generally higher values for these metrics compared to non-reference sites (Fig. 4 a–d). Total abundance (range 257–6038) and the relative abundance of EPTCO (0 – 0.84) did not reveal any clear pattern between sites (Fig. 4 e–f).

The dominating taxa in degraded rivers were Oligochaeta, Polychaeta, the gastropod families Thiaridae, Physidae, Planorbidae, Viviparidae and Pachychilidae, and one or more representative of the hirudinean families Salifidae, Erpobdellidae and Hirudinidae. A few ETCO were normally also present in degraded rivers, typically represented by Baetidae (E), Caenidae (E), and Libellulidae (O). Many EPTCO were normally present in reference rivers, typically represented by Leptophlebiidae (E), Heptageniidae (E), Ephemerellidae (E), Polymitarcyidae (E), Perlidae (P), Dipseudopsidae (T), Helicopsychidae (T), Polycentropodidae (T), Psychomyiidae (T), Hydroptilidae (T), Leptoceridae (T), Pshephenidae (C), Elmidae (C), Hydrophilidae (C), and Gomphidae (O).

3.5. Macroinvertebrate response to degradation

Our integrated approach to assess the cumulative degradation was supported by the PCR analysis undertaken on the ASPT and EPTCO indices and the environmental variables collected. Variance explained along PCA axis 1 represented degradation going from pristine forest sites with natural leaf litter in the upper part of river network to river sites

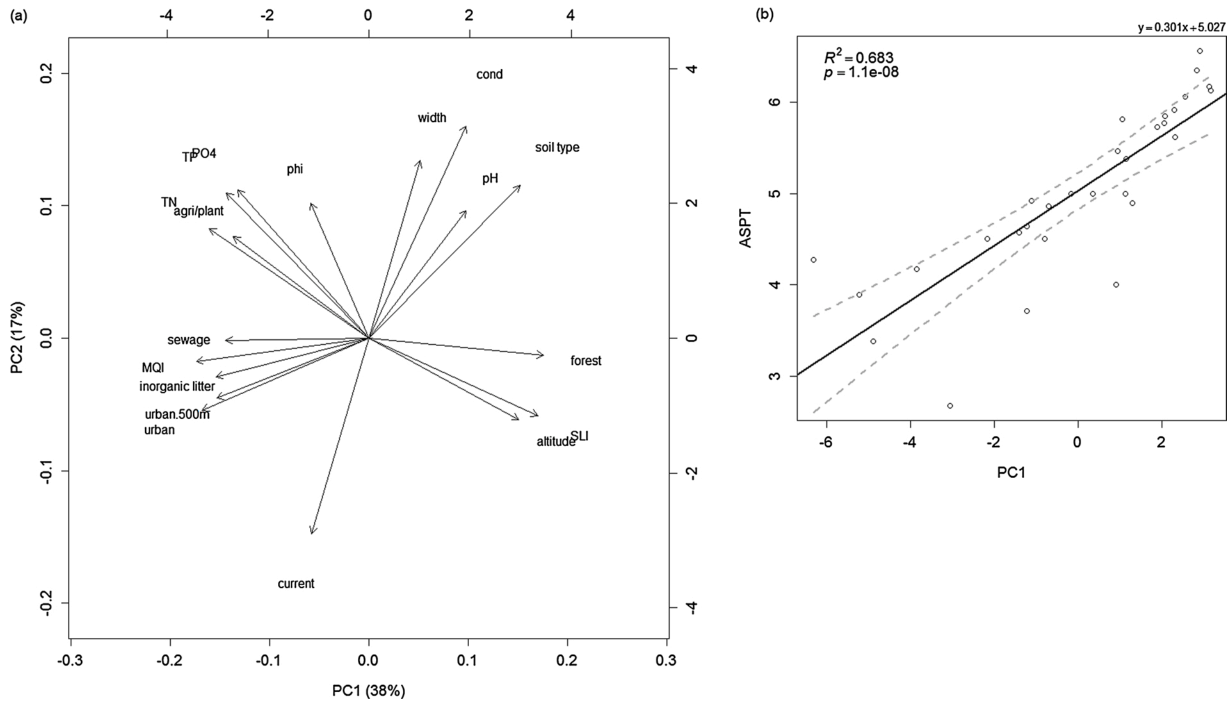

Fig. 5. a) Ordination (PCA) for selected physico-chemical parameters in the variation space formed by the two principal components (PC1 and PC2), and b) principal component regression (PCR) showing the ASPT index's response to PC1. 95 % confidence limits are shown.

heavily influenced by pollution and hydromorphological degradation related to urban and agricultural land-use (Fig. 5a). Variance explained along PCA axis 2 was primarily related to natural characteristics of soil type and river size. The ASPT index was significantly related to principal component axis 1 ($R^2 = 0.68$, $p < 0.001$), hence showing a high diagnostic capability to this group of stressors (Fig. 5b). Biodiversity expressed by EPTCO likewise declined in response to the pressure gradient ($R^2 = 0.59$, $p = 0.041$).

4. Discussion

We found that applying a river basin management approach to this tropical river network, based on the principles of the EU WFD on setting up a monitoring network, was an effective way to identify pressures and assess environmental status. The intensive land-use practices in the Bago District, dominated by land-use conversion and point source run-offs from urban areas, resulted in homogenizations of riverine macroinvertebrate communities (hypothesis 1), and the applied foreign evaluation indices (ASPT and EPTCO) differentiated sites accordingly (hypothesis 2).

4.1. Degradation of river water chemistry

As shown by our study, nutrients may indicate water quality deterioration in areas where forest has been converted into agriculture fields and plantations (Fig. 5a). However, for the purpose of supporting evaluations by bioindicators, like in the EU WFD, considerable work remains to identify target levels and class boundaries for nutrient pollution because background levels may be dependent on geological characteristics (Lintern et al., 2018) and bioavailability (Withers and Jarvie, 2008). More data on nutrient sources and background levels are therefore needed, and these criteria should eventually be related to biological communities and responses to estimate harmful thresholds (Dodds and Welch, 2000; Dodds, 2006; Poikane et al., 2019).

Sewage inputs to rivers are rapidly consumed by bacteria and other organisms which reduce the levels of oxygen in the water and sediments which are crucial for the survival of macroinvertebrates (Hynes, 1960). Although our analysis indicates that sewage effluents are a major pressure in our study area, we were not able to quantify it for several reasons. Several sites with known sewage inputs had more than 85 % oxygen saturation in daytime (sampling campaign 1), hence indicating non-lethal conditions. During night oxygen levels could become critically low because of non-compensated respiration rates (Mulholland et al., 2005), and 30–40 % lower night-time concentrations were recorded from some sites (T. E. Eriksen, personal observation). Biochemical oxygen demand (BOD) measures the actual oxygen consumption of aquatic microorganisms (Friberg et al., 2010) and is for this reason often used in monitoring to organic pollution (Cerqueira et al., 2008; Cunha et al., 2011; EEA, 2015). However, it was not feasible to conduct a full surveillance of BOD, as well as for bacteria, from our study sites owing to a combination of long travelling distances, poor road infrastructure, the warm climate and lack of appropriate laboratory facilities. Elevated levels of nutrients found at some river sites are also likely to come from agricultural land-use. These may have less direct effect on macroinvertebrate communities as their bioavailability is generally lower than nutrients entering from sewage inputs (Friberg et al., 2010; Withers and Jarvie, 2008).

4.2. Physical degradation of catchment and habitats

The morphological quality index (MQI) indicated that about 1/3 of the river reaches were hydromorphological degraded. Although the results make sense, they should be interpreted with caution as the method was developed for temperate rivers in Europe (Rinaldi et al., 2013). At the catchment scale, it is predicted that a conversion of forests into cultivated and urban areas will alter sediment dynamics and nutrient fluxes to rivers (Owens et al., 2005). Therefore, at the habitat scale, the relatively low amounts of CPOM and high siltation (SLI index) observed in degraded river sites was probably a result of lower leaf inputs resulting from degraded catchments and riparian zones (Studinski et al., 2012), faster decomposition rates in warmer and more eutrophic waters (Kominoski et al., 2015), and possibly also physical weathering and burial by sediment and garbage inputs (Yule et al., 2015). At sites subject to heavy garbage littering, we observed that plastic bags covered a large proportion of the stream bed and in effect clogging the underlying sediments. Kick sampling in these areas revealed that gas had accumulated underneath, possibly methane, hydrogen sulfide, carbon dioxide and/or ammonia following decomposition of organic matter (see Hynes, 1960), indicating a retarded interaction between oxygenated river water and the sediments. For this reason, we believe that plastic pollution may increase the threshold for oxygen amelioration on river fauna following organic pollution.

4.3. Ecological and biodiversity response to degradation

The ASPT index demonstrated high diagnostic capabilities in relation to the pressure gradient and showed that the index can be used to evaluate ecological water quality in this region. The ASPT was designed for use in the United Kingdom (Hawkes, 1998), but has been applied worldwide with and without modifications (Armitage et al., 1983; Dickens and Graham, 2002; Mustow, 2002; Rios-Touma et al., 2014), and has also proven useful for evaluating water quality in tropical rivers in South America and Africa impacted by organic pollution, acting alone or in combination with other stressors (Jacobsen, 1998; Soldner et al., 2004; Wronski et al., 2015). Based on our samples collected only in the dry season, there is a good probability that rivers sites are degraded when the ASPT value is below 5, and values lower than 4 are undoubtedly indicative of strong perturbation. However, given its origin, the index naturally lacks sensitivity scores for several taxa that are common in tropical regions and which may fill similar niches to European indicator taxa. For this reason, several modifications to the BWMP score exist in other Asian countries, such as Thailand, the Hindu-Kush region and Vietnam for the purpose of river biomonitoring (Mustow, 2002; Ofenbock et al., 2010; Forio et al., 2017), although the modifications are sometimes minor compared to the original system. The Asia Foundation proposed a simplified BMWP/ASPT for river biomonitoring in Lao PDR, that require limited taxonomical expertise to operate (TAF, 2017). A modification to this system for Myanmar rivers by adding more sensitivity scores (Ko et al., 2020) looks promising. Furthermore, the Mekong River Commission conducts some biomonitoring in the Mekong River and its tributaries by using a locally modified ASPT index (MRC, 2009). Modifications by adding sensitivity scores for local taxa could be considered in Myanmar. However, because different sensitivity scores have been assigned to the same taxa in many of these modified systems, we believe more data should be gathered from Myanmar before making such modifications.

The decline in EPTCO family richness along the pressure gradient shows that the current practices of intense land-use may be critical also for the biodiversity in this area. Using a family-level richness proxy is likely not optimal because the radiation within families can be high in species rich areas (Guerold, 2000; Bailey et al., 2001; Marshall et al., 2006). However, it may also ameliorate inherent variation in richness metrics (Vinson and Hawkins, 1998), as it is more probable to encounter taxa belonging to higher taxonomical levels (e.g. order and family) compared to lower levels (genus/species) by using a rapid biomonitoring approach. Even so, until more reference data is acquired from various regions and geological conditions, that cover spatial and temporal variation in community composition, target levels for assessments (expected reference values) must be used with caution. We recommend more studies to address the inherent variation to richness and abundance/dominance-based metrics in this area given their global popularity for biomonitoring.

Species and genus level identifications of many macroinvertebrates is not possible in tropical Asia because several groups are understudied, so that new genera and species are continuously being discovered (Kaltenbach and Gattolliat, 2019; Mey and Freitag, 2019; Kaltenbach et al., 2020). Consequently available identification literature is largely incomplete and deficient (Dudgeon, 1999; Boonsoong and Braasch, 2013). Because Myanmar has practically no experience in river biomonitoring using macroinvertebrates, local taxonomical expertise is still limited. The family level indices, ASPT and EPTCO therefore represent two operative metrics to evaluate ecological and biodiversity status of rivers in Myanmar that could be established without too much effort. Ongoing species loss is a major concern in this region (CEPF, 2012). Thus, as more data is collected, we recommend that future studies address biodiversity status in rivers using lower taxonomic levels, such as using molecular methods, because the true diversity is probably very high (Kaltenbach and Gattolliat, 2019), although poorly documented. Furthermore more metrics should be tested and locally adapted to Myanmar to create more robust evaluation systems, such as multimetric systems (Karr and Chu, 1999), as has been the practice for river monitoring in the EU and elsewhere (Hering et al., 2006; Baptista et al., 2013; Shi et al., 2017).

4.4. Perspectives in a biodiversity context

Myanmar, as part of the Indo-Burma biodiversity hotspot, holds a very high overall species diversity (Myers et al., 2000; Brooks et al., 2002) and has for long been noted for its exceptionally high diversity of freshwater organisms (Allen et al., 2012). Degradation of undisturbed nature is taking place at an alarming rate, and estimates for Indo-Burma showed that only 5 percent of pristine habitats remained in 2011 (CEPF, 2012), and by the year 2050, most of the remaining intact vegetation may be lost due to overexploitation and climate change (Habell et al., 2019). By the year 2012, 13 percent of all assessed freshwater species in Indo-Burma were already threatened by extinction, with numbers predicted to increase dramatically. Over the period 2010–2015 Myanmar lost 1.8 % of its forest every year (FAO, 2015). Our study confirms that the conversion of forest to intensive agriculture is now a major threat to the integrity of rivers in Myanmar; a fact recognized by the Global Assessment of Biodiversity and Ecosystem Services (IPBES, 2019). To our knowledge, it is quite unique to find this many tropical lowland rivers (< 75 m a.s.l.) with such a low degree of human degradation. However, without any protective legislation they will soon be gone. An immediate focus should therefore be put on protecting the remaining pristine river ecosystems that are now facing increasing pressures to safeguard aquatic and riparian biodiversity in these extremely species rich ecosystems.

5. Conclusions

Although our results are promising, much work remains to implement a nation-wide system in Myanmar with legally binding actions for ecological water quality. Substantial local resources are required to be able to characterize, monitor, evaluate, and eventually improve the status of water bodies, and such capacity takes time to build. Not only is there a need for more instruments and laboratory facilities, but experts on taxonomy and water chemistry are also urgently needed, in addition to evaluation criteria required to operate such a biomonitoring system in practice. Despite the many challenges, we highly recommend that this work continues. We recommend coming studies to test similar approaches also outside the Bago District, and moreover, that sensitivity scores of the local fauna are considered when more data are available. The primary focus onwards in Myanmar should be to establish status class boundaries for biological and supportive elements. As per the EU WFD, the “high/good” and “good/moderate” class boundaries represent the two most important thresholds as 1) at least good status must be obtained, and 2) water bodies having “high status” must remain in this class. As such classifications are entirely based on observed deviations from natural states, more data from reference conditions are needed to cover different regions, geologies and river typologies to account for natural variation that such elements are often subject to. However, as shown in this study and elsewhere, the ASPT may serve the purpose of separating sites subject to high and no/low perturbations, despite some underlying natural variation, and may therefore serve the purpose of getting started in the use of bioindicators in Myanmar.

Table A1
Taxon list showing the number of registrations based on 30 samples from 25 stream sites in the Bago and Sittaung Sub-basins. Identification level was to family if possible (except for Oligochaeta and Polychaeta). Applied BMWP scores are shown following Armitage et al. (1983) apart from the split taxon Corbiculidae/Sphaeriidae = 3.

Taxon group	Family	Number of samples where taxon was present	BMWP score
Bivalvia	Bivalvia indet.	7	
Bivalvia	Corbiculidae/ Sphaeriidae	15	3
Bivalvia	Unionidae	4	6
Coleoptera	Coleoptera indet.	11	
Coleoptera	Dytiscidae	8	5
Coleoptera	Elmidae	13	5
Coleoptera	Gyrinidae	1	5
Coleoptera	Hydraenidae	1	
Coleoptera	Hydrophilidae	5	5
Coleoptera	Psephenidae	3	
Coleoptera	Scirtidae	3	
Crustacea	Isopoda indet.	1	
Crustacea	Gecarcinucidae	1	
Crustacea	Palaemonidae	17	
Decapoda	Sesarmidae	1	
Diptera	Diptera indet.	11	
Diptera	Ceratopogonidae	24	
Diptera	Chaoboridae	2	
Diptera	Chironomidae	30	2
Diptera	Culicidae	5	
Diptera	Empididae	1	
Diptera	Limoniidae	1	
Diptera	Simuliidae	15	5
Diptera	Tipulidae	1	5
Ephemeroptera	Baetidae	28	4
Ephemeroptera	Caenidae	26	7
Ephemeroptera	Ephemerellidae	2	10
Ephemeroptera	Ephemeridae	12	10
Ephemeroptera	Heptageniidae	5	10
Ephemeroptera	Leptophlebiidae	6	10
Ephemeroptera	Polymitarcyidae	9	
Ephemeroptera	Prosopistomatidae	1	
Ephemeroptera	Teloganodidae	1	
Gastropoda	Gastropoda indet.	3	
Gastropoda	Ancylidae	10	6
Gastropoda	Hydrobiidae	1	3
Gastropoda	Pachychilidae	10	
Gastropoda	Physidae	4	3
Gastropoda	Planorbidae	4	3
Gastropoda	Pleuroceridae	1	
Gastropoda	Thiaridae	16	3
Gastropoda	Viviparidae	5	6
Hemiptera	Hemiptera indet.	2	
Heteroptera	Corixidae	14	5
Heteroptera	Gerridae	4	5
Heteroptera	Nepidae	2	5
Heteroptera	Notonectidae	1	5
Hirudinea	Erpobdellidae	1	3
Hirudinea	Hirudinidae	3	
Hirudinea	Piscicolidae	1	4
Hirudinea	Salifidae	2	
Hydrachnidia	Hydrachnidae	14	
Nematoda	Nematoda	2	
Odonata	Anisoptera indet.	6	
Odonata	Zygoptera indet.	1	
Odonata	Chlorocyphidae	11	
Odonata	Coenagrionidae	8	
Odonata	Gomphidae	16	8
Odonata	Libellulidae	8	8
Oligochaeta	Oligochaeta	30	1
Planipennia	Sisyridae	2	
Platyzoa		2	
Plecoptera	Perlodidae	1	10
Polychaeta	Polychaeta	3	
Trichoptera	Trichoptera indet.	4	
Trichoptera	Dipseudopsidae	1	
Trichoptera	Ecnomidae	13	6
Trichoptera	Helicopsychidae	1	
Trichoptera	Hydropsychidae	17	5
Trichoptera	Hydroptilidae	17	6
Trichoptera	Leptoceridae	19	10
Trichoptera	Philopotamidae	3	8
Trichoptera	Polycentropodidae	2	7
Trichoptera	Psychomyiidae	1	8

CRediT authorship contribution statement

Tor Erik Eriksen: Conceptualization, Methodology, Visualization, Formal analysis, Investigation, Writing - original draft, Writing - review & editing. **Nikolai Friberg:** Conceptualization, Methodology, Formal analysis, Investigation, Writing - original draft, Writing - review & editing. **John E. Brittain:** Conceptualization, Writing - original draft, Writing - review & editing. **Geir Søli:** Conceptualization, Writing - original draft, Writing - review & editing. **Andreas Ballot:** Conceptualization, Methodology, Investigation, Writing - review & editing. **Eirin Årstein-Eriksen:** Investigation, Writing - review & editing, Visualization. **Tomas Adler Blakseth:** Methodology, Writing - review & editing. **Hans Fredrik Veiteberg Braaten:** Investigation, Writing - review & editing.

Declaration of Competing Interest

The authors report no declarations of interest.

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Appendix A

Table A1

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