

Spatial and temporal variation of benthic macroinvertebrates in the Nam Gnom Basin receiving discharged waters from the Nam Theun 2 Reservoir (Lao PDR)

Variabilité spatio-temporelle des macro-invertébrés benthiques du bassin de la Nam Gnom recevant les eaux du Réservoir Nam Theun 2 (RDP du Laos)

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Abstract – In order to assess the impact of water release in the downstream area of the Nam Theun 2 Reservoir (Lao PDR), the spatial and temporal variation of benthic macroinvertebrates was explored. Between 2010 and 2012, five sites were monitored in the Nam Gnom Basin, a tributary of the Xe Bangfai River, receiving the turbinated waters. Repeated-time collections revealed the presence of 109 taxa mainly identified to the family level, attesting to a rich biota. Preliminary results did not show shifts in diversity, population composition, and feeding metrics. Redundancy Analysis indicated that the altitudinal gradient (*i.e.* altitude and altitude-related variables) remained the major environmental factor influencing the macroinvertebrates distribution before water discharge. The presence of a tributary downstream of the release point, and the respect of the natural river inflow can explain the preservation of natural conditions. More samples and a strengthened ecological knowledge of Southeast Asian macroinvertebrates are still required to confirm these preliminary results.

Key words – freshwater invertebrates, Southeast Asia, biomonitoring, hydropower, downstream section

Résumé – Afin d'évaluer l'impact du relâcher des eaux dans la zone aval du Réservoir Nam Theun 2 (RDP du Laos), la variabilité spatio-temporelle des invertébrés aquatiques a été étudiée. Entre 2010 et 2012, cinq sites ont été échantillonnés sur le bassin de la Nam Gnom, un affluent de la rivière Xe Bangfai, qui reçoit les eaux turbinées. Les prélèvements issus du suivi ont permis de mettre en évidence la présence de 109 taxa principalement

identifiés au niveau de la famille, révélant une richesse importante. Les résultats préliminaires n'ont pas montré de changement majeur des métriques de diversité, de composition des populations et des régimes trophiques. L'analyse canonique de redondance indique que le gradient altitudinal (altitude et variables associées) demeure le facteur environnemental prépondérant affectant la distribution des macro-invertébrés devant les eaux turbidées. La présence d'un affluent en aval du point de relâcher ainsi que le respect du débit naturel de la rivière peuvent expliquer la préservation des conditions naturelles. Des échantillons supplémentaires ainsi qu'une meilleure connaissance de l'écologie des macro-invertébrés de cette région restent nécessaires pour confirmer ces résultats préliminaires.

Mots-clés – invertébrés aquatiques, Asie du Sud-Est, biomonitoring, projet hydroélectrique, zone aval

1 INTRODUCTION

In regulated systems, upstream and downstream initial characteristics of rivers are modified *e.g.* thermal and hydrologic regime, sediment transport, channel morphology, and water quality (Ward & Stanford, 1979; Dynesius & Nilsson, 1994; Petts, 1984; Nilsson *et al.*, 2005). These alterations cause changes in assemblage structure of aquatic organisms and bioassessment methods can be used to assess environmental consequences of dams. Macroinvertebrates are widely used for that purpose (Armitage, 1984; Boon, 1988; Moog, 1993; Cortes *et al.*, 1998; Ogbeibu & Oribhabor, 2002). Their ubiquitous occurrence, high species richness, and limited migration patterns provide a large spectrum of responses to environmental changes including short to long-term cumulative effects (Resh *et al.*, 1995).

In Southeast Asia, the Mekong River basin is recognized for its high hydropower potential (Lauri *et al.*, 2012; Ziv *et al.*, 2012). An estimation of 200 hydropower projects are located in the "Greater Mekong Sub-region", which includes Thailand, Cambodia,

Myanmar, Vietnam, Yunan Province (China) and Lao PDR (Souksavath & Nakayama, 2013). In 2009, the Mekong River Commission (MRC) estimated that the majority of the projects in the Lower Mekong Basin were located in Lao PDR (MRC, 2010). Interest in using macroinvertebrates to assess environmental changes is growing in the region such as in Vietnam (Hoang & Bae, 2006; Jung *et al.*, 2008; Hoang *et al.*, 2010) and in Thailand (Mustow, 2002; Getwongsa & Sangpradub, 2008; Thani & Phalaraksh, 2008; Boonsoong *et al.*, 2008). However, in Lao PDR, knowledge and use of benthic macroinvertebrates for bioassessment studies remain scarce (Davidson *et al.*, 2006; Pathoumthong & Vongsombath, 2007).

In the present article, spatial and temporal variability of macroinvertebrates in the downstream section of the Nam Theun 2 (NT2) hydroelectric project (Lao PDR) was investigated. Between 2010 and 2012, five sites were monitored in the Nam Gnom Basin receiving discharged waters from the NT2 Reservoir (Nam Theun Basin). It has been hypothesized that water discharge would lead to taxonomical and functional changes (Jalon *et al.*, 1994;

Vinson, 2001). More specifically, we hypothesised (i) a reduction of the relative abundance of molluscs as discharged waters had lower conductivity (Chanudet *et al.*, same issue) and diversity and richness of molluscs are known to be closely associated with water conductivity (Dillon, 2000; Horsák, 2006) and (ii) an increase of the relative abundance of collectors-filterers which usually dominate assemblages in downstream area of dams (Schlosser, 1992; Malmqvist & Eriksson, 1995).

To test these hypotheses, the total macroinvertebrate diversity of the studied area was estimated and the macroinvertebrate assemblages were compared between reference's sites (sites outside of influence of discharged waters) and impacted sites (sites under influence of discharged waters) through a taxonomical and a functional approach. Finally, spatial and temporal distribution of macroinvertebrates was explored through an ordination method.

2 MATERIAL AND METHODS

2.1 Study area

The NT2 hydropower area is located in the Khammouane Province in central Lao PDR (Fig. 1). The design of the project is characterized by a water diversion from the Nam Theun Basin (sand-stone dominant watershed) to the Xe Bangfai Basin southward (limestone dominant watershed). The Nam Kathang River is a tributary of the Nam Gnom River reaching the Xe Bangfai River few kilometres downstream. The

Nam Kathang River starts after the confluence of two streams: Nam Kathang Noy and Nam Kathang Gnai, flowing into the Regulating Pond, constructed to buffer turbinated waters coming from the Power House. The inflow from the streams in the Regulating Pond is negligible comparing to the one from the Power House (3% vs. 97% on an annual basis). Downstream the Regulating Pond, waters are discharged in the Downstream Channel and in the Nam Kathang River through the Regulating Dam. Water release into the river respects an outflow corresponding to the natural inflow with an environmental minimum flow of $0.2 \text{ m}^3 \cdot \text{s}^{-1}$ while the outflow in the Downstream Channel is in yearly average to $220 \text{ m}^3 \cdot \text{s}^{-1}$. Water release from the Power House started for test in March 2010 and showed a stable regime since April 2010 when commercial operations began. Detailed features of the project are provided in Descloux *et al.* (same issue).

Five stations were sampled within the Nam Gnom basin (Fig. 1 and Fig. 2):

(i) Three sites were sampled in the Nam Kathang sub-basin: NKT1, NKT2, and NKT4. NKT1 was located on the Nam Kathang Noy River and NKT2 on the Nam Kathang Gnai River. Both stations were situated upstream of the Regulating Pond in unregulated rivers where no significant human disturbance was observed. NKT4 was located 3.2 km downstream of the Regulating Pond and upstream of the confluence with the Nam Gnom River. Human activities (cloth washing, fishing, and presence of cattle) were reported at this station.

(ii) Two sites were monitored in the Nam Gnom sub-basin. NGM1 was

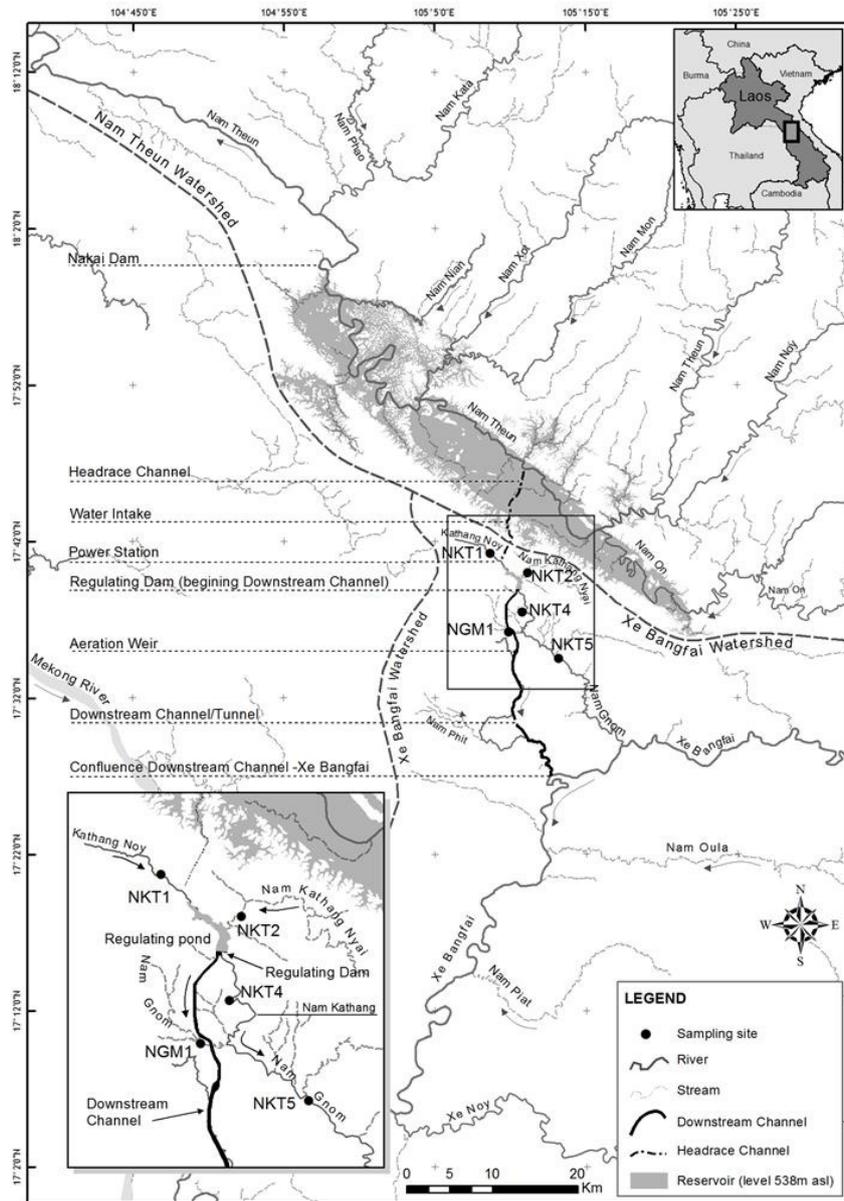


Fig. 1. Location of studies sites in the Nam Gnom Basin.

Fig. 1. Situation géographique des stations prospectées sur le bassin de la Nam Gnom.

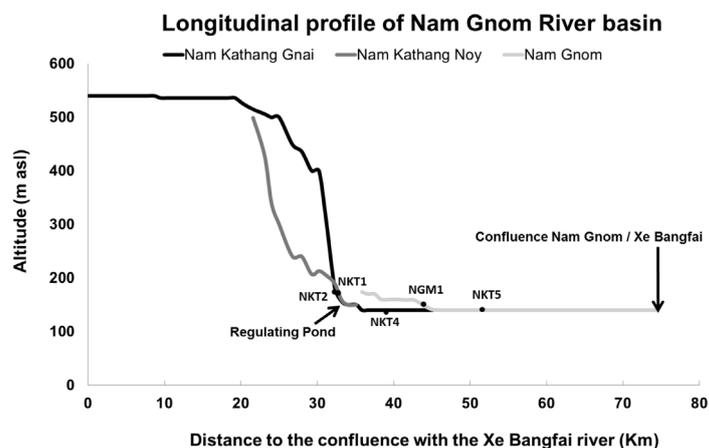


Fig. 2. Location of the station on the longitudinal profile of the Nam Gnom Basin.

Fig. 2. Situation des stations le long du profil longitudinal du bassin de la Nam Gnom.

located 700 m upstream of the confluence with the Nam Kathang River. NKT5 was situated 6 km downstream of the confluence with the Nam Gnom River and 17 km downstream of the Regulating Dam. Both sites were situated downstream of an irrigation dam interrupting the continuum of the Nam Gnom River. Agriculture, presence of cattle, fishing and cloth washing/bathing activities were reported to these stations.

Among the five stations, only NKT4 and NKT5 were influenced by the water release of the NT2 hydropower plant.

2.2 Aquatic invertebrates sampling and identification

Field collections were made during 6 campaigns numbered C1 to C6 from January 2010 to April 2012 (Tab. I).

Samplings were carried out twice a year during the low flow period which is

a key driver of the structure, function and condition of river ecosystems (Rolls *et al.*, 2012). Each site was sampled during the cool dry season in January and at the end of the warm dry season in April.

C1 was sampled 2 months before the beginning of water release from the NT2 Reservoir (April 2010) and NKT4 was sampled only until January 2011 (C3). A preliminary study comparing the station with NKT5 (unpublished results) underlined similitude between both stations. After the campaign C3, NKT4 was abandoned and NKT5 became the downstream reference station. Sampling of NKT1 could not be carried out in April 2010 (C2) because of low water level (<1 cm).

The protocol used corresponds to a multi-habitat sampling method. At each station, defined as twice the bankfull width, eight samples were collected with a surber sampler (area of 1/20 m², mesh size 500 µm) covering proportionally all

Table I. Summary of field campaigns.**Tableau I.** Synthèse des campagnes d'échantillonnage.

Date	Season	Discharging waters from NT2 Reservoir	Code	NGM1	NKT1	NKT2	NKT4	NKT5
January 2010	Cool dry	2 months before	C1	X	X	X	X	X
April 2010	Warm dry	1 month after	C2	X	-	X	X	X
January 2010	Cool dry	10 months after	C3	X	X	X	X	X
April 2011	Warm dry	13 months after	C4	X	X	X	-	X
January 2012	Cool dry	22 months after	C5	X	X	X	-	X
April 2012	Warm dry	25 months after	C6	X	X	X	-	X

habitat types with a share of at least 5% coverage. Samples were preserved with 8% formalin on the field. At the laboratory, aquatic invertebrates' samples were sorted and identified to the family level according to Dudgeon (1999) and Sangpradub & Boonsoong (2006). All biological materials were further preserved in alcohol 75% and were housed in a collection at the Aquatic Environment Laboratory of Nam Theun 2 Power Company.

2.3 Environmental variables

Water temperature, dissolved oxygen (DO), pH, conductivity, and turbidity were directly measured *in situ* using a calibrated HACH HQ40d-multi probe and a HACH 2100P Turbidimeter.

Two physical variables were recorded in the field to characterise habitat:

- (i) Relative abundance of substrates. They were categorized as follows: % of aquatic vegetation (macrophytes, bryophytes, and emerged spermatophytes), % of submerged coarse organic elements (roots, trunks), % of mud (<0.5 mm), % of sand (particle size 0.5-2.5 mm),

% of gravels (2.5-25 mm), % of pebbles (25-250 mm), and % of boulders (>250 mm).

- (ii) Current velocity (V , $\text{cm}\cdot\text{s}^{-1}$). Five classes were identified: $V > 150$, $150 > V > 75$, $75 > V > 25$, $25 > V > 5$ and $V < 5$.

A mapping of habitats at each station is provided Appendix 2.

Mean values, minimum, and maximum of the physico-chemical and habitat variables are shown in Table II.

2.4 Data analysis

To explore taxonomical and functional changes in the macroinvertebrates assemblage, mean values, minimum, and maximum of diversity, composition, and functional metrics were calculated (Tab. III).

Diversity metrics included the taxonomic richness (S = number of taxa), density ($\text{ind}\cdot\text{m}^{-2}$), Shannon-Wiener diversity index ($H' = -\sum p_i \ln p_i$), and Pielou evenness index ($E = H'/\ln S$), where $p_i = n_i/N$, n_i = abundance of the taxon i , N = total abundance.

Composition metrics included the % of Crustacea, % of Annelida, % EPT

Table II. Mean values (minimum - maximum) of abiotic factors measured in the Nam Gnom Basin from January 2010 to April 2012. Altitude, chemical variables and substrates composition were included in RDA.**Tableau II.** Valeurs moyennes (minimum - maximum) des variables abiotiques mesurées sur le bassin de la Nam Gnom entre janvier 2010 et avril 2012. L'altitude, les variables physico-chimiques et les pourcentages de substrats ont été intégrés à l'analyse canonique de redondance (ACR).

	NKT1		NKT2		NKT4		NGM1		NKT5	
	Nam Kathang	Noy Nam Kathang	Nam Kathang	Gnai	Nam Kathang	Nam Kathang	Nam Gnom	Nam Gnom	Nam Gnom	Nam Gnom
Sub-basin										
Influence of NT2 Reservoir	no	no	no	-	yes	yes	no	no	yes	yes
Other human alterations	-	-	-	-	Impoundment, cattle ranching, fishing, washing	Impoundment, irrigation, cattle ranching, fishing, washing	Impoundment, irrigation, cattle ranching, fishing, washing			
Physical variables										
Altitude (m)	183	184	184	184	161	134	134	165	165	165
Width (m)	2 - 6	3 - 10	3 - 10	3 - 10	25 - 30	20 - 40	20 - 40	10 - 30	10 - 30	10 - 30
Depth (cm)	15	20	20	20	30	20	20	30	30	30
Current velocity (cm.s ⁻¹)	5 - 25	5 - 25	5 - 25	5 - 25	5 - 25	5 - 25	5 - 25	5 - 25	5 - 25	5 - 25
Chemical variables										
Water temperature (°C)	26.4 (22.6 - 29.0)	23.6 (18.4 - 29.2)	23.6 (18.4 - 29.2)	23.6 (18.4 - 29.2)	25.1 (21.1 - 31.0)	26.2 (22.0 - 30.3)	26.2 (22.0 - 30.3)	27.6 (22.8 - 34.3)	27.6 (22.8 - 34.3)	27.6 (22.8 - 34.3)
pH	8.3 (8.0 - 8.5)	7.9 (7.6 - 8.4)	7.9 (7.6 - 8.4)	7.9 (7.6 - 8.4)	7.9 (6.6 - 8.5)	8.2 (7.9 - 8.5)	8.2 (7.9 - 8.5)	8.1 (7.5 - 8.4)	8.1 (7.5 - 8.4)	8.1 (7.5 - 8.4)
Conductivity (µS.cm ⁻¹)	215 (192 - 245)	222 (102 - 448)	222 (102 - 448)	222 (102 - 448)	43 (31 - 56)	318 (269 - 369)	318 (269 - 369)	250 (198 - 290)	250 (198 - 290)	250 (198 - 290)
Dissolved oxygen (mg.L ⁻¹)	9.3 (8.4 - 10.2)	8.7 (7.3 - 9.7)	8.7 (7.3 - 9.7)	8.7 (7.3 - 9.7)	10.7 (10.0 - 11.4)	10.0 (6.3 - 14.9)	10.0 (6.3 - 14.9)	9.6 (7.6 - 11.7)	9.6 (7.6 - 11.7)	9.6 (7.6 - 11.7)
Turbidity (NTU)	4.3 (2.3 - 8.1)	4.2 (1.6 - 8.3)	4.2 (1.6 - 8.3)	4.2 (1.6 - 8.3)	7.0 (4.4 - 8.5)	8.7 (2.6 - 18.0)	8.7 (2.6 - 18.0)	4.1 (2.4 - 6.6)	4.1 (2.4 - 6.6)	4.1 (2.4 - 6.6)
Substrates composition										
% Aquatic vegetation	5.5 (0 - 16.7)	4.5 (0 - 25)	4.5 (0 - 25)	4.5 (0 - 25)	21.7 (12.5 - 37.5)	0	0	10.4 (12.5 - 25)	10.4 (12.5 - 25)	10.4 (12.5 - 25)
% Submerged coarse organic elements	18.9 (12.5 - 28.6)	15.9 (0 - 37.5)	15.9 (0 - 37.5)	15.9 (0 - 37.5)	13 (14.3 - 25)	12.8 (12.5 - 25)	12.8 (12.5 - 25)	12.5 (12.5 - 22.2)	12.5 (12.5 - 22.2)	12.5 (12.5 - 22.2)
% Mud	13.5 (0 - 25)	4.5 (0 - 12.5)	4.5 (0 - 12.5)	4.5 (0 - 12.5)	4.4 (0 - 12.5)	12.8 (12.5 - 37.5)	12.8 (12.5 - 37.5)	2.1 (0 - 12.5)	2.1 (0 - 12.5)	2.1 (0 - 12.5)
% Sand	10.8 (0 - 37.5)	6.8 (0 - 25)	6.8 (0 - 25)	6.8 (0 - 25)	0	17 (12.5 - 37.5)	17 (12.5 - 37.5)	16.7 (12.5 - 37.5)	16.7 (12.5 - 37.5)	16.7 (12.5 - 37.5)
% Gravels	21.6 (0 - 42.9)	11.4 (0 - 37.5)	11.4 (0 - 37.5)	11.4 (0 - 37.5)	21.8 (25 - 37.5)	14.9 (12.5 - 25)	14.9 (12.5 - 25)	20.8 (12.5 - 25)	20.8 (12.5 - 25)	20.8 (12.5 - 25)
% Peebles	24.3 (0 - 66.7)	36.4 (0 - 100)	36.4 (0 - 100)	36.4 (0 - 100)	39.1 (25 - 71.4)	42.5 (25 - 100)	42.5 (25 - 100)	31.2 (12.5 - 55.5)	31.2 (12.5 - 55.5)	31.2 (12.5 - 55.5)
% Boulders	5.4 (0 - 14.3)	20.5 (0 - 40)	20.5 (0 - 40)	20.5 (0 - 40)	0	0	0	6.3 (12.5 - 25)	6.3 (12.5 - 25)	6.3 (12.5 - 25)

Table III. Mean values (minimum - maximum) of macroinvertebrates metrics measured in the Nam Gnom Basin from January 2010 to April 2012.
Tableau III. Valeurs moyennes (minimum - maximum) des métriques mesurées sur le bassin de la Nam Gnom entre janvier 2010 et avril 2012.

	NKT1	NKT2	NKT4	NGM1	NKT5
<i>Diversity metrics</i>					
Taxonomic richness	43 (36 - 50)	31 (22 - 41)	37 (28 - 46)	39 (32 - 50)	44 (39 - 48)
Density	10.604 (2.370 - 19.540)	3.683 (1.540 - 6.694)	5.156 (2.658 - 8.575)	10.621 (5.880 - 2.658)	12.142 (7.515 - 17.440)
Shannon diversity	2.00 (1.81 - 2.28)	2.07 (1.03 - 2.4)	2.3 (2.07 - 2.56)	2.38 (2.2 - 2.54)	2.25 (1.61 - 2.54)
Evenness	0.61 (0.56 - 0.7)	0.64 (0.31 - 0.74)	0.71 (0.63 - 0.79)	0.73 (0.67 - 0.78)	0.69 (0.49 - 0.78)
<i>Composition metrics</i>					
% EPT	23.7 (8.7 - 44.1)	34.8 (7.6 - 55.8)	34.4 (29.1 - 43.9)	37.8 (27.9 - 55)	27.9 (12.5 - 43.3)
% Others insects	48.5 (29.1 - 73.7)	39.8 (9 - 55)	47.2 (37 - 54.6)	51.4 (37.6 - 69.5)	49.8 (40 - 63)
% Molluscs	14.5 (0.3 - 46.8)	24.3 (0 - 83.4)	13.2 (12.9 - 13.4)	6.2 (2 - 11.1)	13.2 (6.1 - 18.4)
% Annelida	13.2 (0 - 39.6)	1 (0 - 4.5)	4.6 (3.3 - 5.8)	4 (0 - 13.4)	7.2 (0.4 - 20.2)
% Crustacea	0 (0 - 0.2)	0 (0 - 0)	0 (0 - 0)	0.1 (0 - 0.3)	0.9 (0 - 4.1)
% Minor groups	0 (0 - 0.1)	0.2 (0 - 0.8)	0.5 (0.1 - 1.3)	0.5 (0 - 1.5)	1 (0.2 - 2.5)
<i>Feeding metrics</i>					
% Shredders	2.2 (1.1 - 3)	4.7 (0.5 - 10.2)	2.6 (1.3 - 4.7)	3.3 (2 - 6.2)	1.5 (1.1 - 2.4)
% Scrapers	28.6 (12.8 - 71.4)	47.2 (17.4 - 85.4)	22.5 (16.4 - 30.4)	25.5 (8 - 45.1)	18.7 (11.4 - 22.8)
% Collector-gatherers	54 (6.2 - 76.8)	36.1 (4.3 - 70.1)	42.1 (31.5 - 53.2)	48.7 (23.3 - 75.1)	63.5 (56.4 - 68.2)
% Collectors-filterers	4.5 (1.2 - 9)	5.4 (2.7 - 11.9)	15.5 (11.7 - 21.9)	7.7 (2.9 - 13)	8.6 (2.2 - 16)
% Predators	10.8 (3.9 - 22.6)	6.5 (4.5 - 8)	17.3 (6.6 - 25.1)	14.8 (9.9 - 26.7)	7.7 (3.2 - 12.3)

(Ephemeroptera, Plecoptera and Trichoptera), % of other insects (*e.g.* Coleoptera, Hemiptera, etc.), % Molluscs, and % of minor groups (*i.e.* Hydracarina, Nematomorpha and freshwater planarians).

Feeding metrics included the % of five functional feeding groups (FFG). FFG were assigned according to Merritt *et al.* (2008) and defined according Cummins & Klug (1979) as follows:

- (i) Scrapers (Sc) which consume algae and associated material;
- (ii) Shredders (Sh), which consume leaf litter or other Coarse Particulate Organic Matter (CPOM particles >1 mm);
- (iii) Collector-gatherers (Co-Ga), which collect Fine Particulate Organic Matter ($0.45 \mu\text{m} < \text{FPOM}$ particles <1 mm) from the stream bottom;
- (iv) Collectors-filterers (Co-Fi), which collect FPOM from the water column using a variety of filters;
- (v) Predators (Pr), which feed on other consumers.

In addition, a species-accumulation curve (method exact, function “specaccum”) and the estimator of species richness Chao 1 (function “specpool”) were used on the whole taxonomic data set to estimate the representativeness of the sampling and estimate the diversity of the benthic macrofauna inhabiting the Nam Gnom basin.

A constrained ordination was conducted to explore spatial and temporal organization of sites and macroinvertebrates assemblage. A previous Detrended Correspondence Analysis (DCA) (function “decorana”) displayed short gradient lengths (<4 standard

units) indicating that a linear model was the most valuable (ter Braak & Smilauer, 1998). A Redundancy Analysis (RDA) (function “rda”) was hence processed. Rare taxa (density <0.1%) were previously discarded (Appendix 1). Environmental data set included altitude, chemical variables and substrate composition (Tab. II). We also tested inter-annual (Year) and intra-seasonal (January vs. April) variation. All data were $\log(X+1)$ transformed with the exception of percentages, for which arcsin transformation was used (Legendre & Legendre, 1998). Statistical significance of the environmental and macroinvertebrates association was verified with anova ($P < 0.05$; 9,999 Monte Carlo permutations). Forward selection (function “ordistep”) was used ($P < 0.05$; 9,999 Monte Carlo permutations) to determine significant environmental variables.

Data were analysed using R statistical software (R Core Team Development, 2013) and package Vegan (Oksanen *et al.*, 2007). Temporal evolution of S, density, % Molluscs, and % Co-Fi were plotted with GraphPad Prism® 6.0 (GraphPad Software, San Diego, USA).

3 RESULTS

3.1 Environmental variables

Sampling sites represented a range of low altitude (134-184 metres above sea level; Tab. II and Fig. 2) streams (channel width: 2-40 m, water depth: 15-30 cm; Tab. II). Pebbles and gravels were the most common substrates in

the Nam Gnom basin. Sand were mainly found in stations downstream (NGM1 and NKT5) while large boulders were widely represented at NKT2 (mean = 20.5% of the total substrates).

The predominance of discharged waters coming from the NT2 Reservoir was confirmed by the analysis of the conductivity (Tab. II). In the Nam Kathang Noy (NKT1) and in the Nam Kathang Gnai (NKT2) average conductivity was $215 \mu\text{S}\cdot\text{cm}^{-1}$ and $222 \mu\text{S}\cdot\text{cm}^{-1}$ respectively, whereas it dropped down to $43 \mu\text{S}\cdot\text{cm}^{-1}$ at NKT4, few kilometres downstream of the Regulating Dam. After the confluence with the Nam Gnom River (NGM1, mean conductivity = $318 \mu\text{S}\cdot\text{cm}^{-1}$) the conductivity increases to an average value of $250 \mu\text{S}\cdot\text{cm}^{-1}$ (NKT5). Discharged waters did not alter the other parameters which showed close values between upstream and downstream sites. During the six campaigns, all sites showed relatively high DO (mean for all sites and campaigns = $9.5 \text{ mg}\cdot\text{L}^{-1}$; S.D. = 1.7), temperature (mean for all sites and campaigns = $25.8 \text{ }^\circ\text{C}$; S.D. = 3.8), alkaline pH (mean for all sites and campaigns = 8.1; S.D. = 0.4) and low turbidity (mean for all sites and campaigns = 5.4 NTU; S.D. = 3.7).

3.2 Macroinvertebrates survey

3.2.1 General patterns of macroinvertebrates in the Nam Gnom basin

A total of 109 macroinvertebrates taxa were collected in the Nam Gnom basin from January 2010 to April 2012 (Appendix 1). During the six sampling campaigns 69, 72 and 78 taxa were collected at NKT2, NGM1, and NKT5

respectively. At NKT1, 77 taxa were collected during the five sampling campaigns and 55 at NKT4 during the three sampling campaigns. The species-accumulation curve processed in the Nam Gnom Basin did not reach asymptote (Fig. 3) indicating that more samples would be required to estimate the total richness. The richness estimator Chao 1 points out the possible presence of 139 taxa (S.D. = 17.3) within the entire Nam Gnom basin. Consequently, sampling covered around 78.4% of the potential total number of macroinvertebrates' taxa.

Minimum and maximum values of taxonomic richness and density were observed in sites unaffected by the NT2 project suggesting an important natural variability (Tab. III). 22 taxa and $2,370 \text{ ind}\cdot\text{m}^{-2}$ were collected at NKT2 (January 2011) whereas 50 taxa and $19,540 \text{ ind}\cdot\text{m}^{-2}$ were collected at NKT1 (January 2012) (Figs. 4a and 4b). Lowest diversity ($H' = 1.03$) and evenness ($E = 0.32$) indices were also observed at NKT2 in April 2010 (Tab. III). On the other hand, maximum values of diversity ($H' = 2.56$) and evenness ($E = 0.79$) indices were both observed downstream the regulating dam at NKT4 (Tab. III). At this site, taxonomic richness and density decreased after the beginning of water discharge (Figs. 4a and 4b) but the same pattern was also observed in sites unaffected by the NT2 project. For example, between January 2010 and January 2011, taxonomic richness varied from 31 to 22 taxa at NKT2 and density from $12,920 \text{ ind}\cdot\text{m}^{-2}$ to $5,880 \text{ ind}\cdot\text{m}^{-2}$ at NGM1. At NKT5, taxonomic richness remained stable during the study period (Fig. 4a) whereas density increased (Fig. 4b)

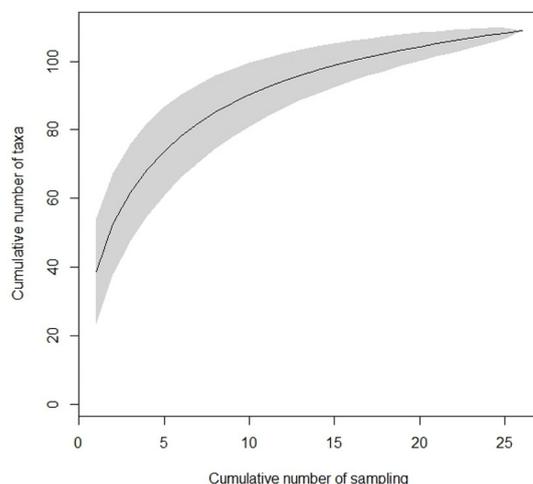


Fig. 3. Species-accumulation curve by cumulative number of sampling (8 surber samples/site) for all stations and dates sampling ($n = 26$). Shaded area indicates confidence intervals from standard deviation.

Fig. 3. Courbe d'accumulation de la richesse taxonomique en fonction du nombre d'échantillonnage (8 prélèvements au surber/site) sur l'ensemble des stations d'étude et des dates d'échantillonnages ($n = 26$). La portion grisée représente l'intervalle de confiance de l'écart type.

from 8,093 ind.m⁻² (January 2010) to 15,105 ind.m⁻² (April 2012).

3.2.2 Taxonomic composition

Diptera (37.3%), Ephemeroptera (23.6%) and Mollusca (12%) were dominant in samples whereas Crustacea (0.2%) and Plecoptera (1.3%) were the less represented. Trichoptera (20 families) and Coleoptera (16 families) were the richest groups followed by Hemiptera (13 families) and Mollusca (13 families; Appendix 1). Insects remained dominant at all sampling sites representing between 66.3% (NKT2) and 89.7% (NGM1) of the macroinvertebrates community. The EPT group (Ephemeroptera, Plecoptera, and Trichoptera) constituted an important part of the community (Tab. III) ranging from

23.7% (NKT5) to 37.8% (NGM1) but Plecoptera remained generally scarce (<1%). Beside insects, molluscs were the second most important group reaching 24.3% at NKT2. At all sampling sites, annelids showed low relative abundances except at NKT1 where they reached 13.2%. Minor groups and crustaceans accounted for 1% or less in the Nam Gnom basin.

Surprisingly, relative abundance of molluscs did not decrease at NKT4 and NKT5 after receiving poor-mineralised waters (Fig. 4c, Tab. II). Populations remained identical at NKT4 during the whole study. In January 2010, relative abundance of molluscs was 13.4%, while it was 12.9% in April 2010 and 13.2% in January 2011. At NKT5, population of molluscs increased after receiving discharged waters. Three

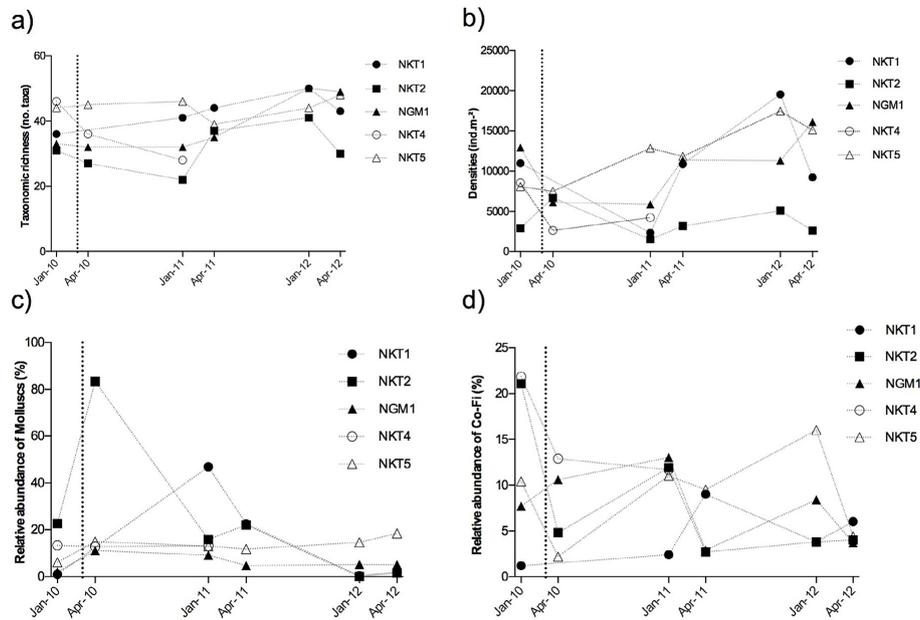


Fig. 4. Temporal variation of taxonomic richness (a), densities (b), relative abundance of molluscs (c) and relative abundance of collector-filterers (d) in the Nam Gnom basin. Vertical line corresponds to the beginning of water discharged from the Nam Theun 2 Reservoir. White filled labels correspond to sites under influence of discharged waters.

Fig. 4. Variation temporelle de la richesse taxonomique (a), de la densité (b), de l'abondance relative des mollusques (c) et de l'abondance relative des collecteurs-filtreurs (d) sur le bassin de la Nam Gnom. La ligne verticale indique le début du turbinage des eaux du Réservoir Nam Theun 2. Les labels blancs correspondent aux sites sous influences des eaux turbinées.

months before the beginning of hydro-power operation (January 2010), molluscs represented 6.1% of the community. Just after the water discharge (April 2010), molluscs represented 14.9% and 18.4% two years later (April 2012).

3.2.3 Functional feeding groups

The collector-gatherer group (Co-Ga), ranging from 36.1% (NKT2) to 63.5% (NKT1), was dominant at all sampling sites except at NKT2 where scrapers (Sc) dominated (47.2%)

(Tab. III). Sc was the second most important dietary group. Minimum average Sc value was observed at NKT5 (18.7%). Other FFG were lower in the Nam Gnom basin. Average contributions of Predators (Pr) and shredders (Sh) did not exceed 17.3% and 4.7% respectively. Collector-filterers (Co-Fi) appeared more abundant in sites influenced by discharged waters, especially at NKT4 where they reached 15.5%.

Although average proportion of Co-Fi appeared to be higher in sites influenced by discharged waters, these

populations were halved at NKT4 after the beginning of water release (Fig. 4d). In January 2010, Co-Fi represented 21.9% of the community whereas they represented 11.7% one year after. At NKT5, Co-Fi remained stable during the study period despite a high seasonal variability.

3.2.4 Environmental factors influencing macroinvertebrates communities

The Redundancy Analysis (Figs. 5a and 5b) explained 23.63% of the total variance and resulted in a significant model ($P = 0.005$). Axes 1 and 2 accounted for 37.68% of the explained variation. Axis 1 explained 25.85% (eigenvalue 6.109) and axis 2 11.83% (eigenvalue 2.797) of the variation fit.

Five environmental variables were retained in the RDA model by the forward selection procedure: Altitude ($P = 0.005$), Year ($P = 0.005$), % Aquatic vegetation ($P = 0.005$), Conductivity ($P = 0.01$) and % Boulder that had a marginal effect ($P = 0.0486$) (Fig. 5a).

The first canonical axis associated positively with Altitude ($r = 0.768$), % Aquatic vegetation ($r = 0.088$), % Boulder ($r = 0.295$) and negatively with Conductivity ($r = -0.354$) and Year ($r = -0.232$) supports a clear altitudinal gradient (Fig. 5a) whereas the association between axis 2 and the environmental variable Year ($r = 0.594$) suggested a temporal gradient. Furthermore, the axis 1 makes a clear distinction between the two sub-basins of the Nam Gnom and the Nam Kathang Rivers. NGM1 and NKT5 (Nam Gnom sub-basin) were located on the negative part and were opposed to NKT4, NKT1 and NKT2 (Nam Kathang sub-basin).

Taxa projection (Fig. 5b) indicated that the Potamanthidae family (Ephemeroptera) was the main contributor of the negative part of RDA axis 1 ($r = -1.147$) and the Hydrophilidae family (Coleoptera) ($r = 0.423$) the main contributor of the positive part. The Pomatiopsidae family (Gastropoda) was the main contributor of the negative part of the axis 2 ($r = -0.769$) and the Leptophlebiidae family (Ephemeroptera) the main contributor of the positive part ($r = 0.506$). Most macroinvertebrates taxa occurred in the negative part of axis 1 and were associated with lowest altitudinal sites.

4 DISCUSSION – CONCLUSION

In addition to the initial bioassessment objectives, this study provided valuable inventory of benthic macroinvertebrates families inhabiting a little studied area of Southeast Asia. A total of 109 macroinvertebrates taxa were identified in the Nam Gnom Basin attesting of a rich biota. Prospecting Northern Vietnam, Jung *et al.* (2008) reported cumulative richness from 61 to 91 families of a wide range of habitat including rivers in national parks. Furthermore, general patterns of diversity were consistent with regional literature. Trichoptera, Hemiptera, and freshwater molluscs were among the most diverse groups. This observation is in line with rivers of the Mekong Basin, known to host one of the highest diversity of these groups (Bogan, 2008; De Moor & Ivanov, 2008; Polhemus & Polhemus, 2008; Strong *et al.*, 2008). On the other hand, the low representation of Plecoptera and Crustacean was predictable.

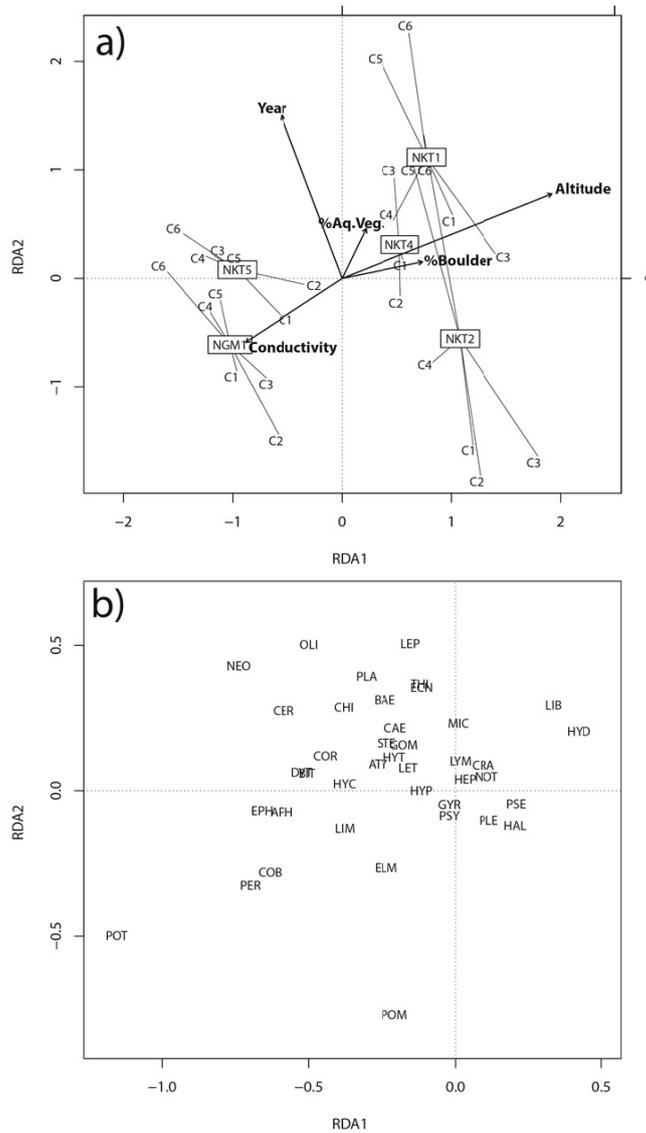


Fig. 5. Axes 1 and 2 of the redundancy analysis (RDA) ordination diagrams. a) Sites-environmental biplot showing significant environmental variables following forward selection. The mean position of the sites was located at the weighted average of corresponding sites. b) Taxa projection. Full taxa names can be found in Appendix 1.

Fig. 5. Axes 1 et 2 de l'analyse canonique de redondance (ACR). a) Graphique sites-variables environnementales significatives selon la procédure de sélection ascendante pas à pas. La position des sites correspond à la position moyenne pondérée des sites correspondants. b) Projection des taxa. Le nom complet des taxa est disponible en Annexe 1.

Plecoptera are known to be scarce in tropical streams (Vinson & Hawkins, 2003) and the surber gear underestimates shrimp abundance due to their high mobility (Ramirez & Pringle, 1998).

Response of macroinvertebrate assemblages to water discharge indicated no evident reduction of diversity indices neither a shift in composition community. At all sites, the typical dominance of Chironomidae and Baetidae was observed (Pinder, 1986; Suren, 1994; Galdean *et al.*, 2001; Ferrington, 2008). The sensitive families *e.g.* Ephemeroptera which are known to negatively react to environmental stress (Azrina *et al.*, 2006; Boonsoong *et al.*, 2009), did not show a decline in population among the upstream stations of the Regulating Pond and the downstream stations receiving water discharge. Surprisingly, mollusc's populations didn't decrease in sites located after water discharge. The high representation of macrophytes habitat (Tab. II) at NKT4 and NKT5 could explain the persistence of mollusc populations. Vegetation composition is a key factor explaining the variation in mollusc species (Horsák & Hájek, 2003).

Furthermore, no functional group changes were apparent. At all sites, the dominance of collector-gatherers was observed, except at NKT2 where scrapers dominated due to the presence of large boulders (>2 m) with low surface heterogeneity. This habitat was poorly colonised by insects' taxa but was largely covered by freshwater snails feeding on an abundant periphyton (*e.g.* Pomatiopsiade; Appendix 1). These results suggested that the capacity to process organic matter was

not altered by water release. In addition, the shredders group, represented by few taxa (Tab. III), known to decrease under increasing perturbation (Boonsoong *et al.*, 2009), did not show relevant differences between the upstream and downstream stations. However, the use of the functional feeding groups (FFG) determined for temperate taxa and the family level assignment of FFG has clear limitations (Tomanova *et al.*, 2006, Ramírez & Gutiérrez-Fonseca, 2014). FFG studies at species-level and regionally based, would have allowed more precise conclusion.

In addition, redundancy analysis showed that altitude remained the main driver affecting the distribution of macroinvertebrates taxa in the Nam Gnom Basin before water release. Furthermore, no association among impacted sites was observed. The other environmental factors retained by the forward selection procedure are closely related to altitude. For instance a negative correlation between conductivity and elevation is generally observed (Wilcox *et al.*, 1957; Rundle *et al.*, 1993) and the positive correlation between altitude and the percentage of boulders reflects the sediment transport implying the presence of larger rocks mainly in the upstream part and finer sediments in the downstream part. This altitudinal distribution of habitats is underlined by a clear differentiation of the taxa distribution. For instance, Potamanthidae was the main contributor of the negative part of RDA axis 1 (lower altitude, higher conductivity). The knowledge on the ecology of this family supports this finding as nymphs are known to inhabit downstream sections such as

fourth-order stream (Munn & King, 1987). Then, our results are in accordance to literature that underlined that most macroinvertebrates taxa showed a positive correlation with lower altitudinal sites (Jacobsen, 2004). Inter-annual variation appeared to be a variable playing a significant role on the macroinvertebrates population structure and composition. Pomatiopsidae showed the highest correlation with the temporal gradient suggesting a turnover of populations in accordance to Attwood & Upatham (2012) who identified natural growth-decline cycles in Thailand and central Lao PDR.

Then, two main factors may explain the relative conservation of natural conditions:

- (i) An environmental minimum flow respecting the natural inflow. Flow regime has a strong influence on the biodiversity of rivers and importance of keeping the flow as close as possible to the natural regime is attested (Stanford *et al.*, 1996; Poff *et al.*, 1997) and confirmed by the interest in restoring natural flow regime to recover biotic integrity all around the world (Sparks, 1995; Petts, 1996; Galat *et al.*, 1998; Sparks *et al.*, 1998; Dudgeon, 2000; Robinson *et al.*, 2003; Attwood & Cottet, same issue).
- (ii) The presence of a tributary downstream of the release point. Tributaries are known to structure longitudinal biotic patterns (Rice *et al.*, 2001) and their importance in regulated rivers are recognized (Petts & Greenwood, 1985; Stevens *et al.*, 1997). According to the serial discontinuity concept (Ward & Stanford, 1983), recovery of large regulated rivers downstream from a dam is limited by relative tributary size. Here, the Nam Gnom River allows to recover biotic integrity of the Nam Kathang River.

Finally, the preliminary results of aquatic invertebrates bioassessment in the Nam Gnom basin allows to draw a general trend of communities in this region. All results of our study are based on a bioassessment at the family-level. Using family level identification is recommended in case of incomplete taxonomic knowledge (Thorne & Williams, 1997) and is qualified as sufficient to assess environmental disturbance (*e.g.* in Thailand; Boonsoong *et al.*, 2008). Recent studies conducted in tropical areas showed that family-level resolution successfully detect anthropogenic impairment (Dedieu *et al.*, 2015). However, genus-level would have provided more accurate information on water integrity (Lenat & Resh, 2001) and on environmental factors influencing communities (*e.g.* differentiation in the FFG, species-level sensitivity). This approach would have given more confidence to guide management decisions (Rosenberg *et al.*, 1986). Even no significant conclusion could be raised due to the few available data before water release, the study allows to strengthen the local knowledge in terms of macroinvertebrates population and environmental factors that could influence them. Bioassessment is at its early stage in Lao PDR. Additional taxonomical and ecological knowledge of benthic macroinvertebrates in the tributaries of the Mekong River are still required to develop robust

bioassessment tools, particularly promising in this fast-growing region.

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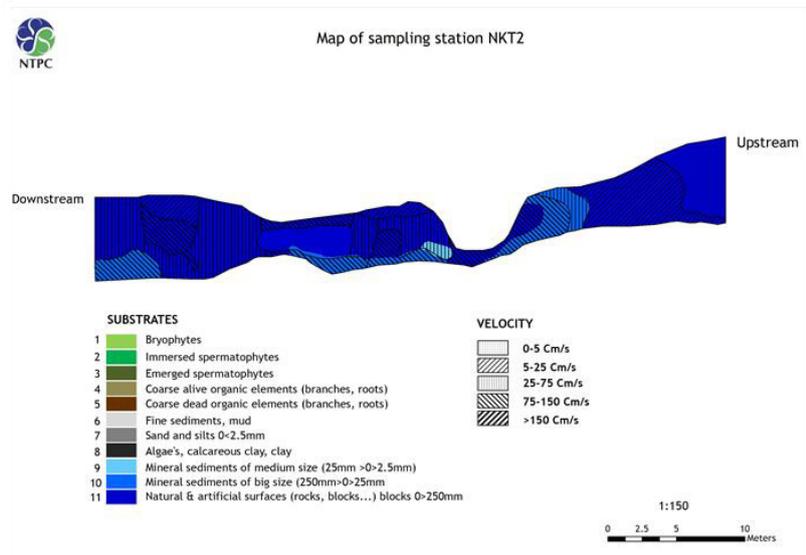
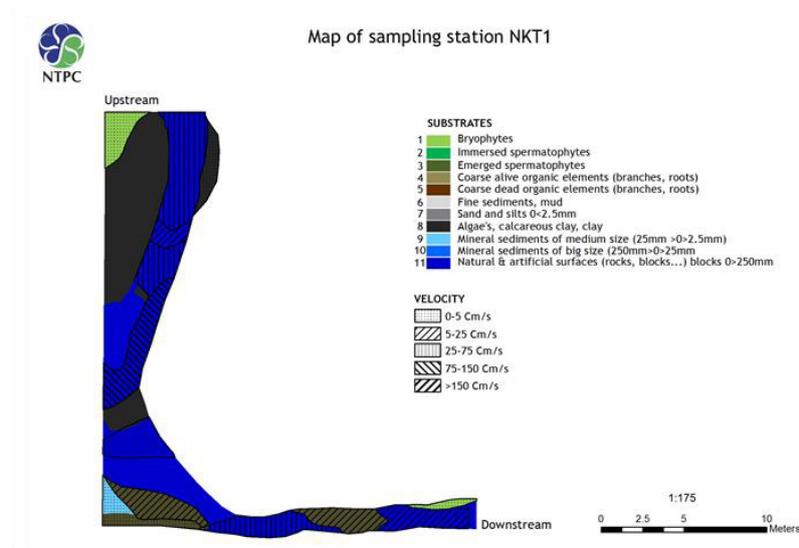
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Appendix 1. Continued.
Annexe 1. Suite.

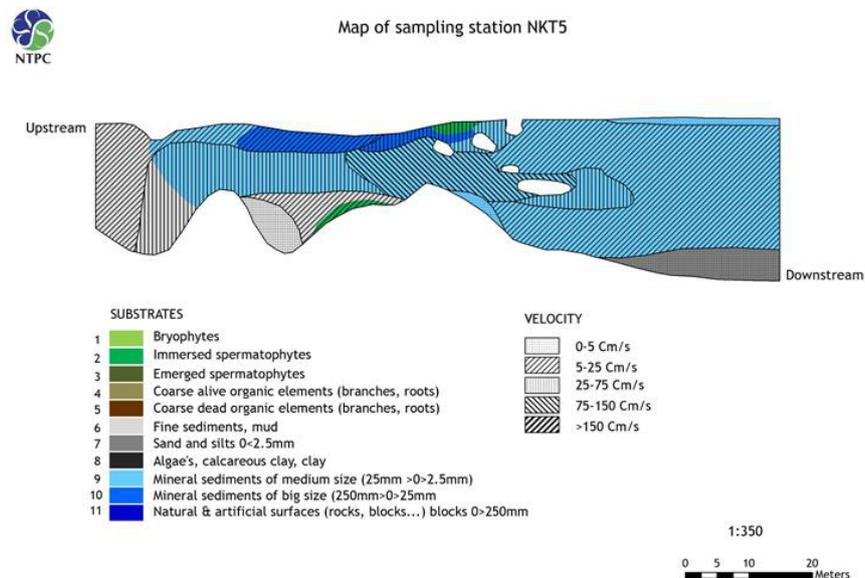
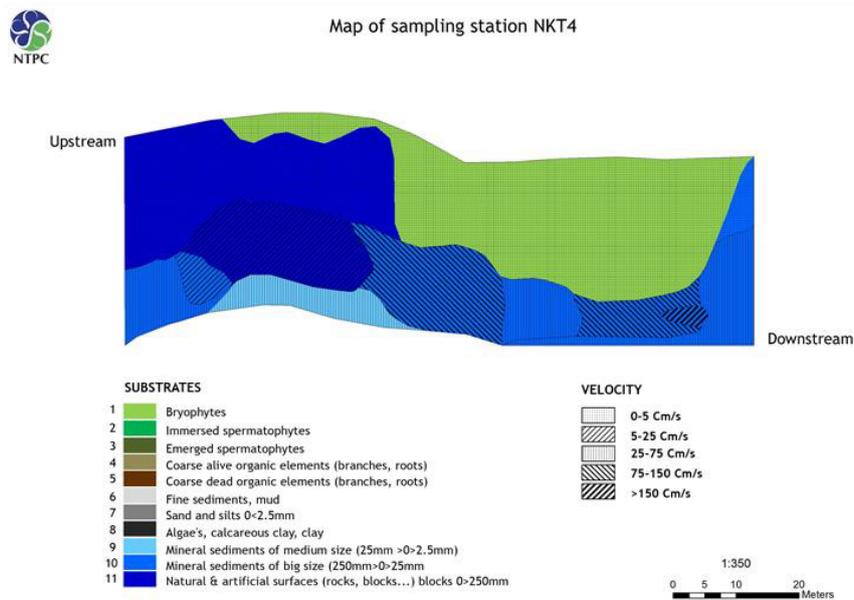
	FFG	RDA Code	MKT1						MKT2						MGM1						MKT4						MKT5							
			C1	C3	C4	C5	C6	C1	C2	C3	C4	C5	C6	C1	C2	C3	C4	C5	C6	C1	C2	C3	C4	C5	C6	C1	C2	C3	C4	C5	C6			
Hemiptera	Aphelocheilidae	Pr	83	13	38	0	3	8	0	0	8	20	3	1178	454	406	383	68	128	30	245	903	49	5	170	403	53	130	0	0	0	0	0	0
	Coixidae	Pr	0	3	8	5	165	0	146	0	15	48	50	3	6	100	143	313	243	48	140	9	13	50	285	53	40	125	0	0	0	0	0	0
	Gerridae	Pr	0	0	5	10	5	0	0	0	8	8	0	0	3	0	0	0	3	3	0	0	16	0	3	0	0	0	0	0	0	0	0	0
	Helotrephidae	Pr	0	0	3	0	0	0	0	0	0	28	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	Hydrometridae	Pr	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	Leptopodidae	Pr	0	3	5	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	Mesoveliidae	Pr	0	0	0	23	3	0	0	0	0	13	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	Micronectidae	Pr	0	0	0	155	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	Naucoridae	Pr	9	3	10	0	5	0	0	0	0	5	0	13	0	0	0	0	0	3	3	0	2	3	0	0	0	0	0	0	0	0	0	0
	Nothoerectidae	Pr	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	Phoridae	Pr	77	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	3	3	0	2	3	0	0	0	0	0	0	0	0	0	0
	Velidae	Pr	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	Chermidae	Sh	157	6	68	40	108	12	40	6	5	15	0	63	0	23	3	35	0	43	93	23	16	0	16	0	23	5	0	0	0	0	0	0
	Aesimidae	Pr	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	Amphiterygidae	Pr	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Calopterygidae	Pr	0	0	0	5	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
Chironomidae	Pr	0	0	0	15	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
Coenagrionidae	Pr	0	0	20	0	0	0	0	0	11	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
Chironomidae	Pr	0	20	0	5	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
Chironomidae	Pr	29	5	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
Chironomidae	Pr	34	0	68	48	1475	12	43	0	0	0	0	55	37	77	30	58	20	8	3	14	20	5	33	0	20	8	0	0	0	0	0	0	
Chironomidae	Pr	0	0	0	10	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
Chironomidae	Pr	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
Chironomidae	Pr	281	0	65	213	165	4	46	8	3	10	5	0	0	0	0	0	0	3	43	3	29	7	15	20	5	0	0	0	0	0	0	0	
Chironomidae	Pr	6	5	3	50	0	0	0	0	0	0	0	0	0	0	0	0	0	0	25	23	0	0	0	0	0	0	0	0	0	0	0	0	
Plecoptera	Pr	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
Trichoptera	Nemouridae	Sh	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
	Perleidae	Pr	3	0	8	8	0	16	6	3	0	0	785	174	103	86	165	383	5	13	0	0	0	0	0	0	0	0	0	0	0	0	0	
	Benedictidae	Co-Fi	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
	Brachycentridae	Co-Fi	0	18	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
	Dipseuropsidae	Co-Fi	3	3	8	3	3	12	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
	Ecnomidae	Pr	0	0	0	0	0	80	0	0	13	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
	Glossosomatidae	Sc	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
	Coarctidae	Sc	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
	Helicopsychidae	Sc	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
	Hydrobiosidae	Co-Fi	109	30	128	688	518	360	97	175	18	178	50	908	240	397	78	790	223	775	65	29	369	15	320	50	458	143	0	0	0	0	0	
	Hydropsychidae	Sc	14	45	63	95	170	28	160	0	35	20	28	255	6	151	43	10	118	78	105	106	68	125	245	88	95	53	0	0	0	0	0	
	Hydroptilidae	Sh	54	35	23	218	80	128	14	45	63	433	173	34	54	53	83	245	28	13	29	44	10	153	180	80	110	0	0	0	0	0		
	Leptoceridae	Sc	23	0	0	0	13	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
	Melaneridae	Sc	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
	Odonotidae	Sh	0	0	20	0	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
	Phlebotomidae	Co-Fi	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
	Phygadeuonidae	Sh	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
	Polycentropodidae	Pr	0	0	0	0	0	20	48	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	

Appendix 2. Substrate types and current velocity mapping of the stations monitored in the Nam Gnom basin.

Annexe 2. Cartographie des types de substrat et des vitesses du courant des stations suivies sur le bassin de la Nam Gnom.



Appendix 2. Continued.
Annexe 2. Suite.



Appendix 2. Continued.
Annexe 2. Suite.

