Assessment of the ecological quality of tropical creeks and other important aquatic habitats in a landscape dominated by teak plantations under Norteak Nicaragua S. A.'s management in the municipalities of Boaco, Camoapa and Matiguás

Report prepared for Norteak Nicaragua S. A.

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1. Introduction

Assessing the environmental health of watersheds is of the utmost importance, since watersheds represent a social, economic, and ecological unit. Creeks are a very important component of these systems, as they play a key role in the inter-connectivity of rivers network, therefore permitting the exchange and transport of water, sediments, nutrients, wildlife, and, sadly, pollutants. That means that on a larger scale, the quality of creeks is an indicator of the wellbeing of watersheds. If in good conditions, hydrographic systems provide critical services such as drinking water, productive fisheries and opportunities for outdoor recreation. Ecologically speaking, they are very productive and crucial habitats for many species since they provide a large diversity of microhabitats. They may even inhabit subservient species (species that are strongly linked to this specific ecosystem and can hardly survive without it). The environmental quality and our quality of life is thus directly linked to conservation efforts of watersheds.

The degradation of watersheds is considered the primary threat to the ecological integrity of tropical streams (Winemiller, Agostinho, & Caramaschi, 2008). Degradation affects the long-term survival of fish communities vulnerable to deforestation and conversion of land to agriculture, amongst others. Tropical stream fish use very diversified ecological niches and their main source of food is aquatic invertebrates along with seeds, fruits and flowers from terrestrial plants. Rich riparian areas are therefore a determinant factor in stream quality (Winemiller et al., 2008). The use of bioindicators is also a great method of assessing habitat quality in streams. Studying benthic invertebrates gives information on the bottom of the food chain and reveals any significant organic contamination of the water. However, these techniques are new and under developed in most countries of Central America. To this day, knowledge on the ecology and the taxonomy of aquatic macroinvertebrates in Central America is very incomplete (Sermeño-Chicas et al., 2010). The streams of Nicaragua are unrecognized and understudied. Nevertheless, many naturalists have observed that the high taxonomic groups of aquatic fauna resemble their European and North American relatives, which are well known (Fenoglio, Badino, & Bona, 2002). As a result, the use of biomonitoring in Central American creeks is possible, even though local biases remain to be accounted for.

The general aim of this study is thus to provide a detailed assessment of the ecological quality of creeks largely influenced by Norteak’s activities to allow for proper management of the resource. We described and studied the habitat potential of several creeks, documented the fish communities present and characterized the macroinvertebrate benthic community. This information offers a solid base on which to establish surveying criteria to detect potential positive and negative impacts of plantation establishment, management, and exploitation.

2. Description of Study Area

The present study was carried out in five creeks located in three neighboring municipalities (Boaco, Camoapa and Matiguás) on the border between the department of Boaco and the department of Matagalpa (figure 1). All the farms are in the large watershed of Río Grande de

Matagalpa and in a mountainous area where average temperatures are cool (22 to 25º C), and the rainfall is high and uniform (1600 mm to 2,000 mm / year) (INIDE-MAGFOR, 2013). The area is in a place of transition between a monsoon climate (Am) and a hot and sub-humid climate with rains in summer according to the modified Köppen system (Hernández, 2017). It is dominated by tropical humid forest (ENACAL, 2019).

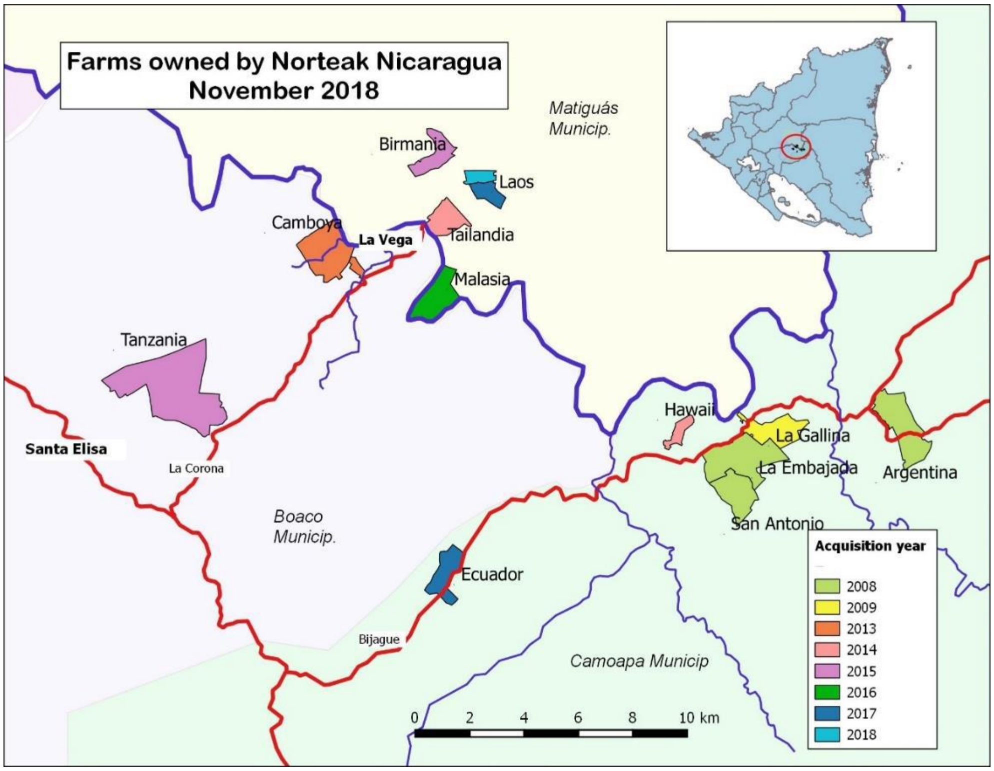

Figure 1 Location of Norteak's farms in the municipality of Boaco, Camoapa and Matiguás. The study sites were established in San Antonio, La Gallina, Tanzania, Laos and Birmania.

The municipality of Camoapa and the municipality of Boaco together have 80.8% of the cattle (186,598 head of cattle) present in the department of Boaco, which contributes significantly to the low forest cover of the area (INIDE-MAGFOR, 2013). Local people generally have low education levels and live in high poverty. Hunting and fishing is common and may have a significant influence on biodiversity in the area.

The selection of the creeks to be evaluated was based on their size, location, and accessibility. Narrow creeks were selected to facilitate field work and allow for comparisons between creeks. Small creeks also meant smaller watersheds and, therefore, a more direct influence from Norteak's activities. Location was an important factor to offer a complete image of creeks in different regions occupied by Norteak's plantations. Two study sites were established per municipality where Norteak has established plantations. Finally, accessibility was the final factor influencing specific site selection within plantations. Easily accessible sites were favoured. All measurements were taken in October.

Table 1 Location and sampling dates of each study site and the registered precipitations of the last 72 hours prior to the field visit based on Norteak's precipitation data.

Date	Farm	Creek	Station	Latitude	Longitude	Normal precipitations (mm)*	Precipitations 72h (mm)
2020-10-01	San Antonio	2541	1	12.60493	-85.19075	19.1	6.4
2020-10-08	La Gallina	3191	2	12.62217	-85.17331	19.1	7.2
2020-10-14	Tanzania	9531	3	12.63480	-85.36969	23.9	14.5
2020-10-15	Tanzania	9531	4	12.63622	-85.37714	23.9	22.0
2020-10-26	Laos	8611	6	12.70036	-85.27431	16.8	14.8
2020-10-27	Birmania	8511	7	12.71131	-85.29164	16.8	14.8

*The normal precipitations were determined by calculating the average precipitation registered for in a day of October in each region in the years 2018, 2019 and 2020. The result was multiplied by 3 to compare it with the precipitations of the last 72 hours prior to the field visit. This makes it possible to judge if the precipitations were normal, high or low. The normal precipitations of the last two stations were estimated with only the years 2019 and 2020 as there was no data for the year 2018.

3. Methodology

3.1 Creek characterization

A detailed description of each site was done within a 25 m segment set using a 50 m measuring tape. The measures taken focused on major habitat components for fish and important hydrological features (annex 10).

First, basic information such as the dimensions of the station segment were observed. The width was measured at the highest water line on each side of the creek. It was measured every five meters in order to obtain an average. The minimum and the maximum width were also noted. The same method was used for depth (every five meters starting from 0 m to 25 m and minimum/maximum depth). The slope was estimated with one observer standing at one end of the segment and the second standing at the other end. The first observer would note the level at which his eyes would set on his colleague while looking straight ahead. The height of the observer's eyes minus the height of where his eyes set gave the elevation difference over 25 m which was then converted to slope percentage (figure 2).

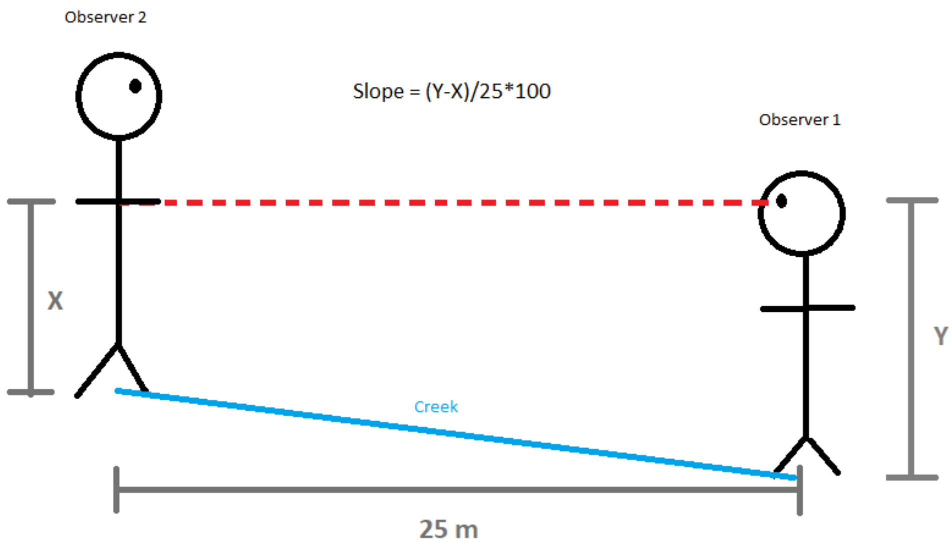

Figure 2 Representation of the method used to measure the slope of each creek

The temperature was measured with a thermometer, and the pH was taken with pH strips. The color of the water was evaluated with the Munsell chart of colors[1] (except when translucid). Transparency was established as either non-transparent or transparent, based on whether the riverbed was visible or not. Since walking into the creeks could resuspend bottom sediments, color and transparency were determined before entering the water.

The current speed was estimated at 0 m, at 12.5 m and at 25 m with a chronometer, a measuring gallon and a leaf. The leaf was released in the water as the timer would start. The distance traveled by the leaf in a given time was then converted to meters per second. That method was found to be effective due to the absence of wind in the study sites. Oranges are a recommendable alternative to the leaf to avoid wind bias since they have a buoyancy similar to water while still floating. The water debit was then obtained by multiplying the width, the depth and the current speed at 0 m, at 12.5 m and at 25 m.

That characterization continued with measures of elements known to affect habitat quality for aquatic fauna. The shading of the creek was estimated relative to various sources of shade (aquatic or riverine vegetation, or another source). The time of the estimation was recorded since the angle of the sun rays may influence shading. The type of substrate found on the riverbed was also estimated on a scale from mother rock to fine particles such as sand and limon. The quantity of organic debris was also considered. The types of riverbank were evaluated according to the following categories: rock, tree and bush cover, herb cover or naked ground. The presence of components such as shelters, thresholds, stream pits, watercourse catchments, spawning grounds, water springs, culverts, trails and other habitat modifiers were also examined as these are all indicator of the habitat quality of the creek for aquatic life. Finally, the presence of potential problems such as non-natural erosion, trash, obstacles for fish access or others were recorded.

[1]Consult the Munsell chart online at http://www.andrewwerth.com/color/?fbclid=IwAR1Hlfd7JR-84Y8hZBOJBzZoy_8BSAo39M7D3Vvotf6lhwFQgZqdXNT2aGY

The characterization was completed with a 200 meters walk downstream on each site in order to have a full picture of the creek, its topography and larger scale characteristics. During this walk, major obstacles in the water flow were noted, dominant microhabitats were observed, and a general appreciation of the creek was given.

3.2 Fish community and other aquatic groups

An active search was performed in each studied segment to establish a preliminary list of fish species and other aquatic organisms for each creek. The fishing effort was not standardized but was consistently approximately 1.5 hours. Fishing was done within 200 m upstream or downstream from the selected segment used for creek characterization and was considered opportunistic. Two fishing methods were used: A D-shape fishing net and a large mesh net.

The D-shaped fishing net was used to catch fish hidden near the water banks, within root systems, in areas with high vegetation content and other cumbersome areas of the small creeks. Quick swipes were performed in murky waters, water pools, submerged root systems, along eroded water banks and in any other relevant fish microhabitat. The net had mesh holes of 1 mm (figure 3).

Figure 3 D-shape net used for opportunistic fishing

The large mesh net was used in more open segments of the creeks where organic debris was not too abundant within the water. The net measured 7 m by 4.5 m and had 4 mm wide mesh holes. Its placement was determined by a quick visual analysis of strategic spots based on the presence of fish and the absence of organic debris, large rocks and eroded banks that could impede proper use of the net. It was placed at the bottom of the water and set down with rocks found on the site so fish would swim on top before being caught. The sides of the net were held above the water (high enough to prevent fish from jumping above it), except for the side facing upstream (figure 4). We would then walk straight into the water, upstream from the net creating disturbances in the water and tapping the surface of the water with sticks to force the fish to flee in the direction of the net. The person scaring the fish would quickly lift the part of the net still underwater, forming a fully closed chamber with the net and making it impossible for fish to escape. The mesh net was then gradually lifted by the edges as the rocks used to hold it down in the creek were removed, making sure no fish were hurt in the process.

Figure 4 Mesh net setup in the creek studied in Laos farm (Kevin Gauthier, 26-X-2020)

One by one, the captured fish were taken out of the bucket in which they were kept and examined on a wet tray made of glass with ledges preventing the fish from jumping out and harming themselves. A measuring ruler was placed below the glass tray to measure the fish (figure 5). One observer was responsible for taking pictures of the specimens as the second one was collecting the rest of the data. Dirty fish were rinsed off by the person responsible for taking pictures with a wash bottle filled with creek water.

Each fish was photographed and given a unique identification number; its standard and total length were measured; the capture method was noted as well as whether the specimen was kept for identification purposes or freed; a visual health analysis was performed.

Standard and total lengths are standardized measurements used in taxonomy and for statistical analysis (health, age, etc.). Total length corresponds to the length of the fish from the tip of the mouth to the tip of the caudal fin. The standard length, on the other hand, corresponds to the length from the tip of the mouth to the tip of the caudal peduncle (figure 6).

Figure 5 Representation of the setup for measurements of captured fish

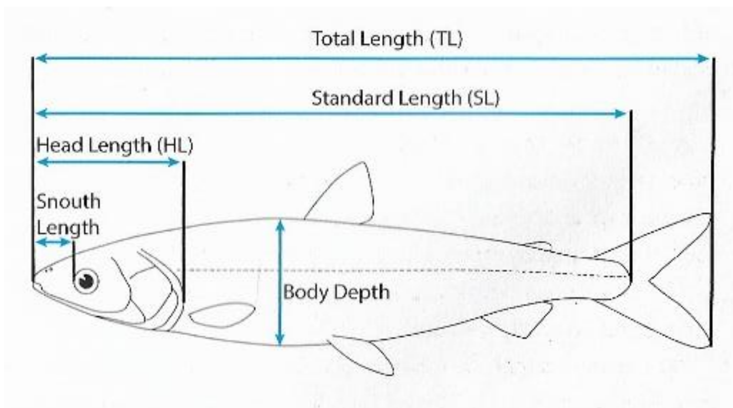

Figure 6 Measurements of total and standard length, figure taken from van der Sleen & S. Albert (2018, p.23)

Specimen collection was limited to reduce the potential ecological impact of the study and to avoid unnecessary cruelty. Fish samples were limited to one fish per visually distinct fish species for every study site. Samples were considered necessary to proper identification considering the limited literature surrounding fish diversity in Nicaragua.

Any other aquatic organism observed during the study was photographed and documented.

3.3 Ecological evaluation of the creeks based on aquatic invertebrates

Benthic species are useful indicators of water and habitat quality in aquatic ecosystems. Both abundance and diversity reveal useful information for a complete ecological evaluation of creeks. The benthos was therefore collected twice in the creek characterization segments of each creek following a standardized method. One sample was collected in lotic water, and the second sample was collected in lentic water. The method enabled us to document a wide diversity of benthic species in a way that allows for comparisons between creeks and time frames.

Lotic sampling sites were selected based on the presence of ripples and the type of sediments (figure 7). Lotic sites with mother rock were not selected since we were looking for invertebrates that use deeper substrates. Lotic sites had to have at least 5-10 cm deep of movable sediments. Sites with rocks larger than 15 cm in diameter were excluded from potential sampling sites since they impeded proper sampling.

Figure 7 Typical lotic sites appropriate for sampling (Stéphanie Villeneuve, October 2020)

Lentic sampling sites were selected based on the absence of visible current and the presence of sediment accumulation (figure 8). Sites with organic detritus accumulation were favoured.

Figure 8 Typical lentic sites appropriate for sampling (Stéphanie Villeneuve, October 2020)

A round entomological net with a diameter of 37 cm and mesh holes of 1 mm was placed in the water facing the current by a first person also responsible for monitoring the sampling time. A second person would then rub the rocks and various substrates of the riverbed vigorously for 60 seconds between 0 cm and 50 cm in front of the net opening, sending sediments along with the macroinvertebrates into the net (figure 9). The sediments contained in the entomological net were then transferred into a tray, where the observers sorted all the macroinvertebrates into an identified sampling vial containing 70 % alcohol to kill and preserve the collected organisms until analysis. To assure preservation of the samples, the vials were kept in a dark environment until further analysis.

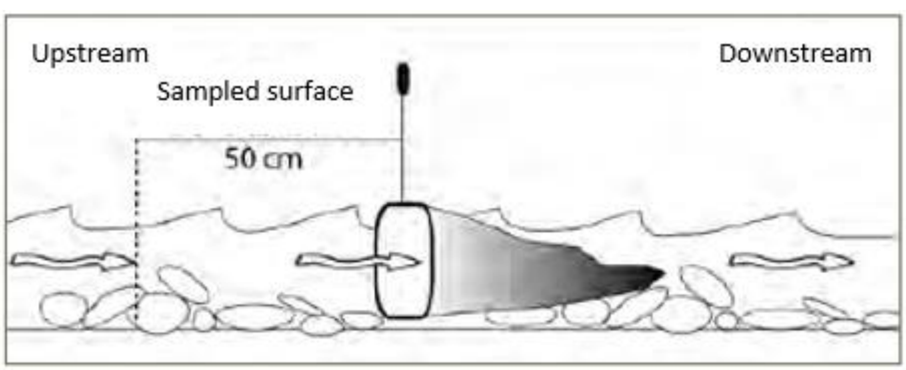

Figure 9 Disposition of the entomological net and delimitation of the sample area, image adapted from MDDEFP (2013, p.11)

Figure 10 Field setup for the first separation treatment of benthic organisms from riverbed substrates (right) and entomological net used for benthic sampling (left) (Stéphanie Villeneuve, October 2020)

A quick opportunistic hunt of macroinvertebrates was performed in the remaining microhabitats of the segment used for the creek characterization to increase species detection and have a more complete picture of the biological community of each creek. The samples collected were preserved and analysed with the same methods as the standardized benthic samples.

Specimens were identified using a KERN OSF 524 binocular microscope capable of a 30X magnification.

We decided to use two bioindicator methods to compensate the possible biases linked to low understanding of tropical macroinvertebrates in creeks. The indicators were calculated using only the standardized lotic and lentic samples of each creek. Both bioindicators used test for organic contamination of the water and habitat degradation for typically fragile groups. The first method used was the EPT analysis. This method uses the abundance of Ephemeroptera, Plecoptera and Trichoptera (the three most sensitive orders of aquatic insects) in relation to the total abundance of aquatic invertebrates to qualify the quality of the water. An example of the calculation necessary to obtain this indicator is presented in Annex 6. In short, it consists of dividing the abundance of the three sensitive orders presented above by the total abundance of all aquatic invertebrates collected and multiplying this factor by 100 to obtain a percentage. The percentage is then compared with the table of water quality, also presented in Annex 6. Since the three important orders of this method typically need a lot of dissolved oxygen to thrive, they tend to decline significantly when organic contamination reduces the amount of available oxygen in the water.

The second method used was the FBI-SV-2010 indicator which gives a number relative to the tolerance to organic contamination to each taxonomic group (lower numbers are related to more sensitive families and organisms with a wide tolerance range are assigned high values). The FBI values are then calculated as an average of the tolerance values of all the families in the sample relative to their abundance (see Annex 7 for calculation example). It basically consists of multiplying the assigned tolerance value of a family by its abundance (number of individuals sampled) and dividing the result by the total abundance of aquatic invertebrates collected. You then add the values obtained for each family to get the final index value which can be compared in the table of water quality presented in Annex 8. This indicator is adapted for use in El Salvador. The tolerance value associated to each family should be adapted to different regions of the world for appropriate use. However, considering the proximity of El Salvador and lack of indicators adapted to Nicaragua, we considered this indicator relevant. Since it associates tolerance values to most invertebrate families, it was considered more precise than alternative indices.

Another indicator that was considered was the BMWP – CR. It is similar to the FBI-SV-2010 indicator, but it was quickly discarded as an option because of the more complex sampling method it requires and its lack of consideration for the relative abundance of each taxonomic group.

4. Results

4.1 Creek characterization

The creeks studied are all permanent. Fish were present in all the studied segments. Table 2, shown below, indicates that the width of the segments ranged between 1.62 m and 9.50 m. Note that at the third station, the riverbed was composed of a relatively flat slab of mother rock making for a significant difference in the waterline width of that day and the width of the highest waterline. The average depth of the creeks was between 0.15 m and 0.29 m and their slope was between an approximate 2 and 7 %.

The only creek where the riverbed was not visible was the one at station 2, making it non-transparent. The color of the water varied from station 1 to 3 and it was colourless for the three other stations.

The current speed and water debit were stronger at station 2. Station 1 and 7 both had particularly weak water debits. Note that the water debit was only measured once in station 3 due to the morphological particularities of the riverbed composed of mother rock. We decided to evaluate the water debit at the most U-shaped point of our segment to avoid inaccurate estimations due to the unusual shape of the water flow.

The water temperature varied between 21.5^{o}C and 25^{o}C, the first two stations being the coldest. Finally, water pH was between 6 and 6.5, which is fairly neutral.

Table 2 General characterization of the segments studied per creek (physicochemical parameters)

Measurements	Creek					
	Station 1	Station 2	Station 3	Station 4	Station 6	Station 7
Average width (m)	1.62 (0.13 – 2.95)	2.10 (1.14 – 3.10)	9.50 (7.14 – 12.77)	3.34 (2.40 – 4.14)	2.28 (1.37 – 3.10)	2.20 (1.55 – 3.20)
Average depth (m)	0,21 (0.01 – 0.63)	0.15 (0.04 – 0.24)	0.27 (0.05 – 0.48)	0.18 (0.01 – 0.50)	0.24 (0.03 – 0.51)	0.29 (0.02 – 0.61)
Slope (%)	5	7	4	–	2	low*
Transparency (%)	Transparent	Non transparent	Transparent	Transparent	Transparent	Transparent
Color	10G 5/2	2.5Y 6/2	10Y 5/2	Colorless	Colorless	Colorless
Average current speed (m/s)	0.23 (0.02 – 0.53)	1.23 (0.81 – 1.69)	0.49 (0.14 – 0.74)	0.06 (0.03 – 0.08)	0.07 (0.04 – 0.10)	0.12 (0.01 – 0.34)
Average water debit (m^3/s)	0.01 (0.01 – 0.01)	0.26 (0.14 – 0.39)	0.05 –	0.04 (0.03 – 0.06)	0.04 (0.03 – 0.05)	0.02 (0.01 – 0.04)
Water temperature (°C)	21.5	22	25	24	24	23
Water pH	6.5	6.0	6.0	6.0	6.5	6.5

* the slope of the station 7 could not be evaluated using our habitual method because the creek associated to this station is curved, therefore we could not see each other from the beginning of the station to the end of it. Thus, it was evaluated by visual approximation.

Shading seems to be abundant, therefore, vegetation cover appears to be very good (figure 11). Station 3 was the only one with the presence of aquatic plants, which is probably linked to its more important exposure to sunshine. This is linked to its rocky riverbed, which naturally widens its course and prevents full coverage from nearby vegetation. The coverage of the water seems to limit primary productivity, which is normal for small mountain creeks.

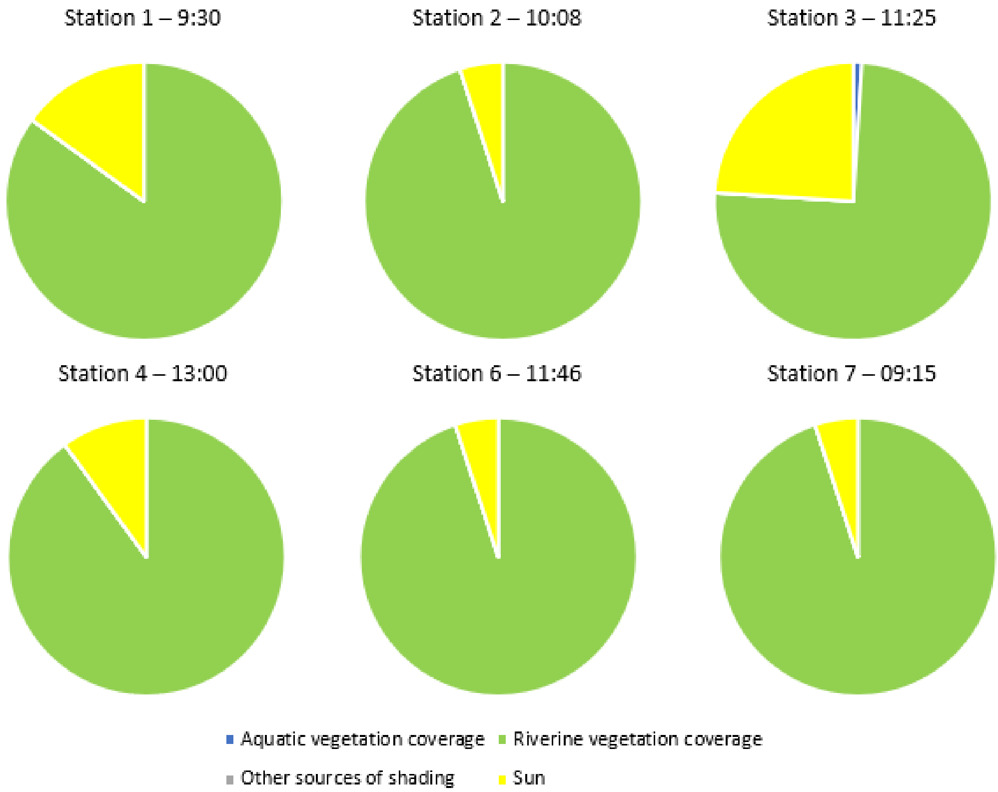

Figure 11 Percentage of coverage by sources of shading per station (the time of the observation was noted in case of significant bias in shading estimations)

The type of substrate in each creek seems to vary; although three stations are dominated by 1 mm to 5 mm sediments, the three others have different dominant substrates (figure 12). As stated before, station 3 has an abundance of mother rock. Additionally, three stations (station 2, 4 and 7) out of six contain visible accumulations of fine sediments (<1 mm).

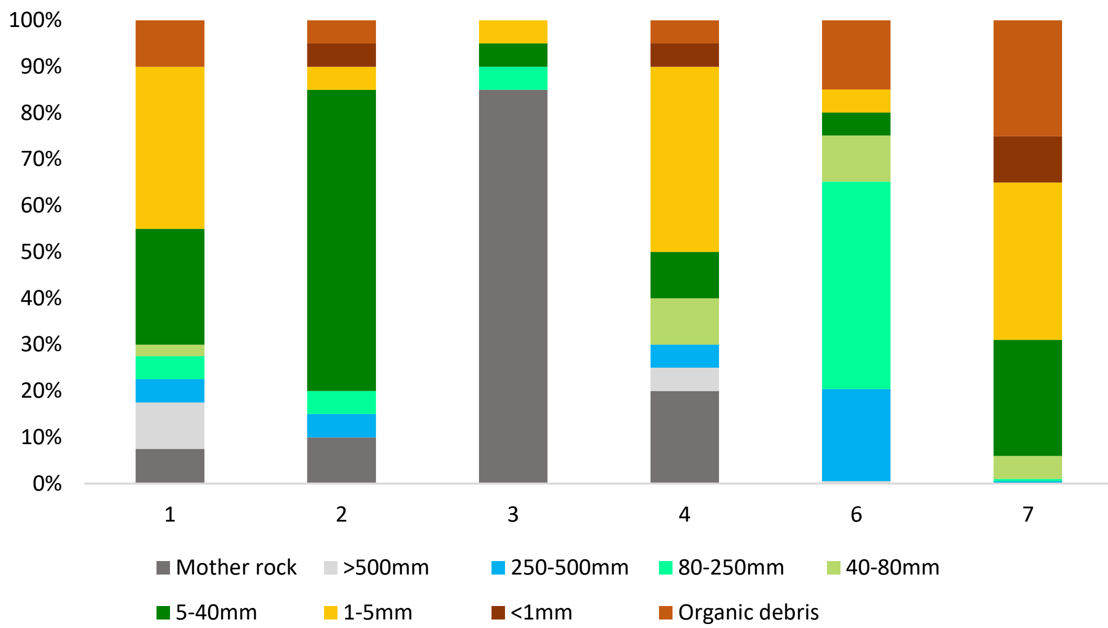

Figure 12 Percentage of riverbed occupied by different substrate sizes, per station

Riverbank composition analysis shows that station 7 is the only one with riverbanks dominated by naked ground (figure 13). All other creeks had a riverbank dominated by trees and bushes. Those trees and bushes protect the banks from erosion risks with their roots (figure 14).

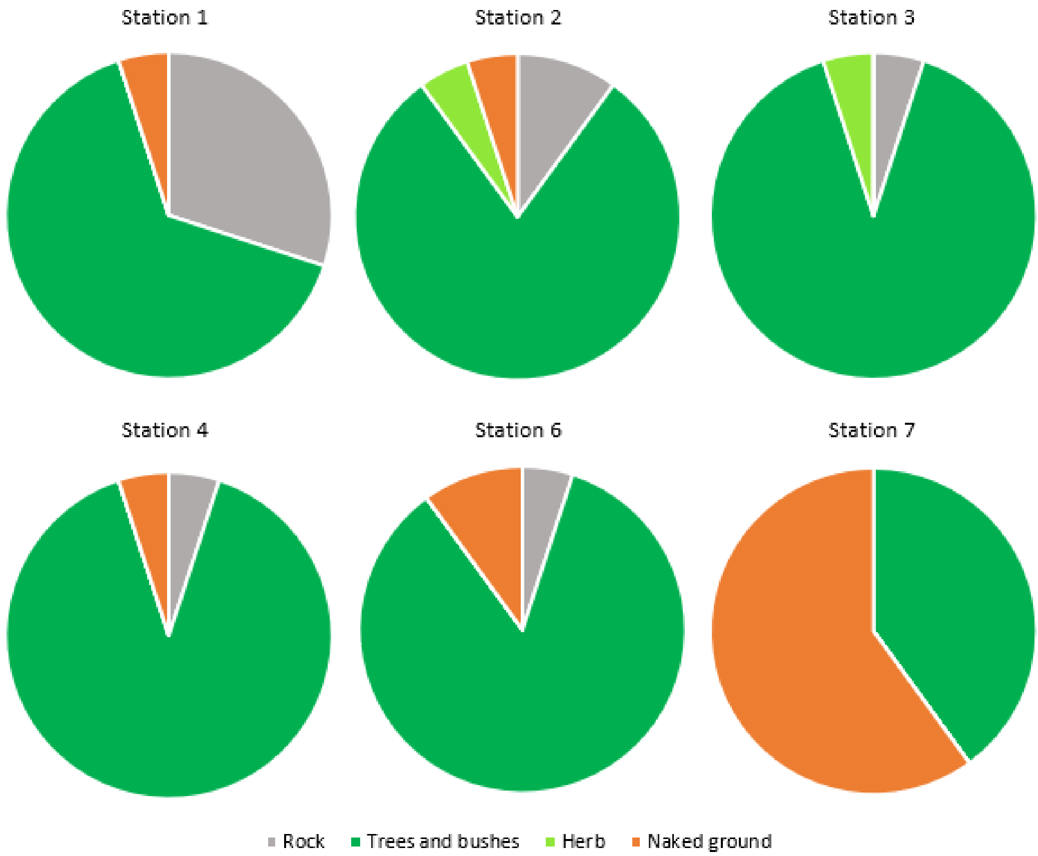

Figure 13 Percentage of banks covered by different types of riverbank, per station

Figure 14 Example of a tree's roots supporting the soil of the riverbank of the station 6 (Stéphanie Villeneuve, October 2020)

The presence of certain structures that may affect the biodiversity is shown in the table 3 below. In every station, shelters and thresholds were present, and no culvert was observed. Most of the stations had stream pits. The majority of the stations did not have watercourse catchments and were not disturbed by trails. Trails and culverts were present elsewhere on the farms.

Signs of erosion because of cow passage were observed in station 1. Also, the presence of a barbed wired fence passing through the creek at the end of station 7 is relevant to keep in mind as it may cause water catchments or hurt local fauna if improperly maintained. The fence marks the border between Norteak's farm and another farm, owned by a cattle farmer.

Table 3 Types of structures present in the different studied stations (presence = green, absence = red)

	Station					
Presence of:	1	2	3	4	6	7
Shelter	green	green	green	green	green	green
Threshold	green	green	green	green	green	green
Stream Pit	green	red	red	green	green	green
Watercourse catchment	green	red	red	red	red	red
Spawning ground	green	green	green	green	green	green
Water spring	red	green	red	green	red	red
Culvert	red	red	red	red	red	red
Trail	red	green	red	red	red	red

The observation of adequate spawning grounds depends on the species discussed. For mollies (*Poeciliidae* sp.), we consider each creek to be a proper spawning territory, given that this group of fish is viviparous. For convicts (*Amatitlania nigrofasciata*), the presence of a flat surface on which males can dig up a pit, such as rocks covered with gravel, is sufficient for the couple to lay their eggs (Mindy Nelson, 1995; Piron, 1978). The reproduction of guapotes (*Parachromis* sp.) seems to follow similar conditions (van den Berghe, López Pérez, R. McKaye, & K. McCrary, 1999). As for *Cynodonichthys isthmensis*, it appears as though peat moss or dark gravel is an appropriate spot for laying their eggs (Garman, 1895). Reproduction of this species and of swamp-eels (*Synbranchidae* sp.) is, however, very poorly documented.

Other observations were also documented. Natural obstacles for fish access were observed in the first three stations (high thresholds). In the second station, there was trash in the segment. Station 1 and 2, displayed a lot of crab holes on the banks (figure 15). Station 3 had a lot of crabs, but there were not many crab holes likely because the riverbanks were mostly rocky. An abundance of roots coming out of the banks and serving as hiding and/or reproduction spots for fish was observed in station 1, 6 and 7 (figure 16). Station 2 had deep areas that could potentially be used as refuge in dry periods. We found a young South American snapping turtle (*Chelydra acutirostris*) as well as a dead Cat-eyed snake (*Leptodeira* sp.) near station 3 (figure 17). This station was between two waterfalls of over 2 meters. Snails were abundant in every station and a caridean shrimp (*Caridea* sp.) was captured in station 2.

Figure 15 Crab holes observed on San Antonio's farm (Stéphanie Villeneuve, 01-X-2020)

Figure 16 Roots on riverbanks in Laos's farm (Stéphanie Villeneuve, 26-X-2020)

Figure 17 Dead Cat-eyed snake (*Leptodeira* sp.) and South American snapping turtle (*Chelydra acutirostris*) (Stéphanie Villeneuve, 14-X-2020)

4.2 Fish

Six different fish taxa were found in the studied creeks (annex X). Among those, *Synbranchidae* sp., was only found in the first station (table 4). Guapotes (*Parachromis* sp.) were only observed in stations 6 and 7. Station 3 only had mollies (*Poeciliidae* sp.), and station 4, which belongs to the same creek as station 3, only had two species. Mollies were the only fish found in every station. Finally, station 6 was the only station in which there were no fish with apparent signs of sickness.

Table 4 Number of fish captured in each creek per taxon and percentage of sick individuals

Taxon	Creek					
	Station 1	Station 2	Station 3	Station 4	Station 6	Station 7
Amatitlania nigrofasciata	0	7	0	0	6	1
Astyanax aeneus	3	0	0	0	0	1
Astyanax sp.	8	0	0	0	1	1
Cynodonichthys isthmensis	1	8	0	4	0	0
Parachromis sp.	0	0	0	0	1	1
Poeciliidae sp.	7	6	8	13	6	1
Synbranchidae sp.	2	0	0	0	0	0
Total of species	4*	3	1	2	4	4*
Total of individuals	21	21	8	17	14	5
% of sick individuals	71	29	75	59	0	40

**Astyanax aeneus* and *Astyanax* sp. were counted as one taxon due to the likelihood that the remaining *Astyanax* sp. are *Astyanax aeneus*

Mollies heavily outnumbered the other species captured in the studied creeks combined and seem to be the dominant fish species (figure 18). However, it is important to consider that mollies were slightly easier to capture than the other species.

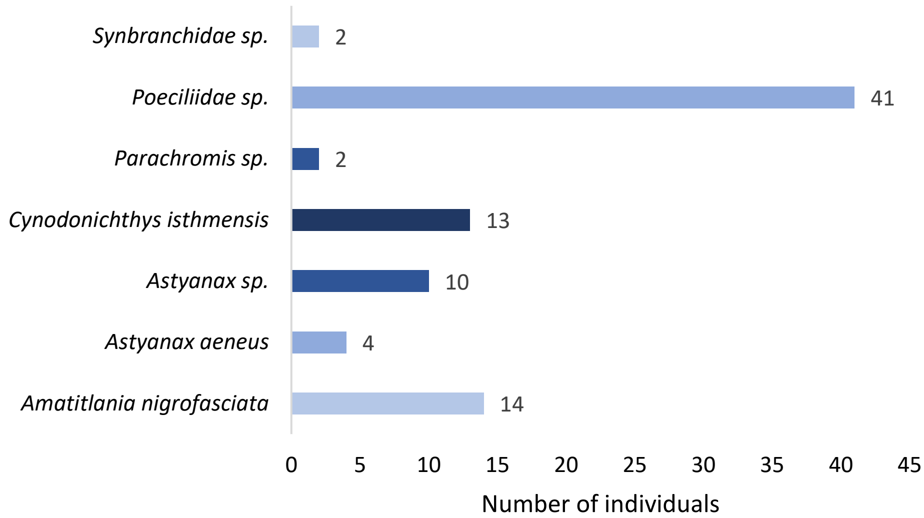

Figure 18 Number of fish found in the studied creeks in October 2020 according to their species (n=86)

4.3 Benthos

As for the benthos collected in each creek, we can see in figures 19 and 20 below that station 1 and 2 were really poor in terms of the quantity of individuals (only 8 in station 2) and in terms of biodiversity (only 7 in both these stations) in comparison to the other stations. Station 3 had almost as much lentic individuals and species as lotic individuals. Station 7 has the highest number of captured individuals (344) as well as the highest number of taxa (30). However, almost all individuals and species were found in the lotic sample. The lentic sample was surprisingly poor in specimens. In total, 792 invertebrates were captured using this standardized method. This represents close to 800 individuals caught in 12 minutes, on a total surface area of less than 6 m^2.

In total, 48 different taxa of aquatic invertebrates were identified in the creeks on Norteak's property. Thirteen of these taxa were only detected through the active hunt (Annex 3 and 4).

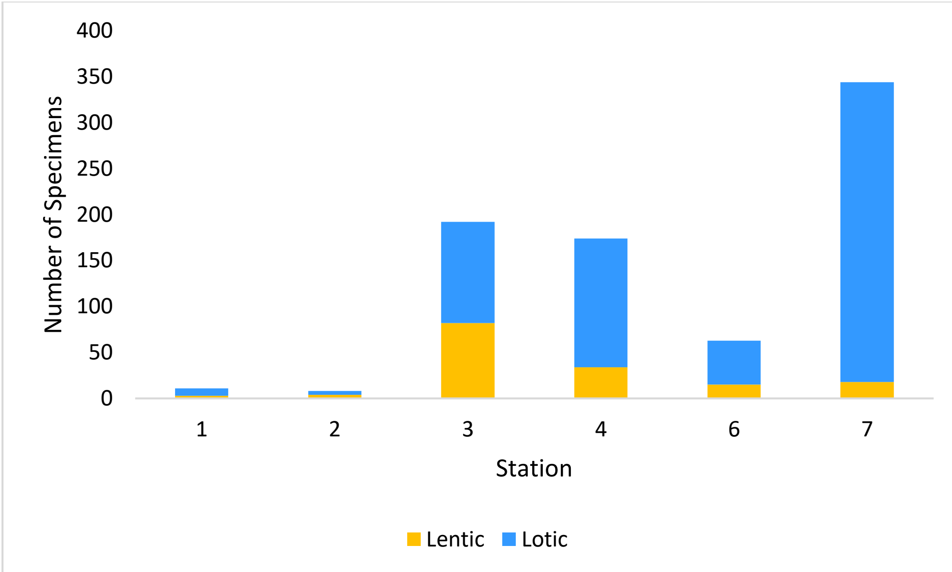

Figure 19 Quantity of specimens collected in each station according to their microhabitat (lentic or lotic), excluding specimens caught by active hunt

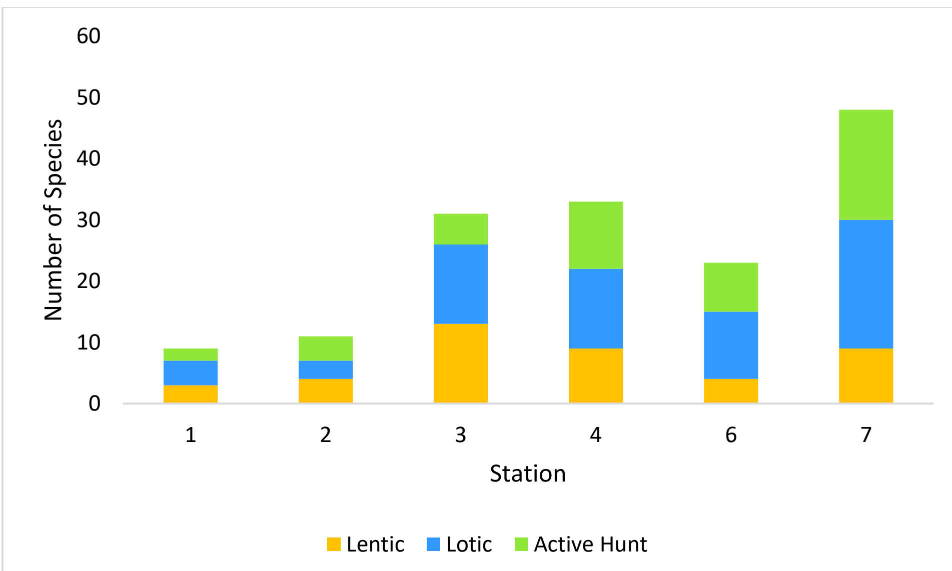

Figure 20 Diversity of aquatic taxa per station according to their microhabitat (lentic or lotic) or the hunting method (benthic catchment or active hunt)

According to the EPT indicator, the water quality of each creek was good (table 5). The FBI indicator (IBF-SV-2010), however, showed that the water quality varied between regular and good. As anticipated, the FBI (IBF-SV-2010) seems to differentiate contamination levels better than the EPT indicator. The first two creeks studied were not evaluated with these methods, since there was not enough aquatic invertebrates collected to perform a reliable analysis.

Table 5 Water quality of each creek according to the EPT indicator and the FBI indicator (IBF-SV-2010), following the analysis of the benthic species collected in October 2020

	Indicator			
Station	EPT Value	EPT Significance	FBI-SV-2010 Value	FBI Significance
3	64,58	Good	5,61	Regular
4	67,24	Good	5,31	Regular
6	66,67	Good	5,57	Regular
7	52,33	Good	4,63	Good

5. Discussion

When performing an assessment of the water quality and ecological state of streams, one must consider the activities taking place in the watershed and determine the most significant threats to the ecosystem. Streams and rivers near agriculture, livestock and forestry activities are vulnerable to potential organic contamination and excess sediments (Moss, 2007). Sediments can become problematic when fine particles clog the substrate, reducing benthic invertebrate habitats (Firmiano, Castro, Linares, & Callisto, 2021). Organic sediments can also become problematic because they lead to lower dissolved oxygen in the creeks, which can prevent more fragile species from inhabiting them (Resh & Jackson, 1993). Sediments in the creeks observed may come from various sources both natural and anthropic. Among these are: crab populations making burrows on the riverbanks, cows creating erosion (especially on the riverbanks), patches of naked ground, strong currents, runoff water from neighbouring lands, man-made roads and trails, steep slopes, and frequent flash floods. Because of the abundance of sources, it is often difficult to pin-point the origins of excess sediments.

Despite this fact, elaborate analysis can lead to strong hypothesis. In our case, our results show that station 2, 4 and 7 had more fine sediments than the other creeks. We think that, regarding station 2, this might be explained by the stronger current increasing erosion of the banks. For station 4, the explanation might reside in the fact that this creek is situated at the bottom of strong slopes. Finally, station 7 had the most important proportion of finer sediments, possibly because of the erosion of its riverbank caused by cows.

Additionally, to sediment inputs, fish are an important aspect to consider when evaluating the ecological value of creeks. The species found and their specific behaviours may indeed provide some valuable information on the creek's conditions. For example, mollies (*Poeciliidae* sp.) were likely the most abundant fish because of their viviparous reproduction behaviour. By avoiding an external egg stage, viviparous fish manage to increase survival rates. Mollies are known for their ecological role for controlling mosquito populations, given that they are omnivorous with a preference for immature stages of mosquitoes (Hess & Tarzwell, 1941). Their presence in Norteak's creeks is possibly helping reduce the transmission of illnesses transferred by mosquitoes. Swamp-eels (*Synbranchidae* sp.) are air breathers, which allows them to thrive in deoxygenated waters and permits them to make short travels over land (van der Sleen & S. Albert, 2018, p.399). Their presence in station 1 alone may therefore be a sign that the water of this site may be deprived of a proper oxygenation. The cyprinodontiformes order is also known to contain air-breather species, which indicates *Cynodonichthys isthmensis* may as well have this ability (Graham, 2011, p.1852). Studies on this species lifestyle have yet to be done. *Parachromis* sp. and *Amatitlania* sp. are both famous for their substantial parental care, which results in a much higher reproductive success (Townshend & Wootton, 1985; van den Berghe et al., 1999). Thanks to extensive parental care, these fish are relatively resilient. The genus *Astyanax* adapted its behaviour to diminish its mortality rates in unstable hydraulic systems by reproducing earlier (their mature size is smaller than most genus from the same family). This allows *Astyanax* to be successful in a river with high frequency of hurricanes (Trujillo-Jiménez, Elias Sedeño-Díaz, A. Camargo, & López-López, 2013). This adaptation may explain their abundance in Norteak's creeks, where flash floods and hurricanes occur.

All the species found in this study are either omnivorous or carnivorous, which means they all eat aquatic insects. This reinforces the idea that aquatic invertebrates are very important in the creeks, as they are at the base of the food chain.

Signs of health issues were observed in most of the fish collected, as shown in the pictures below (figure X). The illness, black spots illness, is very common and does not represent any danger to human health. It is caused by a trematoda parasite that infects aquatic snails in its first life stage. The actively swimming worm then penetrates the skin of fish, its second host, and the fish respond to this intrusion of a foreign organism by surrounding the cyst with black pigmented melanin. Those black spots are often visible to the naked eye. The definitive hosts are fish-eating birds and mammals that complete the life cycle by releasing eggs into the water with feces. It seems as though there are presently two species of those parasites present in the studied creek, given that two forms of black spots were observed (figure 21).

It is commonly known that stress causes depressed immune capacities in fish, therefore increasing the risk of catching an ailment (Schreck, Contreras-Sanchez, & Fitzpatrick, 2001). Stress factors in a natural habitat are diverse and common (predation, quality of water, temperature, sunlight, etc.). The fact that fish collected in station 6 did not present any signs of illness may be related to low stress factors. More investigation is necessary to confirm this preliminary hypothesis.

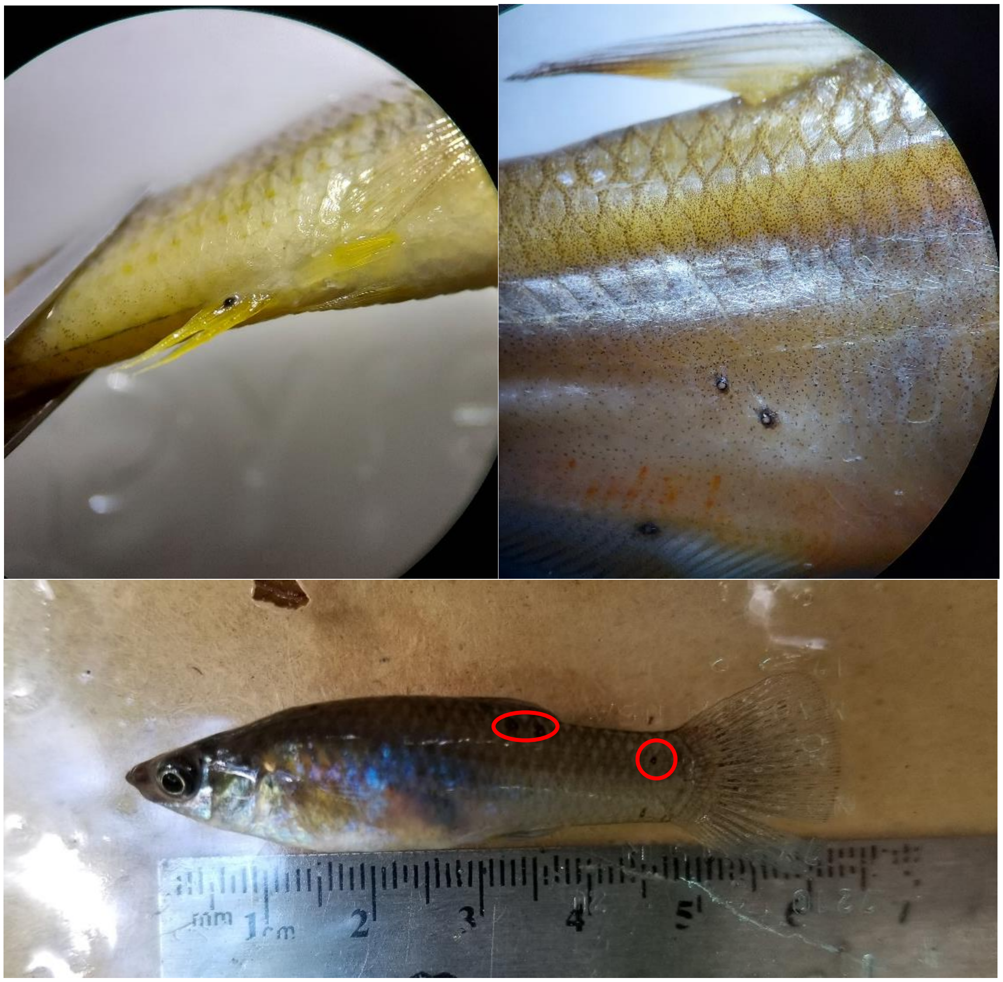

Figure 21 Pictures showing the black spots illness present in the fish collected in Norteak's creek. Top left: bulb-like black spot. Top right: cyst-like black spot. Bottom: Example of infected individual (Stéphanie Villeneuve, October 2020)

Aquatic invertebrate bioindicators can highlight the presence or absence of various stress factors. In this study, we used bioindicators to evaluate potential stresses caused by organic contamination. Stations 1 and 2 were excluded from the analysis because of the low quantity of aquatic invertebrates sampled. This may reflect high stress since abundance was likely lower than the other creeks. However, more sampling would be needed to properly evaluate these creeks using biomonitoring. The analysis of benthic species in the other creeks showed that the water quality varied between regular and good. Regular quality water should be interpreted as water likely containing substantial organic contamination, while good quality water should be interpreted as likely containing organic contamination (Hilsenhoff, 1988). Sources of organic contamination in Norteak's creeks may include laundry soaps, herbicides, inappropriate land use (ineffective riverbank protection), livestock erosion, sediments from road systems, etc. Most of these sources seem to represent minor risks.

The water quality observed through the bioindicators should be considered adequate. However, biomonitoring should be carried out to intervene properly in case of degradation. As Norteak's plantations grow, the most likely sources of contamination are road erosion and runoff waters. As of now, we believe that the quality of the water obtained can be explained by some levels of runoff water from the roads and the plantations during intense rainfalls and by livestock erosion, considering that bovine introduction has not yet been perfectly controlled.

Station 3 represents what could be described as an ideal situation, given that it had high biodiversity across both microhabitats (lotic and lentic). This points to highly functional diversity and ecosystemic resilience (all regions of the creek were favorable to benthic fauna). On the other hand, station 7 showed somewhat surprising characteristics. It appeared to have better water quality than the other creeks according to the FBI-SV-2010 indicator, but a more thorough observation of the creek shows a more complex situation. The FBI-SV-2010 value may indicate low organic contamination, but sediment observations and the distribution of invertebrates within the creek suggest high sediment inputs. Indeed, this station had the most important proportion of fine sediment deposits and the invertebrates sampled were mostly found in the lotic zone showing that fine sediments may reduce habitat availability throughout the creek. This demonstrates that mineral sediments can also have an impact on the biota. We indeed believe, following field observations, that these fine sediments may be the result of cattle trampling of fragile riverbanks increasing hydraulic erosion.

Biomonitoring enables us to understand creeks with a temporal component, while direct measurements of water quality (such as temperature, pH, etc.) tend to miss ephemeral and intermittent contamination. These methods are also often very fast and simple to carry out. However, while performing biomonitoring, it is important to keep in mind that other factors may influence the distribution of the studied species, such as floods, the size and the depth of the creek, turbulence and velocity of the water, predators, the seasons and the type of substrate. In general, the knowledge of the ecology and taxonomy of tropical aquatic invertebrates is still very incomplete. Among other limitations of biomonitoring, we could mention the differences in biological response of different aquatic species to various contaminants and the difficulty of identification of certain groups. In an effort to increase confidence in the bioindication results obtained, it is appropriate to also measure some of the main physicochemical characteristics of creeks that could account for some differences in the results. These main physicochemical components are the altitude, the water temperature, dissolved oxygen concentration, phosphate concentration and the total of dissolved solids (Sermeño-Chicas et al., 2010).

6. Emblematic species

During the study, no rare or particularly vulnerable aquatic species were found. However, some species are noteworthy.

Three fish species caught our attention. Mollies (*Poeciliidae* sp.) were abundant in Norteak's creeks and may serve an important role in the reduction of mosquito populations. Black Convicts (*Amatitlania nigrofasciata*) were also common in the studied creeks. They are noteworthy due to their popularity as aquarium pets. Black Convicts are visually attractive due to their vibrant colors, especially during the breeding season. The last fish that we found to be interesting was the Guapote (*Parachromis* sp.) which can use some of Norteak's creeks as breeding ground. This last species is commonly fished for food by local communities and may contribute to food security in the region. Many other edible species were observed, including crabs and snails, but remain marginally consumed compared to Guapotes.

As for invertebrates, very little is currently known to science about their ecology and the state of species abundance. We did, however, find a group that is interesting to highlight. Plecopteran species are very sensitive to water contamination and therefore likely facing strong declines in Nicaragua. Their presence in Norteak's creeks is good news for the perennity of these little-known critters. They are also interesting for Norteak because of their strong reaction to water contamination, making them excellent bioindicators to follow.

7. Concluding points

- During the study, we found 48 different taxa of macroinvertebrates and 6 different fish taxa.
- Using the EPT indicator and the FBI-SV-2010 indicator, we found that the water quality was regular to good. Regular quality water should be interpreted as water likely containing substantial organic contamination, while good quality water should be interpreted as likely containing organic contamination (Hilsenhoff, 1988).
- The most likely contaminant or source of degradation of Norteak's creeks is organic contamination and excess sediments.
- It is therefore important to properly maintain forest roads and conserve riverine forests in order to reduce the likelihood of contamination.
- The establishment phase and the cutting phase of Norteak's plantations are of higher risk for contamination and therefore a monitoring plan of the creeks should be implemented.

8. Recommendations

1. Include environmental considerations in the maintenance plan of forest roads

Forest roads are a likely source of sediment contamination to rivers in sectors dominated by forestry (Christie & Fletcher, 1999; USDA, 2001). Proper maintenance and checkups considerably help reduce those sediment inputs. Preventive measures are a good way to reduce cost of proper water management. Among these measures, one could consider proper water deviation from roads, the use of enrockment to prevent erosion, construction of sediment barriers and structures dissipating the runoff water's strength.

2. Experimental forest cut

Considering that the final cut of the plantations represents a period of higher risk of contamination for the creeks, we recommend making a test forest cut near a small creek to evaluate if there are any significant impacts. This should be done by estimating the abundance of various sediments following our evaluation method of the riverbed. The EPT indicator method is also important as it would testify to any significant organic contamination. We recommend using the same benthic sampling method as previously described in this report. In addition to these measurements, the temperature of the water, water debit, pH, transparency and color of the water and the composition of the riverbanks should be observed. The previous surveillance methods should be carried out two weeks after the cut to check for immediate repercussions, after six months to verify gradual effects and after a year to obtain a global vision of the impacts following the various seasons. See annex 11 for a recommended field data sheet. This step would enable Norteak to understand potential impacts and develop preventive measures if necessary.

3. Periodic checkups and surveillance during and after plantation establishment and harvesting

In an effort to protect aquatic ecosystems, we recommend basic surveillance of aquatic invertebrates. To maximize understanding of invertebrates, we recommend proceeding to two samplings, one during the rainy season (November) and one during the dry season (April) (Suárez Thelma, 2015). The sampling can be done following the method used in this report. Standardized sampling enables comparisons between years and creeks within Norteak's farms as well as the use of indicator analysis. We recommend Norteak applies an EPT indicator analysis to each sample they collect as a way to follow the water quality through time. We believe this indicator is the most appropriate for Norteak's use because of its simplicity and the lower risk of inaccurate identification.

Sampling should be done at least every five years as a basic monitoring strategy. It is also important to carry out sampling of macroinvertebrates during and after plantation establishment and harvesting. During the establishment phase, sampling should be done two weeks after each herbicide weed removal treatment and one year following the last herbicide treatment. The first sampling should be done two weeks after the final cut to detect any immediate effect, and the second sampling should be done one year after the final cut to document gradual effects.

9. Flooded forest of Bosque de Tanzania

We went to Bosque de Tanzania to do a preliminary description of its flooded forest. This type of habitat is rare and important for many unique species. These environments play a crucial role in carbon sequestration and are particularly vulnerable to human activities (Ferrara, Puhlick, Patterson, & Glover, 2020; Zamora et al., 2020). Because of their biological value, the ecological services they offer and their vulnerability, flooded forests like the one found in Bosque de Tanzania are critical for conservation. To this end, we went and carried out basic observations, measurements, and identified dominant aquatic animals. During our visit, the flooded section was relatively dry with only a small residual creek was present (figure 22). Therefore, measurements focused on this creek.

Figure 22 Photo representing what Bosque de Tanzania typically looks like when flooded (right) (Ove Faubry) and photo of the creek observed in the flooded section of Bosque de Tanzania (left) (Kevin Gauthier, 16-X-2020)

Measurements were taken at the following coordinates: 12.64289, -85.38206. Precipitations 72 h prior to our visit were of 9.7 mm. The average width of the creek was 1.70 m, with a maximum width of 2.25 m and a minimum width of 1.30 m. The average depth was 0.12 m, with a maximum depth of 0.22 m and a minimum depth of 0.07 m. The color of the water was 5G 7/2 ( ), the transparency was 100% and the pH was 6.1. The average current speed was 0.012 m/s and the average debit was 0.002 m^3/s. The residual creek was well protected by vegetation from solar radiation, we estimate vegetation cover at 100%. The vegetation surrounding the creek was typically very dense with abundant vines (figure 23). Roots and fallen vegetation offered numerous shelters for aquatic life within the creek (figure 24).

Figure 23 Photo of typical vegetation covering the flooded section of Bosque de Tanzania (Kevin Gauthier, 16-X-2020)

Figure 24 Photo illustrating the abundance of fallen vegetation within the creek observed in Bosque de Tanzania (Kevin Gauthier, 16-X-2020)

We identified three different types of fish in this habitat (table 6). Fish in this creek were fairly small in comparison with the fish we captured in other creeks in this study. The average total length of all the fish studied in every creek except this one is 4.9 cm (n=95), and the average total length in this creek is 3.6 cm (n=9). This might indicate that the area represents important habitat for younger fish. Only one specimen showed visible signs of sickness (black spots).

Table 6 Characteristics and identifications of the fish captured in Bosque de Tanzania on October 16th 2020 (n=9)

Order	Family	Genus	Species	Average length (cm)	Quantity
Characiformes	Characidae	*Astyanax*	*Aeneus*	3,6	5
Cichliformes	Cichlidae	*Amatitlania*	*nigrofasciata*	3,3	2
Cyprinodontiformes	Rivulidae	*Cynodonichthys*	*Isthmensis*	3,8	2

Various other species were observed (figure 25). The insects observed in the table 7 were collected in the creek and the other species were the result of opportunistic observations in the study area. They represent the most easily observed species of the area. Mammals were detected through their tracks or vocalizations.

Figure 25 Photo of (a) a Gulf Coast toad (*Incilius valliceps*), (b) a convict cichlid (*Amatitlania nigrofasciata*), (c) an Isthmian rivulus (*Cynodonichthys isthmensis*), (d) a Banded tetra (*Astyanax aeneus*), and (e) a Howler monkey (*Alouatta palliata*) found in Bosque de Tanzania (Kevin Gauthier, 16-X-2020)

Table 7 Classification and number of different observed species in Bosque de Tanzania on October 16th 2020

Class	Order	Identification	Spanish name	Quantity
Insects	Coleoptera	Hydrophilini	Coleóptero	4
Insects	Coleoptera	Thermonectus	Coleóptero	1
Insects	Odonata	Libellulidae	Libélula	1
Insects	Hemiptera	Gerridae	Insecto	1
Amphibia	Anura	*Incilius valliceps*	Sapo Costero	N/A
Mammalia	Primates	*Alouatta palliata*	Mono Congo	N/A
Mammalia	Rodentia	*Dasyprocta punctata*	Guatusa centroamericana	N/A
Mammalia	Carnivora	*Nasua narica*	Coatí de nariz blanca	N/A

This unique ecosystem was relatively well preserved. The protection status of this forest certainly helps reduce risks of habitat degradation. However, there is a path crossing the forest that is frequently used by locals that cross on foot or horseback. This path leads to the formation of mud patches that may result in habitat degradation. More studies on the habitat requirements of the species present are needed. This path can also lead to the presence of trash in the forest and its flooded areas. During our visit we indeed observed trash in some places. We hope this unique ecosystem will continue to attract scientists in the future as much remains to be discovered about life in this forest.

10. Preliminary evaluation of the habitat of the American Crocodile (*Crocodylus acutus*) on Camboya Farm

The American crocodile (*Crocodylus acutus*) has been observed multiple times by the local population surrounding Río Grande Matagalpa in the area of Camboya farm. This species is of particular interest due to its high vulnerability to human activities and poaching. It is listed as a vulnerable species under IUCN classification (IUCN Red List, 2009) and it is prohibited to hunt it under the MARENA conservation act. Hence, we evaluated the value of Norteak's conservation area bordering Río Grande Matagalpa for the local American crocodile population.

We evaluated vegetation density and cover along the river edge, availability of loose soil, the slope of the riverbank, abundance of microhabitats favorable for hunting, exposure to waves and erosion. We also observed any signs of presence. Vegetation density is an important habitat component, especially for neonates. It prevents erosion, protects against waves that impede proper breathing and hunting behaviour and favors better food abundance (Thorbjarnarson, 1989). Crocodiles in riverine habitats often construct burrows which offer important protection against predators (including humans) and serve as a refuge for resting and thermoregulation. Burrows are typically large (many meters long) and their construction requires relatively loose soil

(Thorbjarnarson, 1989). In Haiti, crocodiles seem to avoid rocky shores with medium and steep gradients for habitat selection likely because steep slopes are less used by riverine fauna which represents a significant food source (Thorbjarnarson, 1984 in Thorbjarnarson, 1989). Favorable microhabitats for aquatic and riverine life were documented because they serve as important feeding grounds for American crocodiles. Neonates and young crocodiles eat aquatic insects and their larvae, as well as snails, which increase in abundance according to vegetation density. It is believed that young crocodiles may forage submerged vegetation and/or bottom sediments to find invertebrates to eat. As they grow in size, crocodiles eat larger preys with fish becoming their main food source (Thorbjarnarson, 1989). Fish tend to use submerged vegetation as shelter and hunting grounds. A special attention was paid to wave exposure along the river's edge as exposure to wave action represents one of the main factors affecting habitat selection among all age groups (Thorbjarnarson, 1989).

We trod carefully along the riverbank adjacent to Camboya farm between the points 12.68753, -85.32194 and 12.689, -85.32253 (figure 26). Along this segment, vegetation cover was high, representing 100% coverage on the bank and 25% aquatic coverage within the first 5 m from the riverbank (figure 27). The banks had steep slopes which have a low habitat value but had an abundance of diggable soil for burrow constructions. The edges of the river showed low exposure to waves and low levels of erosion. This is probably the result of high vegetation coverage. As seen on a neighbouring farm, removing riverine vegetation exposes the banks to high erosion risks (figure 28). Within our segment, two large submerged trees were observed offering favorable microhabitats for shelter and hunting to local crocodiles (figure 29). At least one sign of presence was observed (basking site) (figure 29). We also noted the presence of many vertebrates, such as a Central American Boa, and multiple reptile tracks (figure 30). These characteristics seem to represent the general aspect of the entire riverbank of Río Grande Matagalpa on the farm, meaning that the riverbank is relatively homogeneous within the farm's borders. It is also worth noting that the river is afflicted by high contamination levels from various sources upstream (ex. plastic litter).

Figure 26 Map of Norteak's farm Camboya, showing the section of the riverbank that was characterized on October 28th 2020 (red line) and the location of Río Grande Matagalpa and Río Congo

Figure 27 Photo illustrating the high vegetation density of Río Grande's riverbank and its steep slope (Kevin Gauthier, 28-X-2020)

Figure 28 Photo of the riverbank on the neighbouring farm where riverine vegetation was removed. Note the steep drop along the shoreline resulting from the high erosion levels. (Kevin Gauthier, 28-X-2020)

Figure 29 (Left) Photo of a basking spot of American crocodiles (*Crocodylus* acutus) in Norteak's protected riverine area of Río Grande Matagalpa. (Right) Photo of a typical microhabitat favorable to crocodiles along the riverine area of Camboya farm. Fallen trees along the banks provide shelter and quality feeding grounds for the species. (Kevin Gauthier, 28-X-2020)

Figure 30 Photos showing high vertebrate use of the riverine habitat of Río Grande Matagalpa; (a) Central American Boa (*Boa imperator*), (b), (c), (d) reptile prints (Kevin Gauthier, 28-X-2020)

In Colombia, crocodiles were reported to move to inundated areas and lagoons adjacent to main water courses during the rainy season. This seems to be a way of avoiding swift currents and the effects of flash floods (Medem, 1981 in Thorbjarnarson, 1989). It is likely that crocodiles in Río Grande Matagalpa share this behaviour due to the vulnerability of the river to flash floods and varying water levels. Considering this, we suspect Río Congo to be an important habitat component within the vicinity of the farm. We presume this river to be a refuge during the rainy season and the hurricane season.

While on the field, our local guide indeed shared his knowledge of previous observations in the low sections of this river. The first few hundred meters after the intersection of Río Congo and Río Grande Matagalpa offer suitable habitat for crocodiles. This section of Río Congo has coffee-colored water enabling individuals to dissimulate themselves in their habitat, dense riverine vegetation, abundant submerged vegetation, and wave exposure is low (figure 31). These characteristics seem to provide optimal habitat, especially for feeding behaviour, and offer shelter to individuals more vulnerable to swift currents.

Figure 31 Photos illustrating Río Congo's main habitat characteristics for American crocodiles (*Crocodylus acutus*); (a) high vegetation density, (b) coffee-colored water and (c) submerged vegetation (Kevin Gauthier, 28-X-2020).

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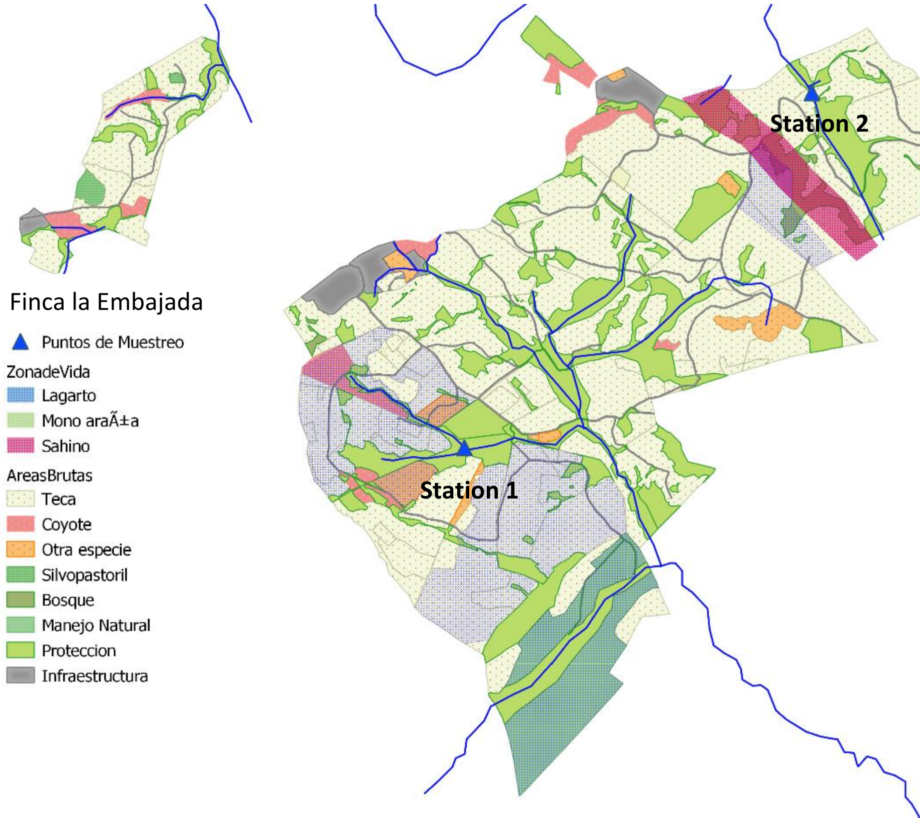
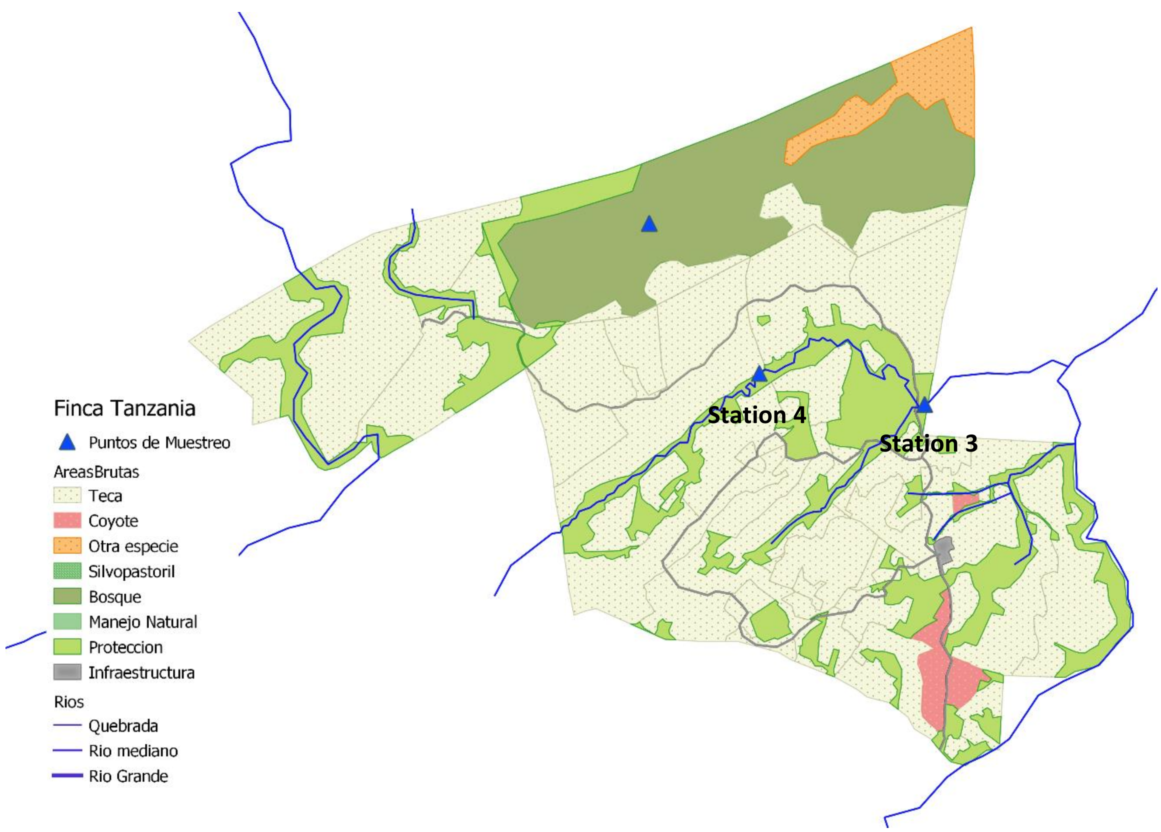
ANNEX 1: LOCATION OF THE STUDY SITES WITHIN EACH FARM IN OCTOBER 2020






Finca Laos
Puntos de Muestreo
AreasBrutas
Teca
Coyote
Otra especie
Proteccion
Rios
Quebrada
Rio mediano
Rio Grande
Station 6


Finca Birmania
Puntos de Muestreo
ZonadeVida
Lagarto
Mono araÃ±a
Sahino
AreasBrutas
Teca
Coyote
Otra especie
Silvopastoril
Bosque
Manejo Natural
Proteccion
Rios
Quebrada
Rio mediano
Rio Grande
Station 7

ANNEX 2: FISH SPECIES OBSERVED IN NORTEAK'S CREEKS IN OCTOBER 2020

Family	Identification	Spanish name	Locations observed	Photos
Characidae	*Astyanax aeneus*	Colirroja (Costa Rica)	San Antonio, Bosque de Tanzania, Birmania	
Characidae	*Astyanax* sp.	Sardinita Mexicana	San Antonio, Laos, Birmania	
Cichlidae	*Amatitlania nigrofasciata*	Carate; Punto rojo (Nicaragua)	La Gallina, Bosque de Tanzania, Laos, Birmania	
Cichlidae	*Parachromis* sp.	Guapote (Nicaragua)	Laos, Birmania	
Poeciliidae	*Poeciliidae* sp.	Topotes y espadas	San Antonio, La Gallina, Tanzania, Laos, Birmania	
Rivulidae	*Cynodonichthys isthmensis*	Olomina (Costa Rica)	San Antonia, La Gallina, Tanzania, Bosque de Tanzania	

Synbranchidae	*Synbranchidae* sp.	Anguila	San Antonio	

ANNEX 3: LIST OF COLLECTED BENTHIC SPECIMENS PER STATION ACCORDING TO THEIR MICROHABITAT, ABUNDANCE AND THEIR IMPORTANCE IN THE BIOINDICATOR CALCULATIONS (N=881), OCTOBER 2020

Station	Microhabitat	Order	Id.	Total	EPT	FBI sensitivity
1	lentic	Diptera	*Diptera* sp.	1		
		Ephemeroptera	*Leptophlebiidae*	1	1	5
		Unknown	*Animal* sp.	1		
	lotic	Diptera	*Diptera* sp.	1		
		Ephemeroptera	*Leptophlebiidae*	1	1	5
		Ephemeroptera	*Tricorhytidae*	3	3	
		Hemiptera	*Veliidae*	3		5
2	lentic	Diptera	*Diptera* sp.	1		
		Gastropods	*Gastropod* sp.	1		
		Odonata	*Aeshnidae*	1		4
		Odonata	*Corduliidae*	1		
	lotic	Ephemeroptera	*Ephemeroptera* sp.	2	2	
		Ephemeroptera	*Tricorhytidae*	1	1	
		Plecoptera	*Perlidae*	1	1	1
3	lentic	Coleoptera	*Psephenidae*	1		
		Diptera	*Chironomidae*	10		8
		Ephemeroptera	*Baetidae*	2	2	6
		Ephemeroptera	*Leptophlebiidae*	8	8	5
		Ephemeroptera	*Tricorhytidae*	38	38	
		Gastropods	*Gastropod* sp.	1		
		Hemiptera	*Belostomatidae*	2		7
		Hemiptera	*Veliidae*	1		5
		Odonata	*Calopterygidae*	1		7
		Odonata	*Gomphidae*	6		7
		Odonata	*Libellulidae*	1		7
		Unknown	*Pseudothelphusinae*	1		
		Unknown	*Turbellaria* sp.	10		5
	lotic	Coleoptera	*Elmidae*	6		4
		Coleoptera	*Psephenidae*	3		
		Diptera	*Simuliidae*	5		6
		Ephemeroptera	*Baetidae*	8	8	6
		Ephemeroptera	*Leptophlebiidae*	56	56	5

		Ephemeroptera	*Tricorhytidae*	5	5	
		Gastropods	*Gastropod* sp.	1		
		Hemiptera	*Veliidae*	3		5
		Odonata	*Coenagrionidae*	9		9
		Plecoptera	*Perlidae*	2	2	1
		Trichoptera	*Hydropsychidae*	4	4	5
		Trichoptera	*Philopotamidae*	1	1	5
		Unknown	*Turbellaria* sp.	7		5
4	lentic	Diptera	*Chironomidae*	10		8
		Diptera	*Diptera* sp.	2		
		Ephemeroptera	*Ephemeroptera* sp.	2	2	
		Ephemeroptera	*Leptophlebiidae*	8	8	5
		Ephemeroptera	*Tricorhytidae*	8	8	
		Hemiptera	*Gerridae*	1		6
		Odonata	*Coenagrionidae*	1		9
		Odonata	*Zygoptera* sp.	1		
		Trichoptera	*Leptoceridae*	1	1	4
	lotic	Coleoptera	*Elmidae*	18		4
		Diptera	*Chaoboridae*	1		
		Diptera	*Diptera* sp.	4		
		Diptera	*Simuliidae*	5		6
		Ephemeroptera	*Baetidae*	5	5	6
		Ephemeroptera	*Ephemeroptera* sp.	28	28	
		Ephemeroptera	*Leptophlebiidae*	60	60	5
		Ephemeroptera	*Tricorhytidae*	4	4	
		Odonata	*Coenagrionidae*	2		9
		Odonata	*Libellulidae*	2		7
		Odonata	*Zygoptera* sp.	2		
		Trichoptera	*Hydropsychidae*	1	1	5
		Unknown	*Turbellaria* sp.	8		5
6	lentic	Ephemeroptera	*Ephemeroptera* sp.	1	1	
		Ephemeroptera	*Leptophlebiidae*	5	5	5
		Ephemeroptera	*Tricorhytidae*	7	7	
		Odonata	*Coenagrionidae*	2		9
	lotic	Araneae	*Araneae* sp.	1		
		Coleoptera	*Elmidae*	5		4
		Coleoptera	*Psephenidae*	1		
		Diptera	*Diptera* sp.	1		
		Ephemeroptera	*Amelitidae*	3	3	
		Ephemeroptera	*Leptophlebiidae*	20	20	5
		Ephemeroptera	*Tricorhytidae*	3	3	
		Hemiptera	*Veliidae*	4		5
		Odonata	*Coenagrionidae*	6		9

		Trichoptera	*Hydropsychidae*	3	3	5
		Unknown	*Bivalve*	1		4
7	lentic	Coleoptera	*Elmidae*	2		4
		Diptera	*Athericidae*	1		
		Diptera	*Diptera* sp.	1		
		Ephemeroptera	*Amelitidae*	1	1	
		Ephemeroptera	*Leptophlebiidae*	1	1	5
		Ephemeroptera	*Tricorhytidae*	9	9	
		Hemiptera	*Gerridae*	1		6
		Odonata	*Gomphidae*	1		7
		Unknown	*Animal* sp.	1		
	lotic	Coleoptera	*Coleoptera* sp.	1		
		Coleoptera	*Elmidae*	116		4
		Diptera	*Diptera* sp.	5		
		Diptera	*Tipulidae*	3		5
		Ephemeroptera	*Baetidae*	5	5	6
		Ephemeroptera	*Leptophlebiidae*	67	67	5
		Ephemeroptera	*Schistonotes*	26	26	
		Ephemeroptera	*Tricorhytidae*	6	6	
		Gastropods	*Gastropod* sp.	2		
		Hemiptera	*Veliidae*	1		5
		Megaloptera	*Corydalidae*	20		7
		Odonata	*Calopterygidae*	1		7
		Odonata	*Coenagrionidae*	4		9
		Plecoptera	*Perlidae*	14	14	1
		Trichoptera	*Hydropsychidae*	19	19	5
		Trichoptera	*Philopotamidae*	32	32	5
		Unknown	*Pseudothelphusinae*	1		
		Unknown	*Turbellaria* sp.	3		5

Annex 4: Macroinvertebrates collected by active hunting (n=154) on Norteak's farms on October 2020 – species only found with this method are in green

Station	Order	Identification	Quantity
1	Odonata	*Libellulidae*	2
	Trichoptera	*Limnephilidae*	1
2	Hemiptera	*Belostomatidae*	1
	Hemiptera	*Gerridae*	1
	Hemiptera	*Veliidae*	3
	Odonata	*Calopterygidae*	1
3	Diptera	*Chironomidae*	1
	Ephemeroptera	*Baetidae*	9
	Ephemeroptera	*Schistonotes*	16
	Hemiptera	*Veliidae*	3
	Odonata	*Zygoptera* sp.	1
4	Coleoptera	*Staphilinidae*	1
	Ephemeroptera	*Baetidae*	2
	Ephemeroptera	*Schistonotes*	3
	Hemiptera	*Belostomatidae*	2
	Hemiptera	*Gerridae*	1
	Hemiptera	*Notonectidae*	1
	Hemiptera	*Pleidae*	1
	Hemiptera	*Veliidae*	1
	Odonata	*Calopterygidae*	1
	Trichoptera	*Limnephilidae*	1
	Unknown	*Turbellaria* sp.	1
6	Diptera	*Culicidae*	5
	Diptera	*Diptera* sp.	2
	Ephemeroptera	*Tricorhytidae*	4
	Hemiptera	*Gerridae*	11
	Hemiptera	*Hebridae*	1
	Megaloptera	*Corydalidae*	2
	Odonata	*Anisoptera* sp.	1
	Odonata	*Coenagrionidae*	2
7	Araneae	*Araneae* sp.	2
	Coleoptera	*Dystiscidae*	1
	Coleoptera	*Elmidae*	1
	Coleoptera	*Helolidae*	1
	Diptera	*Chironomidae*	4
	Diptera	*Culicidae*	5
	Diptera	*Diptera* sp.	1
	Ephemeroptera	*Leptophlebiidae*	7
	Ephemeroptera	*Schistonotes*	2
	Ephemeroptera	*Tricorhytidae*	21

Gastropods	*Gastropod* sp.	1
Hemiptera	*Gerridae*	6
Hemiptera	*Notonectidae*	12
Odonata	*Anisoptera* sp.	1
Odonata	*Calopterygidae*	1
Odonata	*Coenagrionidae*	4
Oligochaeta	*Oligochaeta* sp	1
Trichoptera	*Limnephilidae*	1
Total		**154**

Annex 5: List of other biodiversity observed during the study on Norteak's farms in October 2020

Class	English Name	Spanish Name	Scientific Name	Locations Observed
Insecta	Aquatic beetle	Coleóptero	Hydrophilini	Tanzania
Insecta	Aquatic beetle	Coleóptero	Thermonectus	Tanzania
Insecta	Dragonfly	Libélula	Libellulidae	Tanzania
Insecta	Water skeeters	Insecto	Gerridae	Tanzania
Amphibia	Gulf Coast toad	Sapo Costero	*Incilius valliceps*	Tanzania
Mammalia	Mantled howler	Mono Congo	*Alouatta palliata*	Tanzania
Mammalia	Central American agouti	Guatusa centroamericana	*Dasyprocta punctata*	Tanzania
Mammalia	White-nosed coati	Coatí de nariz blanca	*Nasua narica*	Tanzania
Reptilia	Common northern boa	Boa común	*Boa imperator*	Camboya
Gastropoda	Snail	Caracol	Gastropod sp.	San Antonio, La Gallina, Tanzania, Laos, Birmania
Malacostraca	Freshwater crab	Pseudotelfúsidos	*Pseudothelphusidae* sp.	San Antonio, La Gallina, Tanzania, Laos, Birmania
Reptilia	Snapping turtle	Tortuga mordedora	*Chelydra acutirostris*	Tanzania
Reptilia	Cat-eyed snake	Culebra	*Leptodeira* sp.	Tanzania
Arachnida	Brazilian wandering spiders	Araña bananera	*Phoneutria boliviensis*	Tanzania
Mammalia	Pale-throated sloth	Perezosos de tres dedos	*Bradypus tridactylus*	Laos
Aves	Kingfisher	Martín Pescador	*Chloroceryle* sp.	Laos
Insecta	Wasp	Avispa	*Hymenoptera* sp.	Birmania

ANNEX 6: EXAMPLE OF A CALCULATION SHEET FOR THE EPT INDICATOR, TABLE TAKEN FROM CARRERA & FIERRO (2001, P.43)

CLASIFICACIÓN	ABUNDANCIA (Número de Individuos)	EPT PRESENTES
Anisoptera		
Bivalvia		
Baetidae	25	25
Ceratopogonidae	1	
Chironomidae	15	
Corydalidae	5	
Elmidae	25	
Euthyplociidae	4	4
Gastropoda		
Glossosomatidae	2	2
Gordioidea		
Hirudinea		
Hydrachnidae		
Hydrobiosidae	5	5
Hydropsichidae	30	30
Leptoceridae	10	10
Leptohyphidae	5	5
Leptophlebiidae		
Naucoridae		
Oligochaeta		
Oligoneuridae	2	2
Perlidae	1	1
Philopotamidae	6	6
Psephenidae	5	
Ptilodactylidae	1	
Pyralidae	3	
Simuliidae		
Tipulidae		
Turbelaria		
Veliidae		
Zygoptera		
Otros grupos	8	
TOTAL	153	90
EPT TOTAL ÷ ABUNDANCIA TOTAL	ABUNDANCIA TOTAL	90 ÷ 153 = 0,58 0,58 x 100 = 58%

Calidad de Agua	
75 - 100%	Muy buena
50 - 74%	Buena
25 - 49%	Regular
0 - 24%	Mala

Annex 7: Example of a calculation sheet for the FBI-SV-2010 indicator, table taken from Sermeño-Chicas et al. (2010, p.26)

Grupos taxonómicos	Abundancia	Puntaje	Abd * Ptj	(Abd * Ptj)/Total
Diptera: Chironomidae	136	8	1088	4.217054264
Trichoptera: Hydroptilidae	3	4	12	0.046511628
Odonata: Coenagrionidae	2	9	18	0.069767442
Ephemeroptera: Leptohyphidae	60	6	360	1.395348837
Ephemeroptera: Leptophlebiidae	38	5	190	0.736434109
Gastropoda: Planorbiidae	14	7	98	0.379844961
Decapoda	5	6	30	0.11627907
Abundancia total	**258**	**IBF-SV-2010**		**6.96**

Annex 8: Table of water quality following the calculation of the FBI-SV-2010 indicator, table taken from Sermeño-Chicas et al. (2010, p.26)

VALOR IBF-SV-2010	CATEGORIA	CALIDAD DEL AGUA	INTERPRETACIÓN DEL GRADO DE CONTAMINACIÓN ORGÁNICA
0.00 – 3.75	1	Excelente	Contaminación orgánica improbable
3.76 – 4.25	2	Muy buena	Contaminación orgánica leve posible
4.26 – 5.00	3	Buena	Alguna contaminación orgánica probable
5.01 – 5.75	4	Regular	Contaminación orgánica bastante sustancial es probable
5.76 – 6.50	5	Regular pobre	Contaminación sustancial probable
6.51 – 7.25	6	Pobre	Contaminación muy sustancial probable
7.26 – 10.00	7	Muy pobre	Contaminación orgánica severa probable

ANNEX 9: TABLE OF ASSIGNMENT OF SCORES OR DEGREES OF SENSITIVITY TO CONTAMINATION OF THE DIFFERENT AQUATIC INVERTEBRATES, TAKEN FROM SERMEÑO-CHICAS ET AL. (2010, P.21-22)

Puntajes o Grados de sensibilidad a la contaminación de las aguas	Invertebrado acuático en los ríos de El Salvador	
	Orden	Familia
0	Diptera	Blephariceridae
1	Odonata	Corduliidae
		Platystictidae
	Trichoptera	Glossosomatidae
2	Odonata	Cordulegasteridae
	Plecoptera	Perlidae
	Trichoptera	Calamoceratidae
		Lepidostomatidae
		Odontoceridae
		Xiphocentronidae
3	Blattodea	
	Coleoptera	Gyrinidae
		Lampyridae
		Ptilodactylidae
	Ephemeroptera	Heptageniidae
	Trichoptera	Polycentropodidae
4	Bivalvia	
	Gastropoda	Hydrobiidae
	Coleoptera	Dryopidae
		Elmidae
		Hydroscaphidae
		Noteridae
		Psephenidae
	Hemiptera	Pleidae
	Odonata	Aeshinidae
	Trichoptera	Hydrobiosidae
		Hydroptilidae
		Leptoceridae
5	Acarina	
	Nematoda	
	Planaria	
	Amphipoda	
	Coleoptera	Hydraenidae
		Limnichidae
		Lutrochidae
	Collembola	
	Diptera	Dixidae
		Tipulidae
	Ephemeroptera	Leptophlebiidae

	Hemiptera	Corixidae
		Gelastocoridae
		Mesoveliidae
		Nepidae
		Notonectidae
		Saldidae
		Veliidae
	Lepidoptera	Crambidae
	Trichoptera	Helicopsychidae
		Hydropsychidae
		Philopotamidae
6	Decapoda	
	Coleoptera	Curculionidae
		Scirtidae
		Staphylinidae
	Diptera	Dolichopodidae
		Empididae
		Simuliidae
		Stratiomyidae
		Tabanidae
	Ephemeroptera	Baetidae
		Leptohyphidae
	Hemiptera	Gerridae
		Hebridae
		Naucoridae
	Odonata	Lestidae
7	Hirudinea	
	Gastropoda	Planorbiidae
	Coleoptera	Dytiscidae
		Hydrophilidae
	Diptera	Psychodidae
	Ephemeroptera	Caenidae
	Hemiptera	Belostomatidae
		Ochteridae
	Megaloptera	Corydalidae
	Odonata	Calopterygidae
		Gomphidae
		Libellulidae
8	Diptera	Ceratopogonidae
		Chironomidae
9	Gastropoda	Physidae
	Diptera	Ephydridae
		Muscidae
	Odonata	Coenagrionidae
10	Oligochaeta	
	Diptera	Culicidae
		Syrphidae

Annex 10: Field data sheet used in this study to describe major habitat components for fish and important hydrological features in each creek

Date: ________________ Lat. ________________ Weather: ____________________

Farm: ________________ Long. ________________ Precipitation of last 72h: ______ mm

Creek: ________________ Alt. _____ m Observers: ____________________

Station: ______________ Type of creek: ________________ ____________________

Dimensions of station segment	Minimum	Maximum	Average
Length (m)	-		-
Width (m)			
Depth (m)			

Slope: ______________% Color: ____________ Transparency: ______ m

	0m	12.5m	25m	
Current speed (m/s)	1.	2.	3.	Average:
Water debit (m^3/s)	1.	2.	3.	Average:

Presence of fish: ___Yes ___No Temperature (°C): ______ pH: ______

Vegetation type	Cover (%)
Aquatic	
Riverine	
Other sources of shading	
Time: _____:______	Total shading:________%

Type of substrate	% of riverbed
Mother Rock	
>500mm	
250-500mm	
80-250mm	
40-80mm	
5-40mm	
1-5mm	
<1mm	
Organic debris	

Type of riverbank	% of banks
Rock	
Tree and bush cover	
Herb cover	
Naked ground	

Presence of:	
Shelter	
Threshold	
Stream Pit	
Watercourse catchment	
Spawning ground	
Water spring	
Culvert	
Trail	
Other:____________	

Potential problem	Presence
Non-natural erosion	
Trash	
Obstacles for fish access	
Other:______________	

Photo number	Represented content

Comments:

ANNEX 11: RECOMMENDED DATA SHEET FOR INTERNAL SURVEYANCE OF CREAKS BY NORTEAK

Fecha: ________________ Lat. _________________ Estado del tiempo: _______________

Finca: ________________ Long. _________________ Responsable: ___________________

Quebrada: ____________ Alt. _____ m

Estación: ______________ Precipitaciones de los últimos 72h: ______ mm

Dimensiones del segmento	Mínimo	Máximo	5 mediciones (promedio)
Ancho (m)			1. 2. 3. 4. 5.
Profundidad (m)			1. 2. 3. 4. 5.

Color: ____________ Transparencia: ______ pH: ____ Temperatura (°C): _____

	0m	**12.5m**	**25m**	Promedio
Velocidad corriente (m/s)	1.	2.	3.	
Volumen de agua (m^3/s)	1.	2.	3.	

Tipo de margen de rio	**% del margen**
Piedra	
Árboles y arbustos	
Herbáceas	
Suelo desnudo	

Problemas potenciales	**Presencia**
Erosión no natural	
Basura	
Obstáculos para peces	
Otro: _____________	

Muestra de invertebrados	Tomada
Corriente	
Zona lenta	