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プランクトン工学研究

Report

Practical Science and Environmental Education Workshop in Manaus, Brazil

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1. INTRODUCTION

It is an unequivocal fact that Amazonian tropical forest is the largest remaining primary forest in the world. The ecosystem in the region is extremely complex with high biodiversity (Peres et al. 2010). Conservation and protection of the dynamic forest and river regions is extremely important not only for the natural environments, but also for the economy and social dependence of benefits from such abundant natural environments.

Important natural parameters that affect status of the natural environments include light (natural sunlight), soil, and water, which abundantly exist in the Amazon region. Solar energy is the primary energy source for the majority of living organisms in both terrestrial and aquatic ecosystems, and drives the diurnal and seasonal cycles of biogeochemical processes (Monteith & Unsworth 2013). In particular, in situ light data remains one of the most underappreciated data measurements although having a significant impact on the physical, chemical and biological processes in the ecosystem (Johnsen 2012). Soil provides the fundamental basis for all terrestrial living organisms including the Amazonian forests as well as life-sustaining infrastructure for human society. Water is the most essential single entity to constitute all organisms from a single cell to the earth. Understanding of importance and roles of each factor and interaction of such complex dynamics in the natural environments can serve as fundamental platform for natural scientists, particularly for young scientists such as university students.

The objective of this workshop was to provide hand-on scientific and environmental education for university students in Manaus, Amazonas, Brazil through practical field measurements using the three most important parameters in the natural ecosystem composed of natural sunlight, soil, and water. The workshop was divided into a series of lectures, in situ field sampling, and data processing, analysis and interpretation with the ultimate goal of empowering the undergraduate students with research-centered environmental education and experience of developing international collaboration.

1.1. Practical Science and Environmental Education Workshop

The workshop was conducted at the Instituto Soka Amazônia (ISA) and laboratories of Instituto Federal de Educação, Ciência e Technologia do Amazonas (IFAM), Manaus, Amazonas, Brazil from March 7 through March 10, 2023. The workshop schedule is summarized in Table 1.

Table 1. Workshop schedule

Date	Program	Conductor	Location
March 7	Lecture on Aquatic Ecology	Dr. Yoshiki Takayama	ISA Auditorium
	Lecture on Environmental Optics	Prof. Victor Kuwahara	ISA Auditorium
	Lecture on Soil Science	Prof. Shinjiro Sato	ISA Auditorium
	Environmental optical data collection	Prof. Victor Kuwahara	ISA campus
	Soil sample collection	Prof. Shinjiro Sato	ISA campus
March 8	Water and plankton samples collection	Dr. Yoshiki Takayama	Amazon River
	Environmental optical data collection	Prof. Victor Kuwahara	Amazon River
	Soil pH measurement	Prof. Shinjiro Sato	IFAM laboratory
	Water pH measurement	Dr. Yoshiki Takayama	IFAM laboratory
March 9	Soil available phosphorus measurement	Prof. Shinjiro Sato	IFAM laboratory
	Measurement of suspended solid concentration and plankton observation	Dr. Yoshiki Takayama	IFAM laboratory
	Calculations of data from light, soil, and water samples	Prof. Victor Kuwahara Prof. Shinjiro Sato Dr. Yoshiki Takayama	IFAM laboratory
March 10	Explanation and discussion on calculated data from light, soil, and water samples	Prof. Victor Kuwahara Prof. Shinjiro Sato Dr. Yoshiki Takayama	ISA Auditorium

2. MATERIALS & METHODS

2.1. Light Measurements

Natural sunlight was measured using an Apogee Instruments handheld quantum meter (MX-200X) fitted to a 102 cm water submersible sensor wand (AM-320), and all radiance measurements conducted in spot-mode. The sensor collects cosine corrected quanta (μmol m^{-2} s^{-1}) with a spectral response of (370 – 650 nm) for photosynthetically active radiation (PAR) in the air and water. Measurements were conducted at four sampling stations in the forest (described below), and at four sampling stations in the river (see Fig. 1b). Data collected in the river were corrected using the suggested immersion factor correction by multiplying values by 1.15. Quantum data were measured in triplicate at each location in addition to weather conditions, GPS location and local time (AMT).

Collected data was later processed to calculate diurnal variability [$E(t)$], daily insolation [Q_s], extinction coefficient [K_d] and/or euphotic depth [Z_{eu}] as follows (Kirk 2011):

$$E(t) = E_{noon}\sin(\pi t/N) \quad \text{(Eq. 1)}$$

where $E(t)$ is PAR quanta (μmol m^{-2}) at time t (hours), E_{noon} is PAR quanta at noon, t is hours after sunrise (hours) and N is day length from sunrise to sunset (hours).

$$Qs=\int_0^N E(t)dt \quad \text{(Eq. 2)}$$

where Q_s is the daily PAR insolation (μmol m^{-2} day), N is day length from sunrise to sunset (hours) and $E(t)$ is PAR quanta (μmol m^{-2}) at time t hours.

$$E_z = E_0e^{-kz} \quad \text{(Eq. 3)}$$

where Ez is PAR quanta (μmol m^{-2}) at depth (z) in centimeters (cm), E0 is PAR quanta (μmol m^{-2}) just below water surface and k is the attenuation coefficient (cm^{-1}).

$$Z_{eu} = 4.6/k \quad \text{(Eq. 4)}$$

where Z_{eu} is the euphotic depth, or the depth layer which E0 (PAR) falls to 1% of the surface value and k is the attenuation coefficient (m^{-1}).

2.2. Soil Samples

Soil samples were collected from four different locations within ISA campus: 1) dense upland forest with abundant organic matter (mata densa), 2) thin upland forest with insufficient organic matter (capoeira), 3) upland forest with presence of Indian Black Earth (IBE) or biochar (*Terra Preta de Índio*), and 4) lowland forest with seasonal flooding (baixio). At each sampling location, 3 sub-samples were collected using a shovel from the top 10-15 cm depth and combined in a bag to represent soil sample of the location. The collected soil samples were dried in an oven at 80 ° C for overnight, and sieved by 2-mm sieve for soil pH and available phosphorus (P) analyses.

2.2.1. Soil pH

Fifteen grams of dry soil sample were weighted using an electric balance into 50-mL centrifuge tube and 30 mL of distilled water was added to tube in triplicate. Tubes were shaken vigorously for 10 min manually. Tubes were let sit in tube rack for 10 min, and soil pH was measured using a pH meter.

2.2.2. Soil Available Phosphorus

Soil available P was extracted using Mehlich 1-extracting solution, which was prepared by sulfuric acid (H_2SO_4) and hydrochloric acid (HCl) to make the final solution concentrations of 0.0125 mol H_2SO_4 L^{-1} and 0.05 mol HCl L^{-1} (Mehlich 1953). Five grams of dry soil sample were weighted using the electric balance into 50-mL centrifuge tube and 20 mL of Mehlich 1-solution was added to tube in triplicate. Tubes were shaken vigorously for 10 min manually. Tubes were let sit in tube rack for 10 min, and supernatant was filtered through Whatman No. 1 equivalent filter paper. Phosphorus concentration in the filtrate was determined using P packtest (Kyoritsu Chemical-check Lab. Corp., Japan) by molybdenum blue method. Soil available P was calculated by using the following equation.

$$\text{Soil available P (mg kg}^{-1}\text{)} = \text{P concentration in filtrate (mg L}^{-1}\text{)} \times (0.02\ \text{L}/0.005\ \text{kg}) \quad \text{(Eq. 5)}$$

2.3. Aquatic Environment

The survey was conducted in the center of the Amazon basin where Solimões River and Negro River merge in Manaus, Brazil (Fig. 1a). The water and zooplankton samples were collected from 9:00 to

11:00 on 8th March 2023 during the rising water period (rainy season) at four sites across the rivers: the center of Amazon River (St. 1, S 03 ° 07.3596"S, W 059 ° 54.4180'), Amazon River side of the confluence (St. 2, S 03° 08.0770', W 059° 54.1392'), Solimões River side of the confluence (St. 3, S 03° 07.9152', W 059° 53.9902') and the center of Solimões River (St. 4, S 03° 08.0890', W 059° 53.4420') (Fig. 1b).

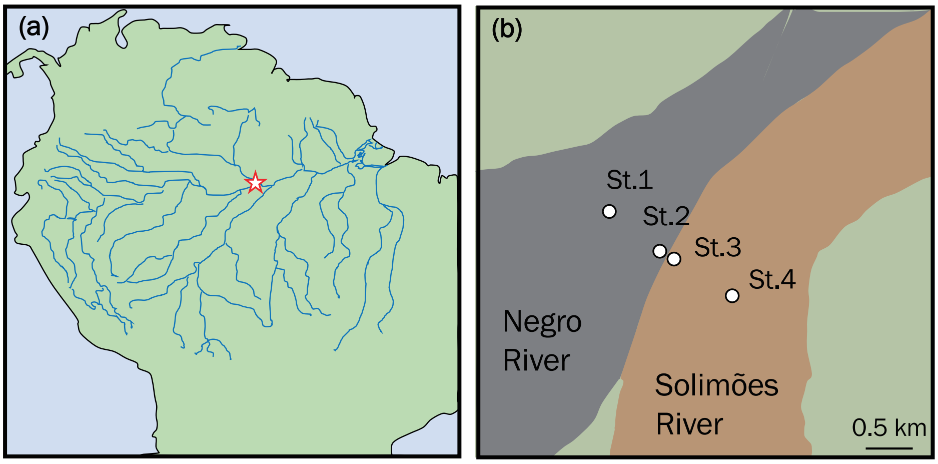

Fig. 1. Location of water and zooplankton sample collection. (a) the Amazon Basin in South America. (b) sampling sites across the two rivers: the center (St. 1) of the Negro River, the confluence (St. 2 & 3), and the center (St. 4) of the Solimões River.

On each sampling site, surface water was collected by a bucket and sieved through 180-μm nylon mesh to remove debris, and then transported to the field laboratory. The temperature of air and river surface water was measured at the time of field sampling by a mercury thermometer (Shinwa Rules Co., Ltd.). Triplicate water subsamples for pH were measured using a pH meter. Three replicate aliquots of water samples for suspended solid (SS) concentrations were filtered onto pre-weighted 1.6-μm glass microfiber filters (GF/A, Whatman Co., Ltd.). The filters were oven-dried at 60 °C for over 24 hours, then weighed by a microbalance. The SS concentration was estimated by the difference in the filter weight before and after sample filtration.

Zooplankton samples were collected by a single vertical haul from the 20 m depth to the surface, using a plankton net (mesh size 100 μm; diameter 30 cm; length 100 cm) equipped with a flowmeter (Rigo Co., Ltd.). The collected samples were immediately fixed in 5% buffered formalin–water solution. Under a dissecting microscope, zooplankton was identified using morphological characteristics and counted into four groups; copepods, cladocerans, fish larvae, insect larvae and others.

3. RESULTS

Fig. 2a, 2b, 2c, and 2d show light measurement on the research boat at the confluence of Negro and Solimões River, soil sample collection in the forest, water sample measurement in the laboratory at IFAM, and all participants and staff in the training workshop.

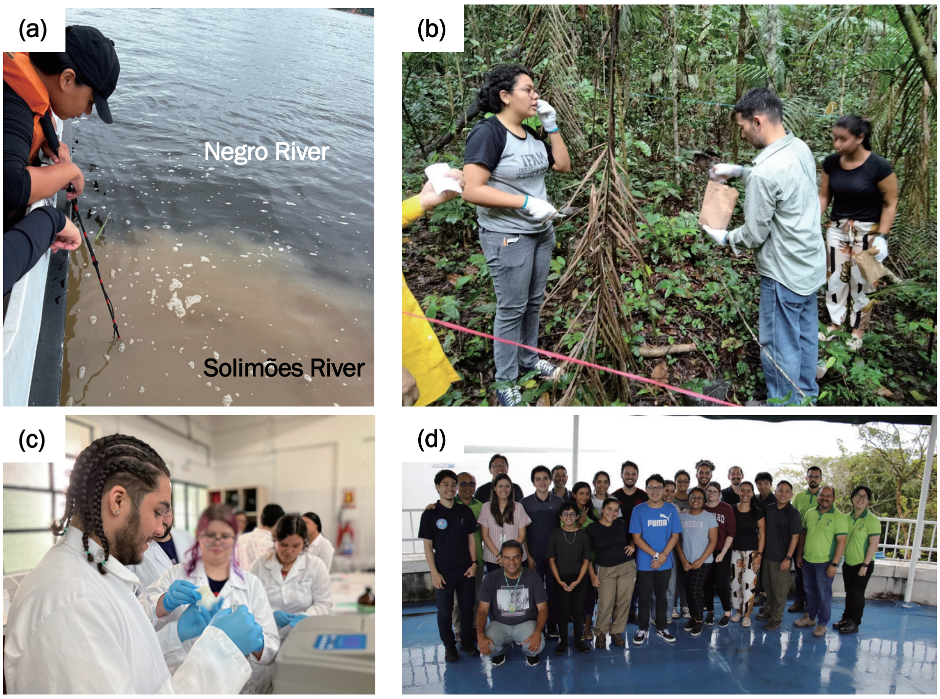

Fig. 2. Images of (a) light intensity measurement on the research boat at the confluence of Negro and Solimões River, (b) soil sample collection in the forest, (c) water sample measurement in the laboratory at IFAM, and (d) all participants and staff in the training workshop.

3.1. Light Measurements: Forest

Weather during quanta measurements in the forest was generally overcast, cloudy and unstable in the afternoon during the survey. Measurements from the capoeira forest station showed ~200, ~50 and ~10 spot-mode quanta (μmol m^{-2} s^{-1}) readings for Direct-Sky, Trail/Canopy and Canopy, respectively (Fig. 3). Using Eq. 1., the potential diurnal variability and solar insolation of solar radiance for each station was modeled assuming atmospheric conditions remained stable for each location (Fig. 3). Similar measurements and calculations were conducted at the mata densa and Terra Preta de Índio forest sampling stations (not shown). All air measurements conducted during the day showed seasonally low and unstable solar radiance, but revealed consistent differences between dense canopy and direct-sky measurements.

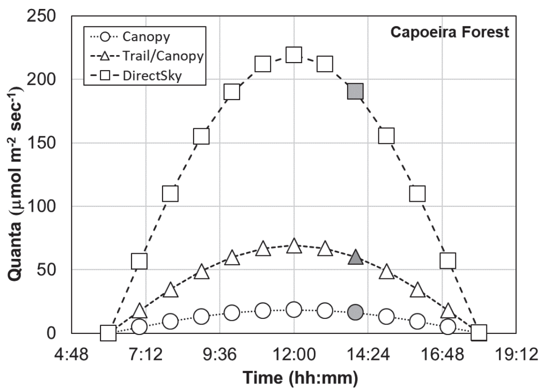

Fig. 3. Diurnal variability of the solar radiance from under the canopy (●), along trail/canopy (▲) & and under direct sky surrounded by canopy (■) based on spot-measurements of quanta (mmol m^{-2} sec^{-1}) during soil sampling at Capoeira Forest on 07-Mar-23 around 14:10. White symbols represent hourly modeled quanta based on spot-measurements (grey symbols) assuming a cloud free day. Weather conditions during the day were cloudy/overcast. Modelled estimate of the daily insolation (Q_s) for the direct sky surrounded by canopy location was 1667 mmol m^{-2} day^{-1}.

3.2. Light Measurements: River

Weather during the river sampling was overcast, partly cloudy and rain throughout the ~30 min survey. Light measurements conducted in the Amazon river showed distinct light attenuation patterns at the three stations sampled (Fig. 4). Depth measurements of quanta conducted at the Rio Negro sector (K_d = 0.103 cm^{-2}; Z_{eu} = 58 cm^{-2}) showed relatively high light transparency compared to the Mixed sector (K_d = 0.132 cm^{-2}; Z_{eu} = 32 cm^{-2}) and Rio Solimoes sector (K_d = 0.231 cm^{-2}; Z_{eu} = 17 cm^{-2}) (Fig. 4). The visually dark color of the Rio Negro (low SS) and light-brown color of the Rio Solimões (high SS) was consistent with the light attenuation patterns observed.

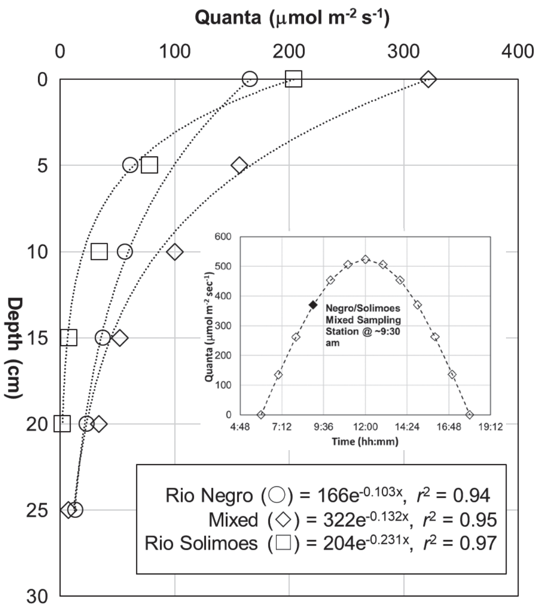

Fig. 4. Diffuse attenuation (cm) of the solar radiance quanta (μmol m^{-2} sec^{-1}) from the Rio Negro (○), Negro/Solimões Mixed (◇) & Rio Solimões sectors of the Amazon River (□) based on spot-measurements at depth on 08-Mar-23 between 09:30 – 10:00 am. Weather conditions during the day was cloudy/overcast. The diffuse attenuation coefficients (K_d) and euphotic depth (Z_{eu}) were 0.103 cm^{-2} (58 cm^{-2}), 0.132 cm^{-2} (32 cm^{-2}) and 0.231 cm^{-2} (17 cm^{-2}), respectively. Modelled estimate of the diurnal variability of solar radiance based on spot-measurement at 9:30 am (◆) is also shown (insert). The daily insolation (Q_s) based on the spot-measurement was 3983 μmol m^{-2} day^{-1}.

3.3. Soil pH and Available Phosphorus

Soil pH in mata densa, capoeira, Terra Preta de Índio, and baixio were 3.80, 3.25, 4.00, and 4.90, respectively (Fig. 5). Soil pH in capoeira was significantly lower and that in baixio was significantly higher than that in the other locations ($p < 0.05$).

Soil available P in mata densa, capoeira, Terra Preta de Índio, and baixio were 5.72, 5.35, 8.39, and 3.33 mg kg^{-1}, respectively (Fig. 5). Soil available P in Terra Preta de Índio was significantly higher than that in capoeira and baixio ($p < 0.05$).

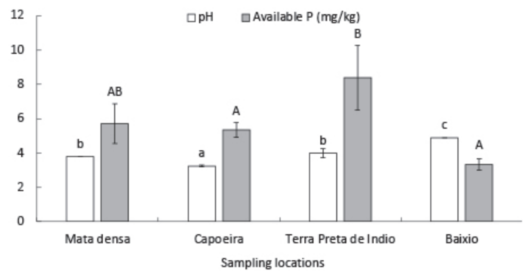

Fig. 5. Soil pH and available phosphorus of 4 sampling locations. The same letters represent no significant differences among different sampling locations for pH (lowercase) and available P (uppercase) (one-way ANOVA, Tukey–Kramer, $p < 0.05$).

3.4. Environmental Parameters Across Two Rivers

The temperature of air and river surface water ranged from 28 to 29 and from 24 to 29, respectively (Fig. 6a). From Negro (st. 1) to Solimões River (st. 4), river surface temperature showed a decreasing trend.

Suspended solid concentration at st. 1, 2, 3 and 4 were 1.8 ± 1.2, 22.6 ± 3.1, 89.8 ± 10.1, 111.4 ± 11.2 g-DW L^{-1}, respectively (Fig. 6b), and the values at St. 3 and 4 in Solimões River were significantly higher than that of st. 3 and 4 in Negro River (one-way ANOVA, Tukey–Kramer, $p < 0.05$). River water pH at st. 1, 2, 3 and 4 were 5.0 ± 0.0, 6.5 ± 0.1, 7.1 ± 0.1, 7.1 ± 0.2, respectively (Fig. 6c), and the value at St. 1 in the center of Negro River was significantly lower than that of other three sites (one-way ANOVA, Tukey–Kramer, $p < 0.05$).

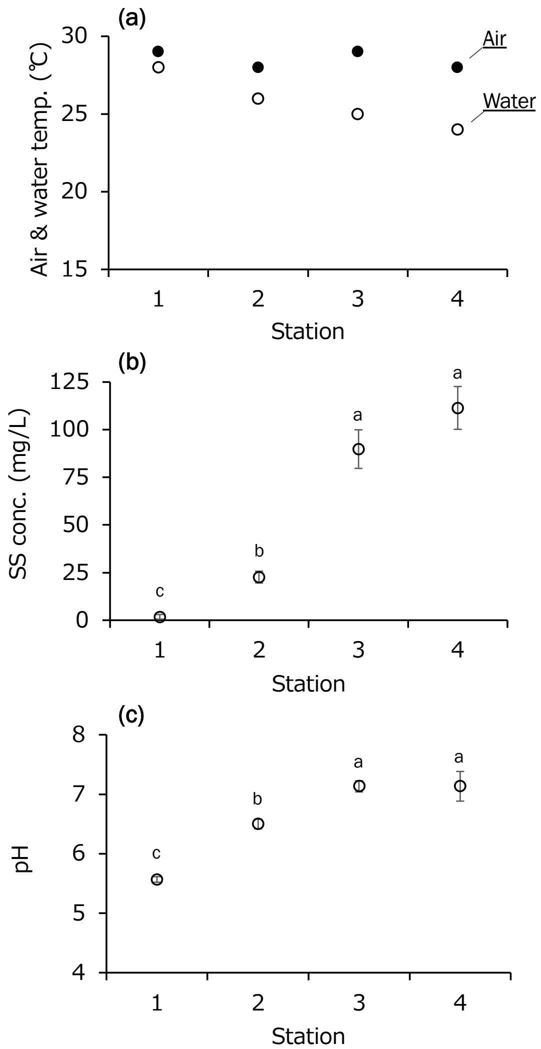

Fig. 6. Spatial variations in (a) air and surface water temperature, (b) suspended solid concentration, and (c) pH at the center (St. 1) of the Negro River, the confluence (St. 2 & 3), and the center (St. 4) of the Solimões River. Error bars show standard deviations (N=3). Letters above plots indicate significant differences (one-way ANOVA, Tukey–Kramer, $p < 0.05$).

The relatively higher abundance (393.9 inds. m^{-3}) of mesozooplankton was observed at st. 2 than at other sites, while the lower abundance (7.1 inds. m^{-3}) was found at st. 4 (Fig. 7a). Cladocerans were dominant, contributing with 28.3–58.0% to the total mesozooplankton abundance at all sites except for st. 4 (Fig. 7b). Copepods were appeared from all sites and contributed 17.2–38.7% of the total abundance.

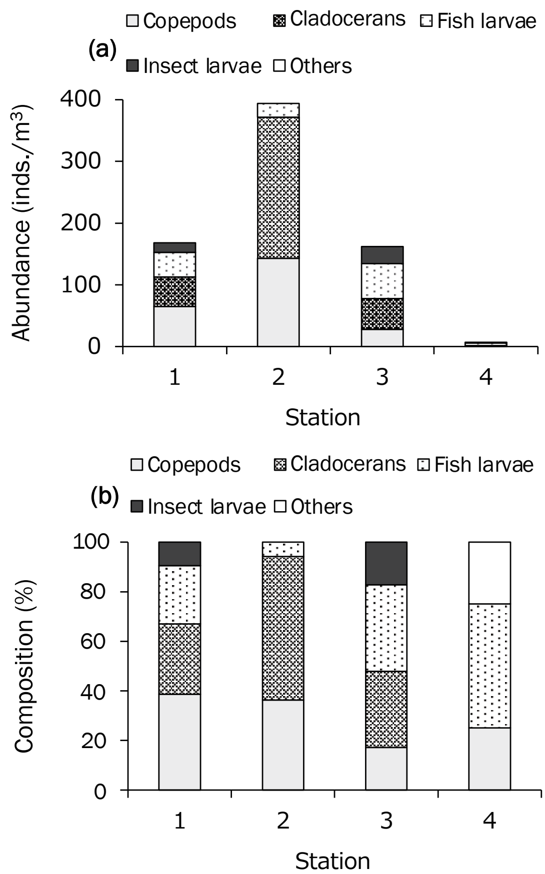

Fig. 7. Spatial variations in (a) abundance and (b) composition of mesozooplankton at the center (St. 1) of the Negro River, the confluence (St. 2 & 3), and the center (St. 4) of the Solimões River.

4. DISCUSSION

4.1. Light measurements

The daily and annual average (climatology) of solar irradiance in Manaus, Brazil is ~460 W m^{-2} and ~1680 kW m^{-2} (Pinker & Laszlo 1992). During the two days of measurements in the forest and river the weather was overcast with rapidly changing cloud conditions and occasional rainfall limiting our measurement capabilities. Although measurements were not under clear-sky conditions, we were able to capture the difference between under the canopy, along the trail/canopy and under open, direct-sky (Fig.3). The results suggest that the vegetation (and soil) during the present study under the canopy and along trails receives less than half of the available solar radiation during the day. However, previous studies have shown solar irradiance above the canopy to be around 70 – 100 times higher than within the canopy that can have a significant impact on the total carbon uptake of new growth versus old growth of the forest (Saleska et al. 2003). In the future, it would be advisable for researchers at ISA to consistently measure solar radiation in conjunction with ongoing tree growth experiments towards evaluating the potential carbon dioxide sequestration potential and contribution of the regional rainforest.

Measurements of natural downwelling quanta attenuation across the Amazonian river (Fig. 1) were extremely interesting due to the significant discrepancy between the Rio Negro (low TSM) and Rio Solimões (high TSM). The 1% light (quanta) penetration depth (cm) in the Rio Negro and Rio Solimões were 58 cm and 17 cm (Fig. 4), respectively. Previous studies of light attenuation in the Amazon River showed similar 1% penetration depth reaching less than 1 m depth in the Rio Solimões (Costa et al. 2013). Further, Costa et al. (2013) reported that both rivers had relatively low concentrations of chlorophyll *a* pigments, while TSM was significantly higher in Rio Solimões and colored dissolved organic matter absorption was significantly higher in Rio Negro, respectively. In the future, it would be interesting to measure the inherent optical water properties (in addition to light measurements) in the river.

4.1. Forest Soil

Soil in the tropical forest including Amazonian forest is generally very acidic, thus soil pH can be as low as 4.0-4.5 (McGrath et al. 2001), which corresponded with soil pH of the upland forest sampling locations in this workshop (mata densa, capoeira, and Terra Preta de Índio). A slightly higher soil pH in Terra Preta de Índio than other upland forest was probably due to the presence of IBE (Glaser and Birk 2012) which was remaining carbonized materials from living and agricultural activities of ancient human settlement. A significantly higher soil pH in baixio was probably due to redox fluctuation caused by seasonal flooding by formation of dissolved ferrous sulfate during reduction, and oxidation of the ferrous sulfate to ferric oxide and sulfuric acid at the soil surface followed by drainage of the acid floodwater (van Breemen 1987).

Soil available P is usually very low in the tropical forest because P is fixed by aluminum (Al)- and/or iron (Fe)-oxide minerals in soil. Available (Mehlich-1 extractable) P in the upland forest sampling locations in this workshop was in similar ranges found in literature (McGrath et al. 2001). Noteworthy was higher available P in Terra Preta de Índio probably caused by the presence of IBE (Glaser and Birk 2012). Even slightly higher soil pH can cause dissolution of Al- and Fe-oxide minerals releasing P to soil.

It appears that soil sampling and analyses of soil pH and available P were properly performed because the standard deviation of both data were very low, particularly soil pH. Since analysis of soil available P was done using P packtest, which was qualitatively measured by blue-color development using chart, the standard deviation of soil available P was relatively high, however still in an acceptable range. Nevertheless, the most important was that both soil pH and available P data showed differences by sampling locations, which is relatively in accordance with hypotheses.

4.3. Aquatic Environmentl

The Amazon River is the largest and most dense river network in the world and discharges continental water to the oceans, which is 20% of the total global amount (Sioli 1984). In the center of the Amazon basin, the muddy white water of the Solimões River meets with the black water of the Negro River, creating a visible boundary. The black water of the Negro River is derived from the high concentration of humic substances, while the white water of the Amazon River is derived from highly suspended inorganic materials (Junk et al. 2015). These characteristics of each river probably made clear differences in pH and SS concentration among the study site in the present study. Surface water temperature in Negro River was relatively higher than Solimões River in the present study, which is congruent with previous research reporting higher temperatures by 1℃ and 0.6℃ in the Negro River (Franzinelli 2011, Nakajima et al. 2017). The warmer water in the Negro River may result from its darker color and slower current speed compared to the Solimões River (Franzinelli 2011). In the present study, mesozooplankton was more abundant in the Negro River and the confluence than in the Solimões River, which is congruent with a previous report by Nakajima et al. (2017). The plankton net used in the present study was not strictly designed for the ichthyoplankton collection (usually a net with a larger mouth and mesh opening is used). Therefore, attention should be given to the fact that our data may have underestimated the abundance of fish larvae.

4.4. Environmental Educationl

Researchers and educators have identified several educational contexts that empowered future scientists (students) towards achieving sustainable development goals (SDGs) (Acevedo-Duque et al. 2023). Although the acquisition of scientific knowledge remains the primary fuel empowering students to contribute to environmental sustainability efforts, hands-on practical experience remains the most efficient source of inspiring participation (Sigahi & Sznelwar 2023). Although the focus of the present study was to share hands-on practical field experience to IFAM undergraduate students and ISA researchers, we were able to receive valuable (select) feedback from the participants as follows,

"The Workshop was an incredible experience in all aspects. First, the interdisciplinary partnership of IFAM, Soka U. and ISA, added new knowledge to the students that attended. The workshop was satisfactory regarding the schedule of the collaboration. I am proud for being selected to the first edition of this event, and grateful for all commitments of the teachers and staff involved for the success." Mr. Bruno da Costa Takaki, IFAM (Student)

"I acquired a lot of knowledge regarding the methods that were used in the environmental analyses, new methods and with simple and efficient equipment, which is necessary when doing a research in the Amazon rainforest interior, for example. Thus, environmental education is present in all the developed activities, showing the importance of maintaining the quality of our terrestrial, aquatic, and atmospheric environment. The teachers are always dialoguing about social and ecological values, with approaches towards the conservation of the environment, a perspective that we must have in order to keep our biodiversity alive and healthy. But, this only depends on us and the knowledge we have." Ana Beatriz

Souza dos Santos, IFAM (Student)

"Brazil, despite being a country with a large amount of cultures, offers few opportunities to interact with people of other ethnicities. In more than seven years of academic life, I have not had any opportunity to participate in events that encourage us to speak in a foreign language. The Workshop made this unprecedented experience possible for me. Besides making me feel great joy for the student's happiness." Mr. Rodrigo Izumi, ISA (Graduate Student)

The overall feedback was helpful in determining the next steps to take in continuing the next workshop planned for FY2023. Further, the international collaboration showed that in-person exchange, particularly after the global pandemic, coupled with environmental education could open the doors for multi-lateral research collaborations beyond student-centered learning activities. Indeed, the most inspiring aspect of the international exchange was the interest in some of the students (and instructors) to pursue graduate school and/or a profession in environmental science. These comments from the workshop participants suggests that the students gained valuable hands-on practical experience that inspired them to contribute to sustainable development goals in the future.

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