Measuring watershed health: training conservation planners how to use biophysical tools for monitoring streams in neo-tropical ecosystems

Watersheds are the natural building blocks of conservation areas. They are the sources of the clean fresh water that is the life's blood of all living things, from the largest mammals to the smallest microorganisms. And because they are also the drainage systems for all activities on the land, their streams and rivers are sensitive and accurate barometers of the ecosystem's health. Yet, despite the critical role that water plays in the life and biodiversity of conserved areas, little is known about the condition of the streams and rivers that convey it to the Andes-Amazon region. The absence of such knowledge has meant that the quality and quantity of fresh water have typically not been included in conservation plans, with the result that landowners, conservation workers, and policy makers have lacked an invaluable tool to help them establish conservation priorities, measure the success of conservation efforts, and calculate the true value of conserved land.

The goal of the Stroud Center's project was to demonstrate the feasibility and value of using information on water quality, water monitoring, and stream health to support and strengthen the Moore Foundation's Andes-Amazon initiative directly – and other Moore initiatives such as the Wild Salmon Ecosystems and the Conservation International initiatives indirectly. Because the Andes-Amazon is the world's largest river basin and the source of 20% of Earth's fresh water, because the area continues to support the world's most abundant biodiversity, all of which is dependent on fresh water, and because the region is under growing assault from a variety of sources, we believe that understanding the condition of its water and preserving the sources of that water, as both habitat and critical resource, must be the foundation of an effective conservation strategy in the Andes-Amazon. Moreover, the fundamental element in protecting clean fresh water is to protect the rain forest, particularly in the headwaters areas and along the stream corridors – for it is from the seemingly insignificant small streams that more than 90% of the fresh water actually comes; and the key to protecting the forest and its inhabitants, large and small, is to safeguard its sources of clean water. Finally, the streams and rivers themselves contain an astounding – and largely unrecognized – level of diversity of plants and animals. Most of them are barely visible to the naked eye, but they underpin all life in the region.

Our plan was simple. First, Stroud Center scientists would carry out an intense field and laboratory effort to produce a credible set of data that described the quality of streams and rivers – both pristine and polluted – in the Andes-Amazon region. Stroud Center educators would translate the technical data the scientists had collected into information and programs that would enable people in the region to understand the issues affecting their water, acquire the tools to protect the sources of that water, and grasp the vital connection between protecting the water and preserving the watersheds from which it comes.

The major premise of the project is that streams in preserved watersheds of the Andes-Amazon region can and should play a dual role in conservation strategies: they are both (1) the habitat for a largely uncharted reservoir of aquatic biodiversity and (2) a yardstick – the natural equivalent of the canary in the coal mine – that can provide:

- a measure of the important ecosystem services (e.g., filtration, treatment, and delivery of fresh water for humans and wildlife) that are being conserved and the value of those services for the health and sustainability of the region and the biosphere;
- a benchmark for assessing on-going efforts to conserve pristine watersheds and restore degraded ones;
- a time stamp for existing conditions that enables policy makers and others to measure the impacts of local development, regional transport of pollutants, and global climate change; and
- the living classrooms for hands-on programs that educate teachers, decision makers, and the general public about the critical need to establish and maintain core preserved areas.

Guided by the above, scientists, technicians, and educators from the Stroud Center, along with collaborators from Florida A&M University and Peru, made several trips to the Andes-Amazon region in 2006 in support of the Moore Foundation's conservation efforts. The teams had two main goals: (1) to create a baseline of scientific data on water quality, stream biodiversity, and stream health that will serve as the foundation for understanding and sustaining on-going conservation efforts in the region; and (2) to craft, test, and implement accessible, easy-to-use, and inexpensive education programs for the people of the region.

It is critical to establish a baseline against which to measure the impact of future changes in the region. Nowhere is this more true than in the frontier areas, such as that around Puerto Maldonado which is undergoing a momentous transformation as a result of ongoing gold mining operations and, perhaps even more importantly, the anticipated construction of a paved highway and bridge across the Madre de Dios. This baseline of freshwater conditions, habitat, and living organisms becomes even more important in the face of global climate change: aquatic organisms are more sensitive to temperature change than terrestrial communities because they are adapted to environments with much less variation in temperature. Hence, these "aquatic canaries" are likely to respond more quickly and with more dire results to small and sudden changes in temperature.

We also believe that it is imperative to engage the local residents in the efforts to understand the complex issues facing their region and its water and to help them become active stewards of their resources. In the course of our work we were often told that conservation is viewed as something "outsiders" are trying to impose on the local communities and that, while the stated benefits of conservation efforts are global in scope, the costs are disproportionately borne by local residents, many of whom are very poor and desperate for economic development. The cash and jobs that are currently generated by gold mining and are anticipated from the construction of the highway and the development of the surrounding countryside are perceived as having more immediate value than the preservation of the rainforest. It is essential, then, to demonstrate the real and quantifiable benefits that will come with the protection of the forest and the sources of fresh water. And those benefits, which include direct economic impacts such as ecotourism, improved public health, and significantly reduced water treatment costs, are substantial.

Accompanied by as many as 39 large black bags of scientific research equipment, the group hewed to a precise but hectic schedule (e.g., see Table 2.1 in Appendix 2) to accomplish its goals. Operating out of five eco-tourist/research stations on the Madre de Dios and Tambopata

rivers, the scientists and educators worked in the field, laboratory, and classroom to accomplish three major outputs:

1. Create and offer training workshops for monitoring biophysical properties of streams and rivers in neo-tropical regions.
2. Test and deploy protocols for measuring and monitoring health in neo-tropical streams.
3. Disseminate training workshop and monitoring research information.

The report that follows describes what the Stroud Center team did with regard to each of those outputs, why we did it, and what we found.

Output 1. Create and offer training workshops for monitoring biophysical properties of streams and rivers in neo-tropical regions.

What we did

During the two-week trip to the Peru in August, Stroud Center scientists and educators worked side by side to get a clear understanding of the local stream conditions and community issues and to pilot test a workshop for students and teachers from Puerto Maldonado, the capital and largest town of Madre de Dios. That workshop served as a prototype for two series of daylong workshops to be given later in the year in Madre de Dios and the Osa Peninsula of Costa Rica.

In October two educators and two scientists from the Stroud Center presented four separate, full-day workshops on water quality monitoring and the ecology of streams and rivers in the Andes-Amazon region. The workshops took place at the ACEER-Tambopata at Inkaterra (ATI) station just downstream from Puerto Maldonado on the Madre de Dios River (Figure 1). In December the group traveled to Costa Rica to give a similar series of workshops at the Fundacion Neotropica on the Osa Peninsula (Figure 2). Presented in Spanish and offered free of charge, the workshops bolstered the conservation efforts of core preserved areas in the neo-tropics, discussed the latest scientific and educational knowledge on issues affecting fresh water, offered practical and affordable methods for monitoring streams and rivers, taught stewardship practices that the participants can both use themselves and transmit to others, and encouraged

Figure 1. Hands-on stream insect sorting and identification by participants of a water-quality monitoring workshop near Puerto Maldonado, Peru.

Figure 2. Learning to identify Costa Rican stream insects at the Fundacion Neotropica on the Osa Peninsula

appropriate conservation policies in the region – particularly the importance of maintaining forest cover. In addition to enhanced knowledge and new communication techniques, the workshop participants received resources, in the form of handouts, CDs, and web links, to help them share their newly acquired information in their communities.

Each workshop began with a lecture on the local and global importance of clean fresh water, the many roles clean water plays in the participants' personal lives and local economies, a basic understanding of the ecology of streams and rivers, and usable ways to determine the health of the water in their communities. The opening lecture set the stage for the rest of the day by framing the issues, providing simple but essential statistical and general information about water resources, explaining the relationship of land use to stream health and the impact of human activities on water quality (e.g., Figure 3), and discussing the critical role that streams flowing through conserved areas play in the region. The workshops were enriched by the findings generated by the Stroud Center's current study, as well as the knowledge the Center has gained from two decades of working in neo-tropical streams in Central and South America.

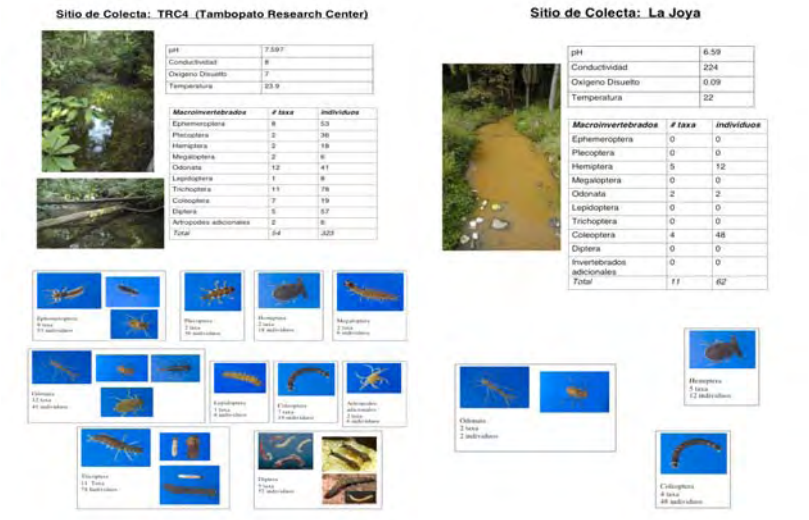

Figure 3. An example of the stream insects that can be found in a 'healthy' stream (on the left) compared to those found in an 'unhealthy' stream (on the right). Both streams are located in the Madre de Dios River basin, Peru.

Workshop participants (see Tables 1.3 and 1.4, Appendix 1) then walked two kilometers to a nearby stream where they waded into the water to perform actual measurements of water quality and to collect macroinvertebrate animals – aquatic insects, snails, crabs, and worms – that provide a biological measure of stream health (Figure 4). The group learned how to make basic

Figure 4. Collecting stream insects for assessing stream health in Peru and Costa Rica

measurements of water flow and chemistry and to collect and identify aquatic organisms. They then returned to the station for lunch, during which an entomologist described the role the stream animals play as indicators of water quality. After lunch, the participants spent a couple of hours in the laboratory, sorting and identifying the animals they had collected that morning. They learned how to assess the health of the stream and watershed by analyzing the abundance and types of species and higher taxa they had found (Figure 5), how to use simple and effective tools to monitor their streams and rivers, and how to compare the clean stream at ATI with others in the region that the Stroud team was studying. The day ended with a boat trip to Inkaterra's Reserva Amazonica and a visit to its famed canopy walk, which was accompanied by a discussion of the critical relationship between the rainforest and the health of the sources of fresh water.

Figure 5. Making the connection between healthy streams (based on the macroinvertebrates found) and healthy watersheds.

As noted above, the December workshops in Costa Rica took place at the Fundacion Neotropica in the town of Rincon on the Osa Peninsula. Rincon lies in the heart of a conservation corridor currently being created between Corcovado and Piedras Blancas national parks, in large part by a grant from the Moore Foundation to The Nature Conservancy. The sparsely populated area is under intense pressure from a project to complete construction (pave/new bridges) of the coastal highway that connects Costa Rica's capital of San Jose with

Panama via the Osa peninsula. Using a format similar to that offered in Peru, the workshops in Costa Rica blended scientific data from Peru and Costa Rica to frame the issues and to provide the appropriate context and perspective for the participants. The workshops were well attended, received excellent reviews, and provided additional feedback on how best to use water quality monitoring data to support and promote conservation in neo-tropical watersheds.

Why we did it

After a number of discussions with people in the region on content, logistics, and potential participants, the Stroud Center's scientific and education staffs decided that the best way to reach the most diverse and significant audiences and to maximize the impact of the work we were doing was to give a series of daylong workshops geared specifically to the following participants: (a) local public- and private-sector decision makers; (b) teachers; (c) conservation planners, non-governmental organization staff members, and university faculty; and (d) eco-tourism guides. This focus enabled us to target our educational efforts at groups that could both use the information in their own work and influence a range of audiences that would ensure a broad dissemination of the issues, information, knowledge, and monitoring techniques. It also enabled us to engage in discussions with local leaders and workers who are currently in positions to make decisions about the use and protection of water resources and with teachers whose students will become the stewards of the future.

Consequently, each workshop was designed not only to teach the participants about freshwater issues, monitoring techniques, and stewardship practices but also to show them how to pass on to others the knowledge they acquired. To do that, we built into the workshops: exercises for teachers to use with their students; information that ecotourism guides can add to their inventory of activities for their clients; ideas that decision makers can introduce into the local political and corporate processes; and practices that conservation planners and NGO staff members can incorporate into policies and programs.

Because conservation efforts are often viewed as something outsiders are seeking to impose on the region, it is imperative to engage the local people in the efforts to understand the complex issues and to become active stewards of their resources. It is much easier for the area's residents to understand the immediate benefits of the cash and jobs being generated by gold mining and anticipated from the construction of the highway and the accompanying development of the surrounding countryside, than the long-term value from the preservation of the rainforest. It is essential, then, to demonstrate the real and quantifiable benefits that will come with the protection of the forest and the sources of fresh water.

What we found

The workshops were well attended and enthusiastically received. We found among the participants a clear and growing recognition of many of the issues that currently face the region and its fresh water – and, even more importantly, of how vulnerable the area is to the enormous changes that are on the way. The most conspicuous current issue is the widespread gold mining in the rivers – a practice that is exposing the entire food chain, from the smallest aquatic organisms to humans, to increasing levels of mercury. But the issue that may ultimately prove

the most cataclysmic is the paving of the Acre-Masuko Highway (connecting Acre, Brazil with Masuko, Peru and ultimately beyond to Cusco), which includes the construction of a bridge across the Madre de Dios River at Puerto Maldonado. While the paved road and bridge, which are expected to be completed within two years, are touted as a commercial transport system that will connect the hinterlands of Brazil and Peru to coastal markets, their most important impact will be to open vast tracts of land to agricultural and other development. In fact, it is estimated that 120,000 hectares will be transformed from rainforest to agriculture, which represents a tenfold increase in the area currently under cultivation. Thus, the watersheds around Puerto Maldonado and along both sides of the proposed highway are clearly watersheds in major transition; and land speculators are actively buying properties in anticipation of the enormous population growth and development already under way in the region. It is imperative that baseline technical information on land use and water quality be acquired in these watersheds before the development occurs so that preservation strategies and development impacts can be accurately measured in the future.

Our workshops also focused attention on the value of the region's streams and rivers – particularly the smaller streams that are the primary sources of water in the overall water system – and the role they play in ensuring clean safe water for drinking, sanitation, agriculture, and industry (including tourism). Drawing parallels to the Stroud Center's six-year study of New York City's drinking water supply system, which covers over 5,000 square kilometers and extends more than 200 kilometers from the center of the city, our presentation demonstrated the critical need to establish a baseline of current water conditions against which to measure future changes and to put good stewardship practices in place before the changes occur.

We found a remarkable thirst for information among the local participants – and a particular interest in the quantifiable economic and health benefits that come with the protection of water resources and the places that produce them. Participants, who ranged from outdoor guides to classroom teachers, from nurses to the president of the country's foremost eco-tourist company, were anxious to learn about threats facing their water sources, methods they can use to monitor the health of their streams and rivers, practices they can afford and implement to promote good stewardship of their resources, and ways to communicate what they have learned to others. Perhaps the most consistent message that came through in both verbal and written commentary on the workshops was "we want more" – more information, more time to learn, more and better tools to make a difference, more workshops in the future for more people to attend. A corresponding message was "we want it now" . . . because, with the rate of change in the region, time is of the essence.

A total of 153 people attended the eight workshops – 31 eco-tourist guides, 34 public and private sector managers, 42 teachers, and 46 conservation workers – with 80 participants in Peru in October and 73 in Costa Rica in December. We provided evaluation forms (see Table 1.5 in Appendix 1), in which we asked the participants to rate the workshops in four categories, using a numerical score of 1 (lowest) to 5 (highest). The average cumulative scores for the 8 workshops ranged from 4.6 to 4.9 (see Table 1.5 in Appendix 1). We also asked for general comments and have attached a compendium of those to this report (see Table 1.6 in Appendix 1).

Output 2. Test and deploy protocols for measuring and monitoring health in neo-tropical streams

What we did

The Stroud Center scientific staff, joined by other collaborators, local technicians, and guides, set up a series of measuring and monitoring stations across the Madre de Dios region. The overall goals were to: (1) measure a suite of physical, chemical, and biological parameters that will enable scientists, conservation workers, and the general public to understand the issues affecting their fresh water and gauge the health of their streams; (2) provide a quick, simple, and effective assessment of the impact of humans on water quality and stream conditions in the region; (3) create a baseline of water conditions against which to measure future changes – from factors ranging from local development to global climate change – in the region's streams and rivers; and (4) establish a set of protocols that will allow people in the region to measure, monitor, and manage the health of their streams.

The Stroud team sampled 31 stream and river sites (Figure 2.1 in Appendix 2) that ranged from pristine – which are primarily in core conservation areas – to those that have been severely polluted by human activities (Table 4 and Appendix 2). The methodology and parameters selected for these studies (see appendices 3-8) were based on those developed by Stroud scientists over the course of their six-year assessment of water quality and stream health for all the streams that provide New York City its drinking water (for results of that study, see http://www.stroudcenter.org/research/nyproject/JNABSPublications.htm)

Why we did it

The water resources of the Andes-Amazon have been little explored – and, in particular, little is known about the quality of water and the integrity of the stream and river ecosystems that supply water to humans and wildlife throughout the region. This scientific deficiency is critical because clean fresh water has become, in the words of E.O. Wilson, the "deciding element on the planet earth." If watersheds are the natural geographic bodies for land conservation efforts in the Andes-Amazon region, then streams and rivers provide the lifeblood for those efforts because: (1) all life in our watersheds needs a daily source of clean fresh water to survive and reproduce; and (2) every aspect of human existence – from agriculture to commerce to health – requires large amounts of fresh water. Yet, the quality and quantity of fresh water has typically not been included in most conservation plans, especially in the neo-tropics. Indeed, the supply of clean fresh water is rarely part of the equation used to calculate the short- and long-term values of conserved land; and water quality and stream monitoring are rarely part of the system used to establish conservation priorities or to measure the success of conservation efforts. As a result, the local population lacks the capacity to understand and monitor the condition of their streams and rivers and the quality of their fresh water.

Thus, the project's intense scientific field effort was intended to provide the foundation on which to build a series of education programs which would distill and translate the technical data into understandable and meaningful information to enable those who live and work in the Andes-

Amazon to assess and monitor their sources of fresh water and to connect that knowledge to the overall conservation effort. Specifically, we have sought to teach those in the region:

- how the health of a local stream affects the physical and economic well being of those who depend on its water;
- how human activities on the land affect the quality and quantity of the water in the streams and rivers;
- how conservation areas protect the sources of clean fresh water;
- how people can manage and monitor the water sources in their communities;
- how the water quality information can be used to determine conservation priorities and measure their success; and
- why they should press local decision makers to implement policies to safeguard the region's clean fresh water.

What we found

A total of 31 stream and river sites were studied (Fig. 2.1, Appendix 2) across six scientific disciplines (Table 2.2; Appendix 2): Biogeochemistry (Appendix 3), Molecular Tracers of Contamination (Appendix 4), Microbial Diversity/Ecology (Appendix 5), Macroinvertebrate Diversity/Ecology (Appendix 6), Nutrient Processing (Appendix 7), and Ecosystem Metabolism (Appendix 8). The detailed technical findings for each discipline are found in their respective appendices to this report and have been provided on our website (http://www.stroudcenter.org/research/MooreFdnPeru/index.htm). The following is a series of questions regarding important conservation issues facing the Madre de Dios region and answers based on insights gained from this study:

1. Can streams and rivers in the region be broadly classified for conservation purposes? If so, are certain characteristics more important than others with regard to conservation?

Answer: Yes on both counts.

a. Streams and rivers fall into three major biogeochemical categories with distinct microbial communities associated with each: (1) Clearwater [low levels of dissolved ions, dissolved organic carbon (DOC) and fine suspended solids (FSS)]; (2) Blackwater (low levels of ions and FSS but high levels of DOC); and (3) Whitewater (intermediate levels of DOC and high levels of ions and FSS). Conservation workers and others can distinguish among these three types visually and/or with simple field instruments.
b. Most small streams in the region have unusually low levels of nutrients but high levels of nitrogen as ammonia. This combination makes them vulnerable to oxygen depletion if exposed to excess nutrient loadings associated with human impact. QLAJOYA, which can support virtually no living organisms and is dangerously toxic to humans, is a case in point.
c. Because of the low nutrients and low in-stream algal production caused by heavy shading, organic inputs from the riparian forests, such as leaves, wood, fruit, and seeds, dominate the food base, metabolism, and type and abundance of aquatic animals of most natural streams in the region. This means that maintaining the quality and quantity of

riparian forest cover is a critical conservation priority throughout the region because it will help sustain the natural food web of the stream.

d. Nutrient uptake in natural forested streams was lower than in most undisturbed temperate streams, which suggests that the local streams are highly vulnerable to anthropogenic impacts from farm fertilizers and sewage inputs.

2. Does the presence and abundance of "keystone" terrestrial animals (jaguars, monkeys, tapirs, macaws) accurately indicate the health of a watershed and its water resources and/or the degree of conservation success in the region?

Answer: Not always.
Watersheds containing an abundance of these keystone animals had streams indicating poor watershed health and/or on-going pollution. For example:

a. A stream (QTRC4) in the "pristine" Tambopata reserve was relatively clean and natural in most respects but had concentrations of one pesticide (Chloropyrifos) that were higher than QLAJOYA, which is heavily polluted by contaminants from Puerto Maldonado.
b. A stream (Q2MIRADORCICRA) in the "pristine" Los Amigos Research Center and Conservation Concession was missing more than half its pollution-sensitive macroinvertebrate species. This indicates severe watershed disturbance, which could be either on-going or historical.
c. A stream (QATI8) associated with the relatively "pristine" watershed near the ACEER-Inkaterra research facility had the second highest concentrations of the herbicide Atrazine measured in this study.

Since the forest food web depends on fresh water, it is likely that such water contaminants will accumulate in the terrestrial food web and place animals in the highest levels (e.g., keystone taxa) in jeopardy.

3. Can the chemistry of the water give us a good indication of water quality and the type of human impacts in the watershed?

Answer. Yes.

a. High ion and nutrient concentrations reflect high human impact in the region (case in point QLAJOYA with the highest level of degradation and human impact as well as the highest ion and nutrient levels).
b. The ratios of ammonium nitrogen to total dissolved inorganic nitrogen (DIN) were higher at sites receiving sewage or manure inputs.
c. Stable nitrogen isotopes ($\delta^{15}N$) of nitrate and of fine particulate organic matter at most agricultural sites revealed enrichment due to both manure and sewage inputs.

4. Are there specific aquatic animals whose presence and abundance indicate overall water/watershed health and potential for supporting viable populations of "keystone" terrestrial animals?

Answer: Yes.

a. Streams containing four out of five of the following aquatic macroinvertebrate groups [crabs (Grapsidae), mayflies (*Campylocia*), stoneflies (*Anacroneuria*), and two caddisflies (*Phylloicus, Triplectides*) always drained watersheds containing keystone terrestrial wildlife.
b. All study streams lacking three or more of the five aquatic groups were associated with highly impacted watersheds.

5. Do water quality data that indicate watersheds incapable of supporting important wildlife also indicate high risk for human health?

Answer: We think so – but on a case-by-case basis.

a. Pesticides found in three streams at levels toxic to aquatic life are also carcinogenic to humans.
b. Fecal steroid ratios indicate that QLAJOYA is contaminated with human waste, and the level of fecal steroids indicates that dangerous human pathogens are also likely to be present.
c. Some streams have high fecal steroid levels of non-human origin, but do not have dangerous levels of human pathogens (e.g., QABEJITA, whose fecal steroid ratio indicates contamination from cattle).

6. Can small pockets of human activity broadly jeopardize watershed health and hence on-going conservation activities?

Answer: Yes.

A very small stream (QINF3), which drained a small banana plantation, contained exceptionally high concentrations of the insecticide Chlorpyrifos and the fungicide Metalayxl, whose negative influences are transported downstream to other parts of the watershed.

7. Does urbanization have a greater impact on water quality than agricultural development?

Answer: Both types of land use can severely impact a stream and often occur together.

a. An urban stream (QLAJOYA) had elevated concentrations of every pesticide and PCB measured and a 100% loss of pollution-sensitive species, both of which indicate severe impact.
b. An agricultural stream (QINF3) had significant levels of insecticides and fungicides and an 80% loss of pollution-sensitive species, both of which indicate severe impact.
c. Stable nitrogen isotopes ($\delta 15N$) of nitrate and of fine particulate organic matter (FPOM) at most agricultural sites show the characteristic enrichment of both manure and sewage inputs.

8. Should best management practices (BMPs) and policies, such as riparian forest buffers, which are common in temperate zones, be an integral part of watershed conservation efforts in neo-tropical watersheds?

Answer: Yes, the region needs policies to protect the riparian forest. However, because they can reduce but not eliminate loss of water quality and impairment of stream health, BMPs should not be used as an excuse to open conserved areas to development.

a. QINF5, whose watershed is partially deforested for cattle and row crop agriculture but has a wide and intact riparian forest along most of its length, had levels of pollution-sensitive taxa comparable to streams in conserved areas.
b. QABEJITA, whose watershed is largely deforested for cattle pasture, was able to retain 40% of its pollution-sensitive taxa and most of its ability to process nitrogen and phosphorus by keeping intact a 5-10 m riparian buffer.
c. Stable carbon isotope (δ13C) of fine particulate organic matter at QKM14 stream suggests that the partial removal of riparian forest along its length has already increased the relative abundance of algae in the stream's food base relative to heavily shaded conserved streams – arguing for a policy to assure the long-term integrity of riparian forest in the region.

9. Beyond global warming and impacts associated with increased UV radiation, are the watersheds, and their human and wildlife populations, at risk from exposure to toxic substances via aerial transport from industrial areas in South America (hence the need to conserve elsewhere in the region to assure success) ?

Answer: This does not appear to be a problem based on our preliminary study

a. PAHs, which are carcinogenic compounds from petroleum and combustion-generated soot, were uniformly low in all streams – and below all USEPA toxicity criteria for water quality. (For perspective, the Stroud Center recently studied 180 streams supplying drinking water to New York City and found 54 of them to have PAH levels exceeding the EPA water quality guidance values.)
b. Pesticides in some study streams throughout the region were undetectable (note: the study did not look for bioaccumulation of pesticides in the tissue of key wildlife.)

10. Do conserved watersheds contain high levels of aquatic biodiversity? Is the biodiversity unique? And can it play a critical role in measuring water quality and watershed health in the region and in gauging conservation success?

Answer: Yes on all counts.

Biodiversity

a. Our 12 most extensively studied small streams contained 204 macroinvertebrate taxa (mostly genera).
b. 50% of the macroinvertebrate taxa were found in only one or two streams – suggesting a high level of both alpha and beta species diversity.
c. The microbial survey, which was the first of its kind in the Amazon basin, revealed distinct bacterial communities in the blackwater and clearwater streams of the conserved areas.

Uniqueness of Biodiversity

a. A significant percentage (> 30%) of macroinvertebrates collected appear to be new species to science.
b. It appears that the microbial community specializing in processing ammonia (a common chemical in Andes-Amazon streams) is not composed primarily of Bacteria (as in non-tropical streams) but rather Archaea (microbes that look like bacteria but are genetically distinct).
c. The failure to detect bacteria in the ammonia processing community of the Andes-Amazon streams, if confirmed, would represent an important and novel observation in the field of microbial stream ecology and would help increase the capacity of microbial ecologists working in the Moore Foundation's marine program to understand what is emerging as an important issue.

Role in water quality monitoring

a. Macroinvertebrate groups sensitive to pollution in the Andes-Amazon region (e.g., mayflies, stoneflies, caddisflies, hereafter the "EPT" group) appear to be similar, but not identical, to those that the Stroud Center has worked with elsewhere in Latin America and the temperate zone.
b. The extinction of pollution-sensitive macroinvertebrate taxa (EPT) from a stream ranged from 100% (highly polluted stream in the town of Puerto Maldonado) to 0% (a pristine stream in a conservation area), with most clean streams in the region losing less than 20% and most polluted streams losing more than 50%.
c. The presence of five aquatic macroinvertebrate animal groups (Grapsidae, *Campylocia* , *Anacroneuria* ,*Phylloicus, Triplectides*) consistently seems to indicate very high water quality.

Gauging conservation success

Loss of EPT taxa indicates that some streams (e.g., Q2MIRADORCICRA) in conserved areas are not healthy and that their watersheds are either suffering from a legacy of previous impacts (and need pro-active restoration) or are in need of better and more widespread protection.

11. Is the approach to water quality monitoring in the Andes-Amazon region and the knowledge gained about its value to help plan, guide, and evaluate watershed conservation applicable elsewhere in the neo-tropics?

Answer: Yes.

For example, on December 17, 2006, Bern Sweeney, director of the Stroud Water Research Center, arrived at "Nectandra," a neo-tropical cloud forest preserve in Costa Rica (www.nectandra.org), just after having finished leading a weeklong series of water quality monitoring workshops as part of a Moore Foundation grant on Costa Rica's Osa Peninsula. He had come to Nectandra at the request of its president, Alvaro Ugalde, and co-founders, David and Evelyn Lennette of San Francisco, CA. The Lennettes had purchased 99.9% of the Nectandra watershed in 1999 and had built and furnished a magnificent education/research center focused on conserving the virgin cloud forest and its jaguars, tapirs, and other native wildlife. Sweeney was asked to confirm the purity of the water of a stream called Quebrada

Verde, in anticipation of using it for education purposes and for growing organic rice as a demonstration of sustainable agriculture. Employing the same macroinvertebrate sampling techniques he had taught his Moore Foundation students the week before, Sweeney determined almost immediately that the stream ecosystem was "dead" as a result of heavily polluted sediments and probably toxic chemicals in its water. These pollutants came from a tiny parcel of upstream land that the Lennettes did not own and which was used for growing ornamental plants for market. In subsequent visits that day to nearby streams whose watersheds were completely within Nectandra's conserved area, Sweeney found water of consistently high quality. Thus, his quick and simple stream survey not only provided a direct measure of Nectandra's conservation success, but it also revealed the immediate need to acquire the tiny piece of the watershed that was being poorly farmed and whose contaminated water and soil was washing into Q. Verde.

Output 3. Disseminate training workshop and monitoring research information.

What we did

The Stroud Center has disseminated – and continues to disseminate – its monitoring and research information in three primary ways:

1. The Series of Workshops in Madre de Dios, Peru, and the Osa Peninsula, Costa Rica. Not only did these workshops directly reach 153 people in two distinct tropical regions, but the participating groups were selected precisely because they are in positions to disseminate broadly the knowledge, information, and techniques they acquired. A fundamental part of the mission of each participating group – eco-tourism guides, public and private decision makers, teachers, and conservation and NGO staff – is to educate people about the importance of natural resources and to show them ways to practice good stewardship.

2. New Collaborations and Initiatives. Our scientific baseline studies and associated education programs and workshops attracted several key people who will collaborate in our effort to increase local, regional, and international recognition of the critical need to understand, protect, and restore freshwater resources. Four brief examples:

    a. Jose ("Joey") Kechlin is the founder and chief executive officer of Inkaterra, Peru's foremost travel company, which has been operating since 1975 and hosts more than 65,000 clients each year. As part of its statement of commitment, Inkaterra promotes "Conservation and scientific research achieved through self-supported sustainable tourism respecting authentic cultural, social and environmental values." In addition to the for-profit corporation, Mr. Kechlin created and operates Inkaterra Association (ITA), a non-profit organization, "which promotes the conservation and protects the biodiversity of the Peruvian Andes and Amazon Rain Forest." Jose Kechlin attended one of our workshops, and at a reception he hosted afterwards for the other attendees, he gave an effective and impassioned plea for the protection of the region's resources and, in particular, of its streams and rivers. As a major employer, the chairman of the Hotel Society of Peru, a member of the board of the National Chamber of

Tourism, and a long-time emeritus board member Conservation International, Joey Kechlin is in a position to help us spread our knowledge on a broad scale and has expressed a strong desire to do so.

b. Nigel Pitman is the science director of the Amazon Conservation Association (ACA) and was the Stroud team's host at ACA's field station at Los Amigos, Peru, which he directs and where we carried out several days of research in August. As a result of our work at Los Amigos and conversations with staff there, Dr. Pitman and several of his employees attended one of our workshops. This, in turn, led to an invitation to help design and implement a baseline study of water quality and sampling stations to be included as part of the new infrastructure that ACA is developing in a large sub-watershed of the Los Amigos research concession of Peru.

c. Rita Colwell is former director of the National Science Foundation and is currently Chairman of Canon U.S. Life Sciences Inc. and a distinguished professor at both the University of Maryland and Johns Hopkins University. She has previously served as Chairman of the Board of Governors of the American Academy of Microbiology and as President of the American Association for the Advancement of Science, the American Society for Microbiology, the Sigma Xi National Science Honorary Society, and the International Union of Microbiological Societies. She is a leading authority on infectious water-borne diseases and is keenly interested in our existing monitoring sites in Peru. Our effort to strengthen neo-tropical conservation efforts with water quality monitoring information has led to an agreement with Dr. Colwell to collaborate on an expanded water-quality monitoring effort that will include infectious diseases of the Andes-Amazon region. The focus of this collaboration is to connect the Stroud Center's measures of water quality and stream health to the incidence of infectious disease organisms, such as cholera, in the water.

d. Many conservation workers, representing both the public and private sectors, attended our workshops in Costa Rica. Some of the participants were in positions that enable them to mandate and/or adjust protocols for environmental protection and conservation. For example, Miguel Madrigal Hernandez, the Director of the Osa Conservation Area for MINAE (Ministerio de Ambiente y Energia), was so impressed with what he learned that he urged his employees and others to attend subsequent workshops during the week, expressed a willingness to endorse the participation of other MINAE workers in future workshops, and requested information that will allow him to add water quality monitoring to the routine task list of the guards employed by the national park system.

3. Website. The third method of dissemination and the one with the broadest reach is our website (http://www.stroudcenter.org/research/MooreFdnPeru/index.htm). Table 1.1 in Appendix 1 lists the information that is currently on our website, which will be updated as new information comes to our attention. We are also working with all the organizations and institutions we invited to our workshops (Table 1.2, Appendix 1) to

provide links to our website. We are emailing all the workshop participants who have email addresses, alerting them to the resources on our website and inviting them to become ambassadors for their watersheds by spreading the word as broadly as possible. And we are working with ACEER, ACA, and other groups to expand our reach, both in the region and beyond.

Why we did it

We believe it is critical to get the results of our information about water conditions and the tools for assessing and monitoring stream health to the broadest possible audience. That was our assumption before we embarked on our work in Peru and Costa Rica, and it was reinforced by our experiences on the ground in both places. Moreover, the results of our research and the lessons of our workshops not only have important implications for the residents of the areas in which we worked, but they are also part of a global discussion about water issues. In particular, the message of the vital connection among the protection of small streams, the health of the water supply, and the preservation of large tracts of intact forestland is one that has been at the center of our research, education, and public outreach for almost two decades. Because of our work with groups across North America and other parts of the world, our website has become a significant source of information on water research, education, and monitoring.

What we found

1. **Issues facing the region.** The Madre de Dios region – and particularly the area of the confluence of the Madre de Dios and Tambopata rivers around Puerto Maldonado – is on the verge of enormous and potentially devastating changes. Supported by both private land development efforts and public policy, the region's population has grown by almost 50% in the last decade and has tripled since 1981. Most of that population growth has taken place in the capital city of Puerto Maldonado, but the rapid expansion of agriculture and gold mining has brought substantial deforestation since 1990. That, however, will pale in the face of the Acre-Musako highway and bridge currently under construction. While much of the discussion around the new road focuses on commercial development, the principal impact will come from the projected tenfold, 120,000-hectare, increase in land under cultivation, which account for 45% of the expected economic benefits of the project. Needless to say, land speculation is rampant in the region. Our research in other areas of the world has demonstrated the clear connection between population growth, deforestation, agricultural activities and road building, and the degradation of a region's streams and rivers. Particularly vulnerable are the small streams, whose critical importance is often overlooked by those who readily see the value of the big rivers, which supply transportation, hydropower, food, recreation, habitat, etc. But small and medium-sized streams provide more than 90% of the overall water supply, and their protection is essential to the health of the entire system.

2. **Ecosystem services.** While the economic value of gold mining, agriculture, timber extraction, and tourism are well understood, the substantial economic benefits of clean fresh water are often overlooked. When, for example, we asked the students who came to our August workshop where their fresh water came from, the answer was "the treatment

plant." Moreover, the participants in the fall workshops had very little understanding of the importance of protecting small streams, the vulnerability of Puerto Maldonado's water supply due to the location of the drinking water intake pipe, the relationship of forest protection and clean water, or the availability and use of accessible methods for monitoring water. But they were anxious to learn about the quantifiable value of clean water to human and animal health, to the eco-tourism industry through the protection of food sources, water supplies, and wildlife habitat, and to reducing the costs of treating drinking water and cleaning industrial equipment.

3. **Human health.** From mercury contamination to water-borne diseases to sanitation problems, the connection of clean water and human health was very much on the minds of those who attended our workshops. Several nurses and six staff members of the Ministry of Health came out from Puerto Maldonado and made clear to us the need to continue the work we were doing, both in assessing and monitoring the water sources and in educating people about the issues.

4. **Major impacts.** There is a great deal of concern about the two major impacts on the region's water: (a) gold mining and the mercury contamination that accompanies it; and (b) the transcontinental highway and the population growth and land development it will bring. The information we conveyed about the current state of large parts of the watershed, the pristine small streams that have to date been little affected by human impact, and the critical role played by the large areas of preserved and protected land made a huge impression on the workshop participants. They asked for more information; they grasped the value of the monitoring techniques they learned in the stream and laboratory; and they requested future workshops to alert and teach others.

5. **Rainforest protection.** There exists a strong connection between protecting the water sources of the Amazon headwaters and preserving its rain forests – a connection that has both ecological and economic components. As the Stroud Center's research in temperate streams has clearly demonstrated, streamside forests play a vital role in protecting a stream's health by enhancing the ability of its ecosystem to process organic matter and pollutants. The deforestation of riparian lands compromises both the quantity and the quality of the stream's ecosystem and reduces its ability to deliver important services to humans. In their most recent study of 16 streams in eastern North America, Stroud scientists found that stream sections flowing through forested areas are wider and shallower than those in meadowlands, their beds are rougher and have more habitat, and water moves more slowly through them. These factors, along with other riparian forest benefits, such as a greater variety of organic food and more natural temperature patterns, produce a richer and more natural ecosystem than do deforested streams, and the increased abundance of bacteria, algae, invertebrates, and fish enables them to better process certain pollutants. Moreover, the streams that the rainforest protects in turn provide essential habitat, food, and water for the flora and fauna of the forest itself. The economic value of this symbiosis – for ecotourism, for human health, for savings in water treatment costs – is enormous, and the costs of compromising the health of the ecosystem would be devastating to the region and beyond.

6. **From local to global: issues for a world in crisis.** Coming on the heels of the 4th World Water Forum in Mexico City on March 21st and 22nd, 2006, which "reaffirm[ed] the critical importance of water, in particular fresh water, for all aspects of sustainable development . . . and underline[d] the need to include water and sanitation as priorities in . . . national sustainable development and poverty reduction strategies," our work in Peru and Costa Rica is part of a global effort to give people and their governments the tools they need to protect the sources of clean water – and the human health that is dependent on that water. We see our work as an important step in an ongoing process to protect one of the world's most critical and vulnerable resources – fresh water – in one of the world's most critical and vulnerable environments – the Amazon rain forest.

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Appendices

Measuring watershed health: training conservation planners how to use biophysical tools for monitoring streams in neo-tropical ecosystems

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Appendix 1. Watershed Education Workshops

James Blaine, Bernard Sweeney, and Kristen Travers

The Stroud Water Research Center is applying its 40 years of experience studying stream and river ecosystems to the development of an integrated scientific research and environmental education project in the Andes Amazon region of Peru. The research laid the foundation for two series of workshops, one given near Puerto Maldonado, Peru and the other on the Osa Peninsula in Costa Rica in October and December 2006, respectively. An overview of these two workshops is presented here. Please visit our website (http://www.stroudcenter.org/research/MooreFdnPeru/index.htm) in order to view all materials relevant to the workshops as summarized in Table 1.1.

To reach the most diverse and significant audiences and to maximize the impact of the work we were doing, the Stroud Center's staff designed the workshops specifically for the following participants: (a) local public- and private-sector decision makers; (b) teachers; (c) conservation planners, non-governmental organization staff members, and university faculty; and (d) eco-tourism guides (an example of Peru workshop invitees provided in Table 1.2). This focus enabled us to target our educational efforts at groups that could both use the information in their own work and influence a range of audiences that would ensure a broad dissemination of the issues, information, knowledge, and monitoring techniques. It also enabled us to engage in discussions with local leaders and workers who are currently in positions to make decisions about the use and protection of water resources and with teachers whose students will become the stewards of the future.

Presented in Spanish and offered free of charge, the workshops bolstered the conservation efforts of core preserved areas in the neo-tropics, discussed the latest scientific and educational knowledge on issues affecting fresh water, offered practical and affordable methods for monitoring streams and rivers, taught stewardship practices that the participants can both use themselves and transmit to others, and encouraged appropriate conservation policies in the region – particularly the importance of maintaining forest cover.

Each workshop began with a discussion on the local and global importance of clean fresh water, the many roles clean water plays in their personal lives and local economies, a basic understanding of the ecology of streams and rivers, and usable ways to determine the health of the water in their communities. The opening lecture set the stage for the rest of the day by framing the issues, providing simple but essential statistical and general information about water resources, explaining the relationship of land use to stream health and the impact of human activities on water quality, and discussing the critical role that streams flowing through conserved areas play in the region.

Workshop participants then visited a nearby stream where they performed actual measurements of water quality and collected aquatic macroinvertebrate animals – insects, snails, crabs, and worms – that provide a biological measure of stream health. The group learned how to make basic measurements of water flow and chemistry and to collect and identify aquatic organisms. An introduction to macroinvertebrate identification and the role the stream animals played as

indicators of water quality was presented prior to a laboratory session spent sorting and identifying the animals the group had collected that morning.

While North America and Europe have a long history of biomonitoring, including well-defined protocols for sampling and analysis, Central and South America are in earlier stages of development, and establishing simple macroinvertebrate analysis tools, such as those widely used in the United States, is difficult when the macroinvertebrate community is not well known.

In Peru we focused on the abundance and types of taxa as indicators of stream health. A comparison of 4 streams in the Madre de Dios draining different land uses illustrated how basic chemical and biological monitoring can help determine future conservation and restoration priorities. In the Costa Rica workshops we also used the Virginia Save Our Streams metrics with fairly good success, although the need to develop simple macroinvertebrate analysis tools remains a priority.

The two series of workshops were well attended; see Table 1.3 for Peru participants and Table 1.4 for Costa Rica participants. Perhaps the most consistent message, which came through in both the formal evaluation (Table 1.5) and the participant comments (Table 1.6) on the workshops, was “we want more” – more information, more time to learn, more and better tools to make a difference, more workshops in the future for more people to attend. A corresponding message was “we want it now” . . . because, with the rate of change coming to both regions, time is of the essence.

We encourage others to utilize the training template and resources provided.

Table 1.1. A listing of the stream water-quality workshop materials and related items as presented on the web at (http://www.stroudcenter.org/research/MooreFdnPeru/index.htm).

Workshop-related Information Category	Specific linked item [1]
Workshop Recruitment	1. Workshop Invitation 2. Press Release
Powerpoint Training Tools Workshop Introduction: Importance of Water Macroinvertebrate Identification	1. Workshop Agenda 2. Peru 3. Costa Rica 4. Introduction to Macroinvertebrates 5. Macroinvertebrate Sorting Sheets 6. Macroinvertebrate Identification Guides
Workshop Outcomes	1. Data Interpretation: Comparison of 4 tributaries to the Madre de Dios, Peru 2. Overview of Water Quality Monitoring 3. Workshop Evaluation 4. Workshop Certificates

[1] Specific web address are not provided as they may be changed in future versions of the webpage.

Table1.2. Invitee list for the Peru neotropical stream water-quality workshops.

I. Conservation workers/University faculty & students
Conservation workers associated with Peruvian and US institutions
Amazon Conservation Association (ACA):
Amazon Center for Environmental Education (ACEER)
Inkaterra Association (IA)
Pro Naturaleza (Peruvian arm of the Nature Conservancy)
Conservation International (Lima offices)
World Wildlife Fund (Lima offices)
Centro Internacional de la Papa
Instituto del Bien Común Lima, Perú
Sociedad Peruana de Derecho Ambiental Lima, Perú
Scientists/students from Peruvian academic institutions
Colegio America
Colegio Universitario Andino
Pontificia Universidad Católica del Perú
Universidad Nacional Jorge Basadre Grohmann
Universidad Nacional Mayor de San Marcos
Universidad Nacional de Trujillo
Universidad Peruana de Ciencias Aplicadas
Universidad de Lima
Universidad del Pacifico
Scientists/students from US academic institutions working in Andes-Amazon region
Cornell University (scientists who track wide-ranging Amazonian mammals and birds)
Environmental Law InstituteWashington, DC
Duke University, Center for Tropical Conservation
Field MuseumChicago, Illinois
Nature and Culture International Del Mar, California
Organization for Tropical Studies Durham, North Carolina
University of Florida, Department of Geography Gainesville, Florida
Wildlife Conservation Society Bronx, New York
II. School Teachers
All science teachers and administrators in the Puerto Maldonado region
III. Conservation guides
Guides associated with the major eco-tourism companies in the Madre de Dios region
IV. Decision Makers / VIP's
Leaders of the following institutions:
Instituto de Investigaciones de la Amazonia Peruana (IIAP) (www.iiap.org)
Instituto Nacional de Recursos Naturales (INRENA)
Local and Regional Dignataries
Governor of Madre de Dios region
Mayor of Puerto Maldondo
Minister of Education

Table 1.3. Participants of the Peru workshops.

	Nombre	Representando a:
HOJA DE ASISTENCIA- 24 de Octubre - Guías turísticos		
1	Wilson Escalante Merma	Inotawa
2	Rocio Martinez	TREES PERU
3	Orestes Silva	
4		Corto Maltes Amazonía
5		Corto Maltes Amazonía
6	Isabel Casero	Japipi- Pro Naturaleza
7	Sonia	Explorer's Inn Lodge
8	Anderson Rengifo	Instituto de Idiomas San Bartolomé
9		WASAI
10		WASAI
11		WASAI
12	Elder	Tambopata Lodge
13		Tambopata Lodge
14	Carlos M. Lara Gonzales	Serpentario
15	Silvia Ramos	CETPRO - Deonisia Herrera Alvarado
16	Heidi Luz Gutierrez Gonzales	Japipi
17	Christophe Giraud	Caiman Lodge
18	Sbila Miranda	Caiman Lodge
20	INKATERRA GUIDE	
21	Wills Flowers	Florida A&M University
22	Kristen Travers	Stroud Water Research Center
23	Bernard Sweeney	Stroud Water Research Center
24	James Blaine	Stroud Water Research Center
HOJA DE ASISTENCIA - 25 de Octubre - Organizaciones públicas y privadas		
1	Eusebio Carpio Chávez	Dirección de la Producción Madre de Dios
2	Olger Mocchco Múñoz	Dirección de la Producción Madre de Dios
3	Edgar Clint Lopez Cornejo	Dirección de la Producción Madre de Dios
4	Carmen R. Sarmiento Pérez	Dirección Regional de Educación Madre de Dios
5	Oscar Chávez Pinto	Dirección Regional de Educación Madre de Dios
6	Mariano Silva	Dirección Regional de Educación Madre de Dios
7	Juan Cruz	Dirección Regional de Educación Madre de Dios
8	Esley Huatangare	Gobierno Regional de Madre de Dios
9	Julio Fernandez Llerena	Dirección Regional de Salud (DISA)
10	Hubert Vera Mendoza,	Dirección Regional de Salud (DISA)
11	Ana Manrique V.	Dirección Regional de Salud (DISA)
12	José E. Sotomayor Rivera	Instituto Sup. Pedagógico NSR
13	Victor H. Díaz Pereira	Instituto Sup. Pedagógico NSR
14	Carmen Quispe	EMAPAT
15	Carlos Angel Murga	San Martin de Porras Hospital (Iberia)
16	Alicia Vicente Aguilar	Hospital Santa Rosa
17	Roberto Rubín de Celis	Hospital Santa Rosa
18	Armando Muñante Del Castillo	SENASA
19	Patricia Condori Yanqui	Unidad de Epidemiología y Saneamiento Ambiental
20	Edgar Cáceres Gallegos	Ministerio Energía y Minas
21	ITA	
22	Wills Flowers	Florida A&M University
23	Kristen Travers	Stroud Water Research Center
24	Bernard Sweeney	Stroud Water Research Center
25	James Blaine	Stroud Water Research Center
HOJA DE ASISTENCIA - 26 de Octubre - Conservacionistas		
1	Victor Velásquez Zea	Asociación para la Conservaciòn de la Fauna Silvestre ACOFAS
2	Elizabeth Chulla	ANIA
3	Justo Rengifo	ATI staff
4	Rocio Pérez Torres	Reserva Nacional Tambopata (INRENA)
5	Dante Gutierrez Luna	Reserva Nacional Tambopata (INRENA)
6	Brummel Casapino Aparicio	IST Jorge Basadre
7	Edith A. Jayahuanca Condori	World Wildlife Fund
8	Lenin Guerra	Dirección Regional de Salud
9	Melina	Dirección Regional de Salud
10	Mara Crippa	Rain Forest Expeditions
11	Alex Mishaja	Comunidad Nativa Infierno
12	Milton Jimenez	ACCA
13	Benjamín Chambi Pacompia	Proyecto Botánico ACCA
14	Wills Flowers	Florida A&M University
15	Kristen Travers	Stroud Water Research Center
16	Bernard Sweeney	Stroud Water Research Center
17	James Blaine	Stroud Water Research Center

Table 1.3. Continued.

	Nombre	Representando a:
HOJA DE ASISTENCIA - 27 de Octubre - Docentes		
1	Ena Bouroncle	Institución Educactiva Básica Regular Dos de Mayo
2	Luis A. Mejía Ramírez	Institución Educactiva Básica Regular Dos de Mayo
3	Nixon Rolín	Institución Educactiva Básica Regular Dos de Mayo
4	Alicia Quispe Medrano	Institución Educactiva Básica Regular Faustino Maldonado
5	Eliseo Bernedo Zerato	Institución Educactiva Básica Regular Faustino Maldonado
6	Orlando Minaya Vargas	Institución Educactiva Básica Regular La Pastora
7	Juan C. Pérez Mendo	Institución Educactiva Básica Regular La Pastora
8	Eliana Rivera	Institución Educactiva Básica Regular Santa Cruz
9	Rafael Vizcarra	Institución Educactiva Básica Regular Santa Cruz
10	Rosario Silva	Institución Educactiva Básica Regular Santa Cruz
11	Roberto Sopla	Institución Educactiva Básica Regular Santa Rosa
12	Tito Alferez Gúzman	Institución Educactiva Básica Regular Santa Rosa
13	Erasmo Pfuño Málaga	Institución Educactiva Básica Regular La Novia
14	Osvino Pacaya	ANIA
15	Julissa Laredo Briceño	Institución Educactiva Básica Regular Santa Cruz
16	Adolfo Motta Montes	Instituto Sup. Pedagógico NSR
17	Jorge Lezama Albarrazin	Sociedad Zoológica de Francfort
18	Raul Aleman Abad	Institución Educactiva Básica Regular Fitzcarrald
19	Jurit Roselló	Institución Educactiva Básica Regular Señor de los Milagros
20	Edith Holgado Espirilla	Institución Educactiva Básica Regular Fitzcarrald
21	Jorge Puma	Institución Educactiva Básica Regular Señor de los Milagros
22	Jhon Eguileta Olazabal	Institución Educactiva Básica Regular José Abelardo Quiñones
23	Bianca Cáceres Sayhue	Institución Educactiva Básica Regular Miguel Garu
28	Licia Silva Ortiz	ACEER Coordinator Pto. Maldonado
29	Wills Flowers	Florida A&M University
30	Kristen Travers	Stroud Water Research Center
31	Bernard Sweeney	Stroud Water Research Center
32	James Blaine	Stroud Water Research Center

Table 1.4. Participants of the Costa Rica workshops.

	Nombre	Representando a:
HOJA DE ASISTENCIA - 12 de Diciembre - Guías turísticos		
1	Maximiliano Mendoza Mendoza	Pueblo Indígena Alto Laguna
2	Félix Montezuma Rodríguez	Pueblo Indígena Alto Conte
3	Pilar Bernal Castro	Guía turística independiente
4	Jose Bogantes Ramírez	Ministerio de Ambiente Golfito
5	Carlos Benavides	Ministerio de Ambiente Piedras Blancas
6	Greddy Porras Ávila	Ministerio de Ambiente
7	David Rodríguez Castillo	Ministerio de Ambiente
8	Adilio Rodríguez Segura	Ministerio de Ambiente
9	Carlos Porras Arguello	Río Claro
10	Mauricio Arburola Salazar	Esquinas Rainforest Lodge
11	Adolfo Montero Chávez	Guía independiente
12	Jose Montiel Montiel	Guía independiente
13	Abner Torres Brenes	Esquinas Rainforest Lodge
14	Annanías Arguijo Arroyo	Guía turístico Drake, Asociación Conservacionista de la Naturaleza
15	Rebeca Quirós Herrera	Guía turístico Drake, Asociación Conservacionista de la Naturaleza
16	Wilberth Sácida González	Guía turístico Drake
HOJA DE ASISTENCIA - 13 de Diciembre - Organizaciones públicas y privadas		
1	Grace Urbina Vásquez	Grupo Rescatando a la Naturaleza Ahora (RANA)
2	Ruth Sandoval Gómez	Grupo Rescatando a la Naturaleza Ahora (RANA)
3	Jonathan Avellán Vega	Grupo Rescatando a la Naturaleza Ahora (RANA)
4	Jason Campos Castro	Grupo Rescatando a la Naturaleza Ahora (RANA)
5	Nínive Salazar Rodríguez	Acueducto Sándalo
6	Ulises Venegas Navarro	Acueducto Sándalo
7	Steven Bell	Friends of the Osa
8	Dennis Vásquez Gallo	Friends of the Osa
9	Marco Hidalgo Chavarri	Corredor Biológico Osa
10	Annia Cordero Méndez	Ministerio de Ambiente (Educación Ambiental)
11	Gerardo Blanco Alvarado	Ministerio de Ambiente (Programa Gestión Comunitaria)
12	Zeidy Miranda Godínez	Asociación Femenina de la Palma (ASOFEP)
13	Miguel Madrigal Hernández	Director Área de Conservación Osa
14	Juan Sánchez Villarevia	Ministerio de Ambiente
15	Walter Aguirre Aguirre	Instituto Costarricense de Electricidad (ICE)
16	Trond Larsen	Director Científico Friends of the Osa
HOJA DE ASISTENCIA - 14 de Diciembre - Conservacionistas		
1	Earl Crews	Santuario Silvestre
2	Jose Barquero Marchena	Cámara Turística de Golfito
3	Ricardo Moreno Ruiz	Instituto Internacional en Manejo y Conservación de Vida Silvestre, Universidad Nacional
4	Concepción López Díaz	Proyectos Varios
5	Yorley Novo Sandí	Colegio La Palma (Directora)
6	Yendry Villalobos Flores	Colegio La Palma (Supervisión)
7	Douglas Abarca Moya	Colegio La Palma
8	Erick Navarro Camacho	Colegio La Palma
9	Ángel Arias Godínez	Proyectos Varios
10	Yeimy Campos Valverde	Asociación Conservacionista Dos Brazos Río Tigre (ACODOBRARTI)
11	Sandra Campos Valverde	Asociación Conservacionista Dos Brazos Río Tigre (ACODOBRARTI)
12	Elizabeth Jones	Bosque Río Tigre
13	Abraham Gallo	Bosque Río Tigre
14	Steve Prchal	Ventanas Corcovado.
15	Guillermo Mulder	Independiente
16	Alfredo Quintero	Proyecto Bambú
17	Adelita Altamirano	Propietaria finca Escondido
18	Helen Schärli	Suital Lodge
19	Carlos Ugalde	Suital Lodge
20	Randall Varela	Instituto Nectandra
21	Julio Sánchez	Propietario
22	Steven Bell	Friends of the Osa
HOJA DE ASISTENCIA - 15 de Diciembre - Docentes		
1	Josefa Gómez	Supervisión Puerto Jiménez
2	Nuria Sibaja	Escuela de Cañaza
3	Ileana Valverde	Escuela de Sándalo
4	Olga Rubí Chavarría	Escuela Dos Brazos Río Tigre
5	Grace Esquivel Chavarría	Escuela de Carbonera
6	Nuria Ortiz Chavarria	Colegio Técnico de Puerto Jiménez
7	Marco Arias Redondo	Colegio Técnico de Puerto Jiménez
8	Grace Nieto López	Escuela La Independencia
9	Albán Fonseca Sequeira	Escuela La Independencia
10	Sor Virginia Aguilar	Escuela Central de San José
11	Sor Olga Acuña	Escuela Central de San José
12	Hazel Quesada Monge	Supervisión Golfito
13	Blanca Jiménez	Escuela Km 20
14	Margarita Núñez	Escuela Ana María Guardia
15	Laura Toruño	Supervisión Golfito
16	Jeffrey Gamboa	Escuela Km 20
17	Gabriela Campos	Escuela Ana María Guardia
18	Cecilia Venegas Méndez	Supervisión
19	Ángela Braun	Fundación Neotrópica

Table 1.5. Worship evaluation form (in Spanish) and summary of participant responses.

Workshop Evaluation form:

Taller de Calidad de Agua

Fecha:

Por favor circule el número que major indique su opinión.

	Completamente de acuerdo		Neutral		En desacuerdo
1. Los contenidos del taller fueron nuevos para mi	5	4	3	2	1
2. Los materiales y las notas proporcionadas fueron útiles	5	4	3	2	1
3. Sería útil contar con entrenamientos adicionales	5	4	3	2	1
4. Esta experiencia de aprendizaje fue valiosa	5	4	3	2	1

Si se ofrecieran nuevos talleres en el tema de agua, ¿cuáles contenidos y/o técnicas serían de su interés?

¿Tiene algún otro comentario respecto a esta experiencia? Por favor escríbalo en este espacio.

Summary of participant responses:

Evaluation Forms 5(Highest) --> 1(Lowest)	Eco Guides Peru (15)	Eco Guides CR (16)	Managers Peru (18)	Managers CR (16)	Teachers Peru (23)	Teachers CR (19)	Cons Wkrs Peru (24)	Cons Wkrs CR (22)
Concepts were new for me	4.7	4.8	4.6	4.8	4.7	5.0	4.2	4.3
Materials & handouts were useful	4.6	4.9	4.8	5.0	5.0	5.0	4.7	4.7
Additional trainings valuable	4.5	4.3	4.8	5.0	4.8	4.4	5.0	4.5
Valuable learning experience	4.5	5.0	4.9	5.0	5.0	5.0	4.7	4.7
Average	**4.6**	**4.7**	**4.8**	**4.9**	**4.8**	**4.9**	**4.6**	**4.6**

Table 1.6. Selected comments from Peru workshop participants.

Guides

- I just want to say that it was very interesting and very informative and I got to learn a lot of new things and hope to be in the next workshop. Thank you very much for giving to us your valuable time.

Public/Private Sector Managers

- I would like a greater projection to the population on the importance of the habitats
- I was delighted to get strategies to control contamination (for those who have no previous experience)
- Very good!

Conservation Workers

- I would love to be able to give a similar workshop to people from local communities -it would help them a lot.
- I would like information directed towards farmers and agriculture near river.
- Thank you for helping me become aware of the importance of helping this resource.
- It was very nice that scientific information of this importance was given to the local population. I hope that this will continue.

Teachers

- The experience was very significant. Thank you very much.
- The experience of finding invertebrates in the stream was something new. And observing the water and oxygen with simple instruments… was all a new experience. Thank you.
- Never could I have imagined that the water had that many insects! The experience was very good, I learned much about water contamination. I want more workshops like this. Thank you Stroud!
- The experience was significant. It showed us the great importance of fresh water and the varieties of insects that can be classified.

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Appendix 2: Project overview and study sites

Bernard Sweeney and James Blaine

Watersheds are the natural building blocks of conservation areas. They are the sources of the clean fresh water that is the life blood of all living things, from the largest mammals to the smallest microorganisms. And because they are also the drainage systems for all activities on the land, their streams and rivers are sensitive and accurate barometers of the ecosystem's health. Yet, despite the critical role that water plays in the life and biodiversity of conserved areas, little is known about the condition of the streams and rivers that convey it to the Andes Amazon region. That lack of knowledge has meant that the quality and quantity of fresh water have typically not been included in conservation plans, with the result that landowners, conservation workers, and policy makers have lacked an invaluable tool to help them establish conservation priorities, measure the success of conservation efforts, and calculate the true value of conserved land.

The intent of the Stroud Center's project was to demonstrate the feasibility and value of using information on water quality, water monitoring, and stream health to support and strengthen the Moore Foundation's Andes Amazon initiative directly – and other Moore initiatives such as the Wild Salmon Ecosystems and the Conservation International initiatives indirectly. The plan was simple. First, Stroud Center scientists would carry out an intense field and laboratory effort to produce a credible set of data that described the quality of streams and rivers – both pristine and polluted – in the Andes Amazon region. Stroud Center educators would then translate the technical data the scientists had collected into information and programs that would enable people in the region to understand the issues affecting their water, acquire the tools to protect the sources of that water, and grasp the vital connection between protecting the water and preserving the watersheds from which it comes.

The project promised three major outputs: (1) the field and laboratory effort sought to "test and deploy protocols for measuring and monitoring health in neo-tropical streams;" (2) the scientific findings would provide the basis for "creating and offering training workshops for monitoring biophysical properties of streams and rivers in neo-tropical regions;" and (3) the Stroud Center would "disseminate training workshop and monitoring research information" as broadly as possible.

In April 2006, the Stroud Center's director conducted a preliminary visit to the Andes Amazon region to inspect and screen a large number of potential study streams and rivers in the Madre de Dios watershed of Peru. Based on that survey, 33 sites were selected for more intensive study later in the year. Figure 2.1 shows the general location of the study area in the Andes Amazon region as well as the specific location of each of the 33 study sites, which ranged from small streams to the Madre de Dios River itself and from pristine streams (primarily in conserved areas) to those severely polluted by human activities.

In August 2006, a team of 14 scientists and educators from the Stroud Center, and a collaborator from Florida A&M University, returned to Peru for an 18-day expedition to sample the 33 sites. The team had two main goals: (1) establish a baseline of scientific data on water quality, stream biodiversity, and stream health that would serve as the foundation for understanding and sustaining on-going conservation efforts in the region; and (2) create, test, and implement accessible, easy-to-use, and inexpensive education programs for the people of the region. Operating out of five eco-

tourist/research stations on the Madre de Dios and Tambopata rivers, the team split into four subgroups, each of which hewed to a precise but hectic schedule (see Table 2.1).

Table 2.2 shows the exact location and local name (where known) of the 31 study sites, as well as the type of sampling performed at each site relative to the six scientific disciplines involved in the project. The detailed technical findings for each study discipline are found in subsequent appendices to this report and on our website (www.stroudcenter.org) as follows:

Appendix 3. Biogeochemistry – inorganic and organic nutrients and contaminants
Appendix 4. Molecular Tracers of Contaminants – organic molecules indicating pollution sources
Appendix 5. Microbial Diversity/Ecology – community structure of stream bacteria and archaea
Appendix 6. Macroinvertebrate Diversity/Ecology – community structure and pollution assessment
Appendix 7. Nutrient Processing – in-stream processing of nitrogen, phosphorus, carbohydrates
Appendix 8. Ecosystem Metabolism – in-stream production and use of organic matter

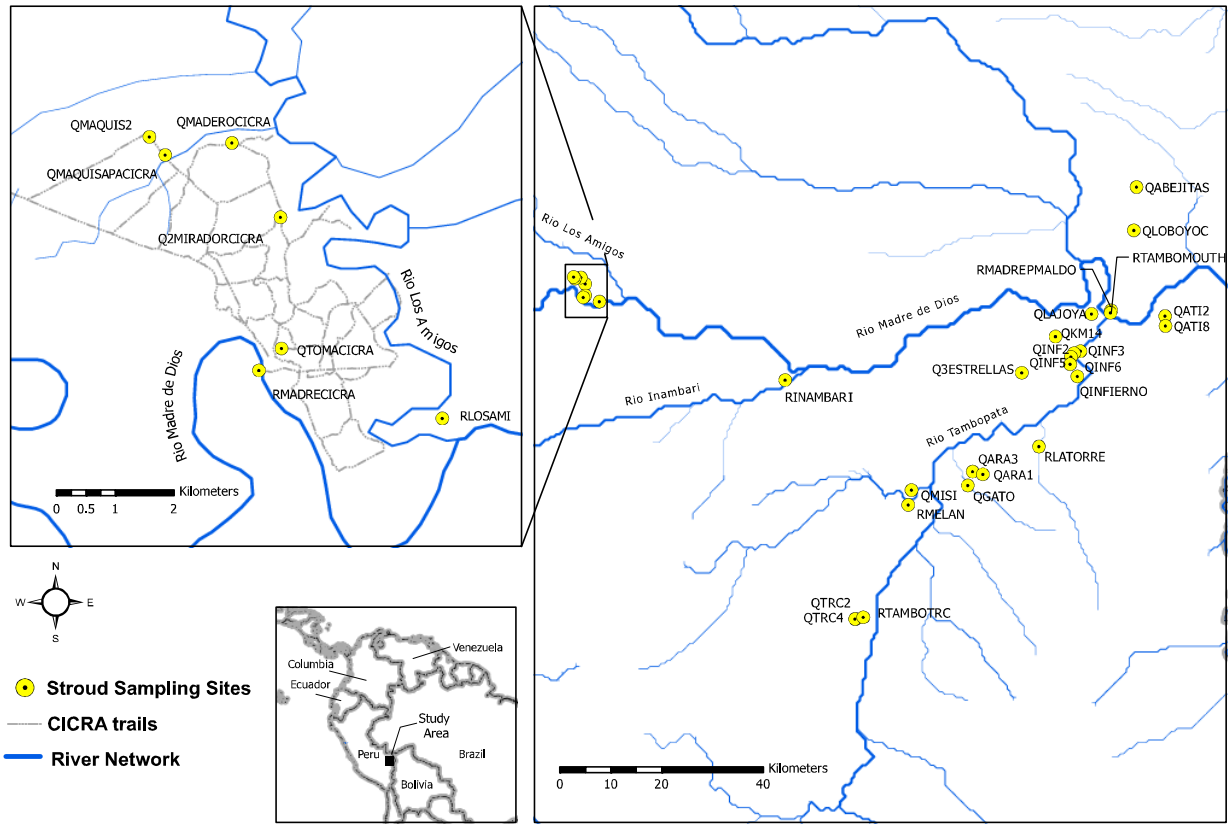

Figure 2.1. SWRC study sites in the Madre de Dios River basin. Refer to Table 2.2 for corresponding site names and type of sampling work conducted at each site.

Table 2.1. Field schedule for the scientific expedition to the Andes Amazon region of Peru, August 14-31, 2006.

	AUGUST																		
	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31
SPIRALING/METABOLISM (Bott, Newbold, Montgomery,Geleskie,Shah,G.Barbieri, K.Stroud n=7)																		
AM	Phila-Miami	Lima-P.Maldo	ATI prep/orien	ATI2	ATI2	ATI2	Move ot P. Maldo	Q. infierno	Q. infierno	Q. infierno	travel P. Maldo to TRC lodge	Q.TRC4	Q.TRC4	Q.TRC4	Q.TRC4	travel TRC to P. Maldo	Fly P. Maldo to Lima	arrive Miami early mornadn fly to Phila by noon
PM	Miami-Lima	travel ot ATI lodge	ATI2	ATI2	ATI2	ATI2	Start setting up at Q. infierno	Q. infierno	Q. infierno	Q. infierno	arrive mid afternoon; unpack; rest	Q.TRC4	Q.TRC4	Q.TRC4	Pack/Rest	Pack /shop	Fly Lima to Phila(during night)	unpack SWRC
sleep	Lima	ATI lodge	ATI lodge	ATI lodge	ATI lodge	ATI lodge	P.Maldo hotel	P.Maldo hotel	P.Maldo hotel	P.Maldo hotel	TRC lodge	TRC lodge	TRC lodge	TRC lodge	TRC lodge	P.Maldo hotel	Airplane	Home
BASEFLOW CHEMISTRY/MICROBIAL ECOLOGY (Gentile, Surma)n=2																		
AM	Phila-Miami	Lima-P.Maldo	ATI prep/orien	Boat to P.Maldo. Sample Tambopata&Madre de Dios on way	Sample to East by car: Q. lajoya, Q.KM14, Q.Tres Estrellas	Sample to North by ferry & car: Q.Laboyoc + 1-2 other streams if interesting	Sample to South by car: Q. Infierno + 4 other streams nearby	Complete filtering & freezing. Organize samples	Car & boat to CICRA lodge; sample some tribs on way	Sample madre de dios &Los Amigos by boat on way to Q.2 mirador CICRA	Sample 3-5 CICRA streams	Boat & Car to P. Maldo Drop off and freeze samples.	sample Refugio1 & Refugio3	Boat to TRC lodge; sample Q. Gato + others on way	sample TRC 2 & TRC 4	Boat to P. Maldo	Final Packing (place frozen samples in coolers) Fly to Lima at noon	arrive Miami early and fly to Phila by noon
PM	Miami-Lima	travel ot ATI lodge	sample & filter ATI2	filter & freeze samples	filter & freeze samples	filter & freeze samples	sampling + begin filtering & freezing	Pack for trip to Los Amigos/ CICRA	filter samples (& Freeze?)	Filter samples (& Freeze?)	Filter samples, pack	Boat to Refugio Lodge Samlple R. LaTorre along way	filter samples (& Freeze?)	unpack; filter samples (& Freeze?)	filter samples/ pack	Pack /shop	Fly Lima to Phila (during night)	unpack SWRC
sleep	Lima	ATI lodge	ATI lodge	P.Maldo hotel	P.Maldo hotel	P.Maldo hotel	P.Maldo hotel	P.Maldo hotel	CICRA	CICRA	CICRA	Refugio	Refugio	TRC lodge	TRC lodge	P.Maldo hotel	Airplane	Home
ENTOMOLOGY (Funk, Sweeney, Flowers) n=3																		
AM	Phila-Miami	Lima-P.Maldo	ATI prep/orien	Finish making up leaf packs	To P. Maldo/unpack/set up in hotel	Sample Q. infierno	Sample Q. tres estrellas	Sample Q. Laboyoc	P. Maldo to Los Amigos (CICRA)	Q. 2 Mirador CICRA	Q. Maquisapa;Q.maquisapa 2	travel CICRA to P. Maldo	sample Refugio 1	Travel Refugio to TRC lodge;sample Q. Gato + others on way	sample Q. TRC2	travel TRC to P. Maldo	Fly P. Maldo to Lima	arrive Miami early mornadn fly to Phila by noon
PM	Miami-Lima	travel ot ATI lodge	Make up lots of leaf packs for use by Entomol as substrates + some for futrue use by use by Licia with students	Sample ATI2	Sample Q. Lajoya	Sample 1-2 more streams near Q. Infierno	Sample Q. KM14	pack/shop	unpack/meet with guides	Q. tomacicra	Q. madero/pack	Boat to Refugio lodge	Refugio	unpack/orient	sample Q. TRC4/pack	Pack /shop	Fly Lima to Phila(during night)	unpack SWRC
sleep	Lima	ATI lodge	ATI lodge	ATI lodge	P.Maldo hotel	P.Maldo hotel	P.Maldo hotel	P.Maldo hotel	CICRA	CICRA	CICRA	Refugio	Refugio	TRC lodge	TRC lodge	P.Maldo hotel	Airplane	Home
EDUCATION (Blaine/Travers) n=2																		
AM	Phila-Miami	Lima-P.Maldo	ATI prep/orien /Meet with Licia Ortiz-Dir of Ed ATI	Finish making up leaf packs	Assist with spiralling	Pilot test Leaf Pack with group of students from P maldo	Move to P. Maldo/Perhaps visit a school in P maldo	help entomol sample Q. Loboyoc	P. Maldo to Los Amigos (CICRA)	Help chem/micro sample madre de dios &Los Amigos by boat on way to Q.2 mirador CICRA	meeting with Nigel etc regarding a workshop later in year	travel CICRA to P. Maldo	assist where needed at Refugio	Travel Refugio to TRC	assist where needed at TRC	travel TRC to P. Maldo	Fly P. Maldo to Lima	arrive Miami early mornadn fly to Phila by noon
PM	Miami-Lima	travel ot ATI lodge	Make up lots of leaf packs for use by Entomol as substrates + some for futrue use by use by Licia with students	Prepare materials for pilot testing leaf pack with class in two days	Assist with spiralling	Finish leaf pack/prep for next day	Meetings with NGO's in P maldo	pack/shop	unpack/meet with guides	help entomol sample Q. tomacicra	pack/further discussions	Boat to Refugio lodge; unpack	assist where needed at Refugio	unnpack/ orient	assist where needed at TRC	Pack /shop	Fly Lima to Phila(during night)	unpack SWRC
sleep	Lima	ATI lodge	ATI lodge	ATI lodge	ATI lodge	ATI lodge	P.Maldo hotel	P.Maldo hotel	CICRA	CICRA	CICRA	Refugio	Refugio	TRC lodge	TRC lodge	P.Maldo hotel	Airplane	Home
Summary lodging	Lima(12); giancarlo and local helper in P maldo	ATI(14) PmaldoHotel(0) CICRA(0) ARA(0) TRC(0)	ATI(14) PmaldoHotel(0) CICRA(0) ARA(0) TRC(0)	ATI(12) PmaldoHotel(2) CICRA(0) ARA(0) TRC(0)	ATI(9) PmaldoHotel(5) CICRA(0) ARA(0) TRC(0)	ATI(9) PmaldoHotel(5) CICRA(0) ARA(0) TRC(0)	ATI(0) PmaldoHotel(13) CICRA(0) ARA(0) TRC(0)	ATI(0) PmaldoHotel(13) CICRA(0) ARA(0) TRC(0)	ATI(0) PmaldoHotel(6) CICRA(7) ARA(0) TRC(0)	ATI(0) PmaldoHotel(6) CICRA(7) ARA(0) TRC(0)	ATI(0) PmaldoHotel(0) CICRA(7) ARA(0) TRC(7)	ATI(0) PmaldoHotel(0) CICRA(0) Refugio(7) TRC(7)	ATI(0) PmaldoHotel(0) CICRA(0) Refugio(7) TRC(7)	ATI(0) PmaldoHotel(0) CICRA(0) Refugio(0) TRC(14)	ATI(0) PmaldoHotel(0) CICRA(0) Refugio(0) TRC(14)	ATI(0) PmaldoHotel(14) CICRA(0) Refugio(0) TRC(0)	Airplane	Home

Table 2.2. Study sites in the Madre de Dios river basin and type of data collected at each site. Biogeochemistry (Biogeochem) included cations, anions, carbon, isotope (C & N), and suspended solids samples. Tracers included molecular tracers and pesticides. Microbial ecology work involved sediment sampling. Macroinvertebrate sampling (Inverts) was both quantitative and qualitative (i.e. hand collections). Ecosystem measurements included both nutrient/carbon cycling (Nutr. Process) and stream metabolism work (Ecosystem).

						Sample Collected					
Major River Basin	Site ID	Site Name	Predominant Watershed Condition[1]	Latitude (dd)	Longitude (dd)	Biogeochem	Tracers	Microbial	Macroinverts	Nutr. Process	Ecosystem
Los Amigos	Q2MIRADORCICRA	Trib to Los Amigos along CICRA trail Segundo Mirador	Forest	-12.54728	-70.09837	✓		✓	✓		
Los Amigos	QMADEROCICRA	Trib to Los Amigos along CICRA trail Maderero	Forest	-12.53578	-70.10594	✓		✓	✓ [2]		
Los Amigos	QMAQUIS2	Trib to Los Amigos along CICRA trail Maquisapa	Forest	-12.53481	-70.11889	✓		✓	✓ [2]		
Los Amigos	QMAQUISAPACICRA	Trib to Los Amigos along CICRA trail Maquisapa	Forest	-12.53761	-70.11644	✓		✓	✓		
Los Amigos	QTOMACICRA	Trib to the Los Amigos along CICRA trail Jean	Forest	-12.56757	-70.09830	✓		✓	✓ [2]		
Los Amigos	RLOSAMI	Rio de Los Amigos at the mouth near CICRA	Forest	-12.57845	-70.07308	✓	✓				
Inambari	RINAMBARI	Rio Inambari at the mouth	Forest	-12.71727	-69.74458	✓					
Madre de Dios	QABEJITAS	Q. Abejitas, Trib to Madre de Dios	Agriculture	-12.37563	-69.12346	✓	✓	✓	✓	✓	✓
Madre de Dios	QATI2	Trib to Madre de Dios at ATI	Forest	-12.60422	-69.07282	✓		✓	✓ [2]		
Madre de Dios	QATI8	Trib to Madre de Dios at ATI	Forest	-12.62165	-69.07238	✓	✓	✓	✓	✓	✓
Madre de Dios	QLOBOYOC	Trib to Madre Dios near Loboyoc	Agriculture	-12.45243	-69.12813	✓			✓ [2]		
Madre de Dios	RMADRECICRA	Rio Madre de Dios near CICRA	Forest	-12.57092	-70.10184	✓			✓ [2]		
Madre de Dios	RMADREPMALDO	Rio Madre de Dios at Puerto Maldonado	Mixed	-12.59377	-69.16848	✓	✓				
Malinowsqui	RMELAN	Rio Malinowsqui at the mouth	Forest	-12.93832	-69.52717	✓					
LaTorre	RLATORRE	Trib to Tambopata at LaTorre	Forest	-12.83478	-69.29586	✓					
Tambopata	Q3ESTRELLAS	Trib to Tambopata near Tres Estrellas	Agriculture	-12.70431	-69.32619	✓			✓ [2]		
Tambopata	QARA1	Trib to Tambopata at ARA	Forest	-12.88405	-69.39518	✓		✓	✓		
Tambopata	QARA3	Trib to Tambopata at ARA	Forest	-12.87934	-69.41312	✓		✓	✓ [2]		
Tambopata	QGATO	R. Gato, Trib to Tambopata	Forest	-12.90395	-69.42187	✓			✓ [2]		
Tambopata	QINF2	Trib to Tambopata near Infierno	Agriculture	-12.66959	-69.23398	✓			✓		
Tambopata	QINF3	Trib to Tambopata near Infierno	Agriculture	-12.66600	-69.22278	✓	✓	✓	✓		
Tambopata	QINF5	Trib to Tambopata near Infierno	Forest	-12.67650	-69.23920	✓			✓		
Tambopata	QINF6	Trib to Tambopata near Infierno	Agriculture	-12.68935	-69.24088	✓					
Tambopata	QINFIERNO	Trib to Tambopata near Infierno	Agriculture	-12.71099	-69.22817	✓			✓		
Tambopata	QKM14	Trib to Tambopata near Puerto Maldonado	Agriculture	-12.64025	-69.26674	✓		✓	✓		
Tambopata	QLAJOYA	Trib to Tambopata at Puerto Maldonado	Urban	-12.60038	-69.20277	✓	✓				
Tambopata	QMISI	Q. Misisipi, Trib to Tambopata	Forest	-12.91185	-69.52127	✓					
Tambopata	QTRC2	Trib to Tambopata at TRC	Forest	-13.14016	-69.62032	✓			✓ [2]		
Tambopata	QTRC4	Trib to Tambopata at TRC	Forest	-13.14010	-69.62111	✓	✓		✓	✓	✓
Tambopata	RTAMBOMOUTH	Rio Tambopata at the mouth	Mixed	-12.59819	-69.16956	✓					
Tambopata	RTAMBOTRC	Rio Tambopata at TRC	Forest	-13.13718	-69.60656	✓			✓ [2]		

[1] Watershed condition is subjective based on field observations and examining aerial photography/satellite imagery.
[2] Hand collections only.

Appendix 3. Biogeochemistry

Anthony K. Aufdenkampe, Charles L.Dow, and Louis A. Kaplan

Executive Summary

- Streams and rivers in the region fall into three major biogeochemical categories that broadly define the ecosystems in those waters. These water types are:
  (i) Clearwater (low levels of dissolved ions, dissolved organic carbon (DOC) and fine suspended solids (FSS));
  (ii) Blackwater (low levels of ions and FSS but high levels of DOC
  (iii) Whitewater (intermediate levels of DOC and high levels of ions and FSS)

- Relative to temperate streams, most small streams in the region have naturally high levels of nitrogen as ammonia, but relatively low levels of other nutrients.

- Human impact to a watershed can increase ion and nutrient concentrations. Although natural variability between sites obscures most human impacts from this one-time survey, some observations are noteworthy:
  - At the most impacted site, QLAJOYA, ion and nutrient concentrations are higher than any other site in this study.
  - The ratios of ammonium nitrogen to total dissolved inorganic nitrogen (DIN) are observably higher at sites suspected of receiving sewage or manure inputs.
  - Stable nitrogen isotopes ($\delta^{15}N$) of nitrate and of fine particulate organic matter (FPOM) at most agricultural sites show the characteristic enrichment due to manure and sewage inputs.
  - Stable carbon isotopes ($\delta^{13}C$) of FPOM suggest that although algae growth is generally not naturally important in the region due to shading by sediment or by forest canopy, removal of either by humans (ponding or cutting) will allow algae to bloom (i.e. QKM14).

Introduction

The chemical properties of natural waters define the environment for an aquatic organism, along with physical properties such as light and physical habitat. Aquatic chemistry is, in turn, defined by the geology of the landscape and modified by the biological systems in that landscape. Therefore, biogeochemical properties and processes provide both controls and clues about aquatic ecosystem structure (Appendices 4 and 5) and function (Appendices 6 and 7). As such, the biogeochemical survey of streams and rivers in the Madre de Dios region of Peru provide critical information valuable to all other components of this project. This survey not only describes the aquatic environments of streams studied more intensively, but puts those more intensive studies into the context of streams and rivers throughout the region.

In addition to providing an environmental context to ecosystem structure and function, biogeochemistry can also be modified by human impacts to the hydrology, morphology, soils, and vegetation of a watershed and by municipal and industrial effluents to stream and

groundwaters. These impacts can result in increased ion (salt) concentrations, increased nutrient concentrations (eutrophication), and decreased dissolved oxygen concentrations. Therefore, the biogeochemical survey also allows for a comparison of the aquatic chemical signatures from different pristine landscapes with the signatures from landscapes that are being used and modified by humans. Furthermore, the survey data provide a baseline from which to assess future human impacts to water resources in the region.

Methods

Field measurements and sampling

The biogeochemistry team surveyed physical, chemical and microbiological parameters at 31 stream and river sites (Figure 2.1, Table 2.1 in Appendix 2), spanning a range of natural water types and a range of human impact. The field measures included temperature, dissolved oxygen, pH, and conductivity. Water samples were also collected for the following laboratory analyses: alkalinity; total dissolved solids (TDS); major anions and cations; nutrients; dissolved organic carbon (DOC) and isotopes ($\delta^{13}C$); biodegradable dissolved organic carbon (BDOC); fine suspended solids (FSS, 0.7-64 µm); fine particulate organic carbon (FPOC), nitrogen (FPON) and isotopes ($\delta^{13}C$, $\delta^{15}N$); coarse suspended solids (CSS, >64 µm); and coarse particulate organic carbon (CPOC), nitrogen (CPON) and isotopes ($\delta^{13}C$, $\delta^{15}N$). CPOC, CPON and coarse-fraction isotopes were measured at 13 of the 31 sites; where not measured, CPOC was estimated as the product of measured CSS and mean measured % organic C.

In the field, portable hand-held meters were used to measure temperature, pH, conductivity, and dissolved oxygen. Most samples for chemical analyses were collected as grab samples, which involved standing in the stream facing into the current, submerging the sample bottle upstream, rinsing the bottle with stream water, and then collecting the water and closing the bottle. Coarse suspended particles were sampled with a 64 µm plankton net that was deployed into the current. The plankton net was retrieved and the particles rinsed to a sample bottle. All sample bottles were closed, placed into a cooler containing ice, and stored cold and dark until they were processed that evening.

Sample processing and analysis

Initial processing of the samples for preservation and transport back to the Stroud Water Research Center was performed within 12 hours of collection in “field laboratories” based in hotels and at field stations. 5-L samples were poured through a 64 µm sieve into a churn sample splitter (Bel-Art), in order to homogeneously subsample suspended sediments for various analyses. Known volumes of water were passed through pre-weighed membrane filters (Millipore HAWP, 0.45 µm pore size) for eventual gravimentric determination of fine suspended sediment (FSS) concentrations, with the filtrate saved for the analysis of dissolved constituents such as the anions, cations, nutrients and alkalinity. Additional known volumes of homogeneously subsampled water were filtered through glass fiber filters (Millipore AP40, ~0.7 µm pore size) for eventual analysis of FPOC, FPON and isotopes, with the filtrate saved for the analysis of DOC and ^{13}C isotopes. Coarse sediments collected on the seive were washed onto preweighed membrane filters (Millipore HAWP, 0.45 µm pore size) for eventual gravimentric

determination of coarse suspended sediment (CSS) concentrations. Filters were air dried or refrigerated, and dissolved constituents were frozen, refrigerated, or chemically preserved, depending upon the specific analyte.

At the Stroud Center, samples were processed and analyzed using predefined and tested standard operating procedures. Frozen filtrates for anion and cation determinations were thawed, filtered through 0.22 µm syringe type filters (Millipore MillexGP) and analyzed by ion chromatography with conductivity detection (Dionex ICS 3000). Samples for DOC and BDOC analyses were filtered through ~0.7 µm glass fiber filters (Whatman GF/F) and dispensed into 40 ml borosilicate vials. DOC samples were analyzed immediately with an Inonics Sievers 900 TOC analyzer equipped with an inorganic carbon removal module. The BDOC samples were inoculated with 400 µl of water from separate collections of site water, the vials capped and sealed with Teflon–lined silicone septa to prevent organic carbon exchange with the atmosphere, and incubated at ~25ºC in the dark for 2 months. At the end of 2 months, the samples were filtered through GF/F filters and analyzed for DOC. BDOC concentrations were calculated as the difference between the initial DOC concentrations and the final concentrations in the BDOC vials. The stable isotope composition of DOC of additional subsamples was analyzed by automated persulfate oxidation and sparging with a OI Analytical Model 1010 DOC-DIC analyzer coupled via a continuous flow GC column interface to a Finnigan DeltaPlus XP Isotope Ratio Mass Spectrometer (IRMS) (St-Jean 2003). The elemental and isotopic composition of fine particulate organic matter (FPOM) trapped onto glass filters and coarse particulate organic matter (CPOM) trapped by the plankton net were analyzed with a Costech 4010 CHNS-O Elemental Analyzer (EA) interfaced in continuous flow with a Finnigan DeltaPlus XP Isotope Ratio Mass Spectrometer (IRMS). The isotopic composition of nitrate was characterized after cadmium reduction to nitrite followed by azide reduction to nitrous oxide gas (McIlvin and Altabet 2005) and subsequent analyses with a Finnigan Gasbench II gas preparation device interfaced in continuous flow with a Finnigan DeltaPlus XP Isotope Ratio Mass Spectrometer (IRMS). All isotope values are provided using the del (δ) scale in units of per mil (‰) relative to Vienna PeeDee Belemnite (VPDB) for carbon and relative to air for nitrogen.

Statistical analysis

A multivariate data analysis method, principal component analysis (PCA), was used to examine whether the study sites could be grouped together based on basic stream chemistry reflecting natural (i.e. geologic) influences. These groups were then compared to a geochemical classification for Amazon rivers based loosely on work by (Stallard and Edmond 1983). The PCA results coupled with the geochemical classification of the sites lead to determining what, if any sites fell outside of these ‘natural’ stream-chemistry settings.

Several iterations of the PCA were run in order to examine and refine the basic stream chemistry inter-relationships among the study sites. The initial run used all sites and included the following chemistry values: anions and cations, nutrients, DOC, BDOC, conductivity, pH, alkalinity, and FSS. Ion concentrations (including nutrients) were expressed in µeq/L with DOC and BDOC expressed in mg/L. All PCAs were run using log-transformed chemistry values, except for pH, with constants added to a few variables to avoid taking the log of zero (e.g. 0.01 µeq/L for Nitrite-N, 0.1 µeq/L for Ammonium-N and 0.001 mg/L for BDOC). A second PCA was run to

more specifically examine geochemical groupings among sites using the following reduced set of chemical parameters: conductivity, alkalinity, pH, FSS, DOC, and K^+. Both of these PCAs were run a second time with a significant site outlier (QLAJOYA) removed, the reasons for which will be discussed in the following section. All PCA results were compared to a geochemical classification of Amazon rivers based on the defined ranges for base cations ($C_b = Ca^{2+} + Mg^{2+} + Na^+ + K^+$) and DOC concentrations.

Results and Discussion

General chemistry (Fig. 3.1) of the streams follows previous observations of low ionic strength and dissolved solids in lowland Amazon streams contrasted with higher ionic strength and greater dissolved solid concentrations for streams and rivers draining the Andes (Stallard and Edmond 1983). Conductivity, a proxy for ionic strength, was very low in some of the lowland streams (<10 μS/cm for QTOMACICRA, QARA3, QTRC4, and QMAQUIS2) and even in the larger rivers measured conductivity was < 120 μS/cm. A notable exception was QLAJOYA where conductivity was 224 μS/cm. Based on field observations for QLAJOYA, it is a lowland stream impacted primarily by sewage inputs and other urban-related influences. Along with measured conductivity, these field observations for QLAJOYA were supported by other chemistry values: exceedingly low dissolved oxygen of 0.09 mg/L and an exceptionally high NH_4-N of 1.2 mg/L that was nearly an order of magnitude greater than measured at any of the other stream sites. Elevated Cl^- concentrations in streams, linked to increased human activity in watersheds both in temperate latitudes (Herlihy et al. 1998) and in the Amazon (Biggs et al. 2002), was also elevated in QLAJOYA relative to the other streams (Fig. 3.1B). Interestingly, NO_3-N concentrations were higher in lowland streams that exhibited overall low ionic strength (Fig. 3.1C).

The PCA results helped to further define general stream chemistry patterns for this study (Fig. 3.2). An initial PCA run using all chemistry values as described in the Methods section (results not provided) showed the QLAJOYA site as a significant outlier. In a subsequent PCA that excluded this site (Fig. 3.2A), the first two factors were able to explain 56% of the variability in the chemistry data. The first factor separated sites along a gradient of increasing ionic strength in one direction and increasing NO_3-N and NO_2-N in the opposite direction. The second factor separated sites based on phosphorus (SRP) and DOC/BDOC concentrations as well as K^+, Na^+ and NH_4-N. These patterns were essentially the same when QLAJOYA was included in the analyses versus when it was excluded. This similarity in PCA results suggests that QLAJOYA chemistry is not anomalous for streams in this region but does in fact define a water-quality endpoint along a non-impacted to impacted stream quality gradient.

Running a PCA with the reduced set of chemistry variables (Fig. 3.2B) was intended to determine whether the sites could be further separated into geochemical classifications of black, clear, or white waters as described by Sioli (1975, 1984) and now in common use throughout the tropics. Specific to these study sites, “Black” was operationally defined as water with low ionic strength, reflected in base cation (Cb) concentrations of < 450 μeq/L and DOC concentration of > 1.5 mg/L; “clear” waters also had low ionic strength plus low DOC (< 1.5 mg/L); “white” waters have high ionic strength (Cb ≥ 450 μeq/L). The corresponding PCA demonstrated that the

Cation (top), anion (middle) and nutrient (bottom) concentrations measured at the sampling sites.

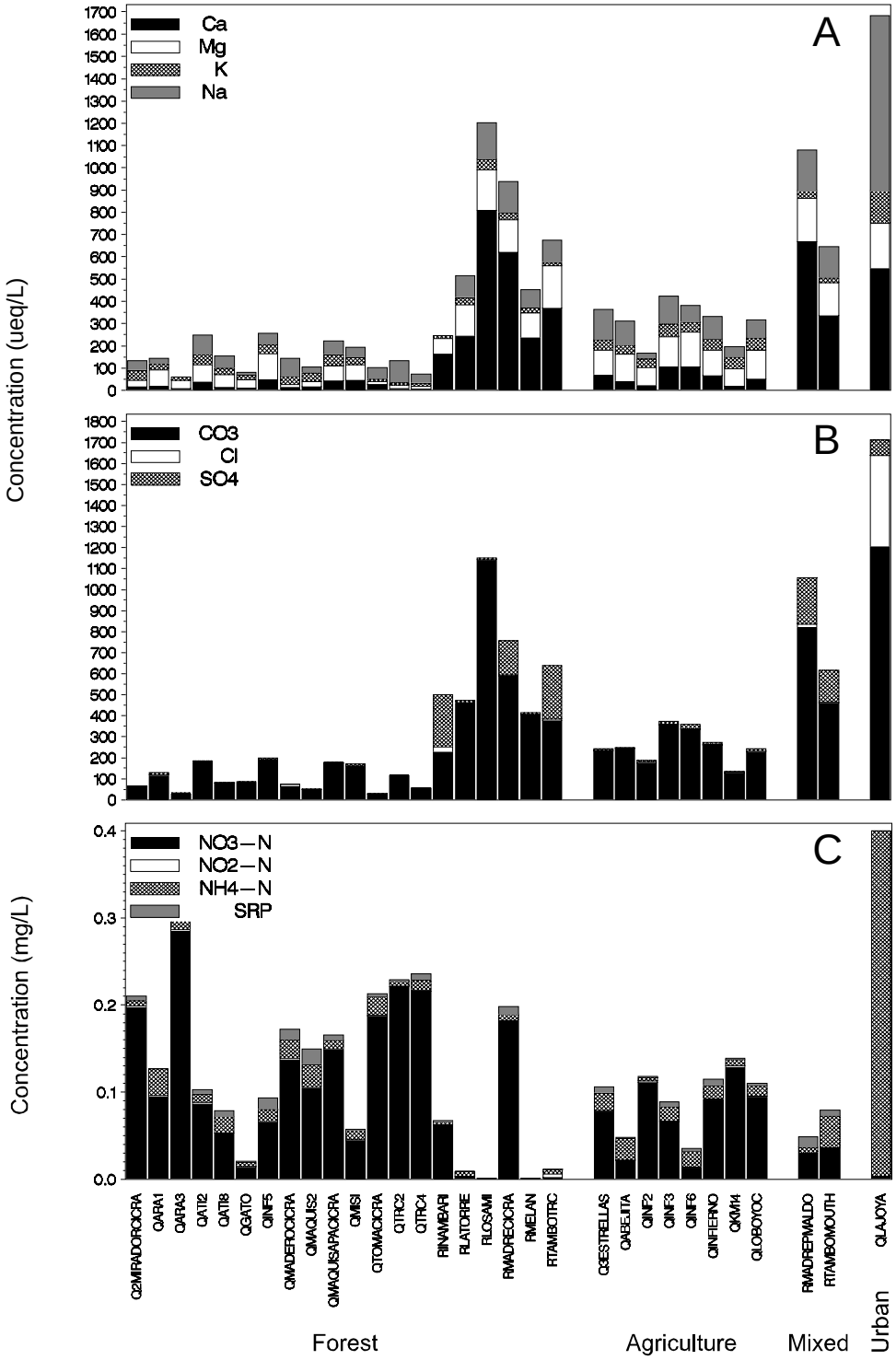

Figure 3.1. Cation (A), Anion (B), and Nutrient (C) concentrations measured in August 2006 at 31 stream sites in the Madre de Dios River basin. The concentration axis for the nutrient plot (C) was truncated at 0.4 mg/L due to the large difference in NH_4-N concentrations between the QLAJOYA site and all others.

Grouping of sampling sites based on ion, nutrient, dissolved & biodegradable organic carbon, and fine suspended sediment concentrations.

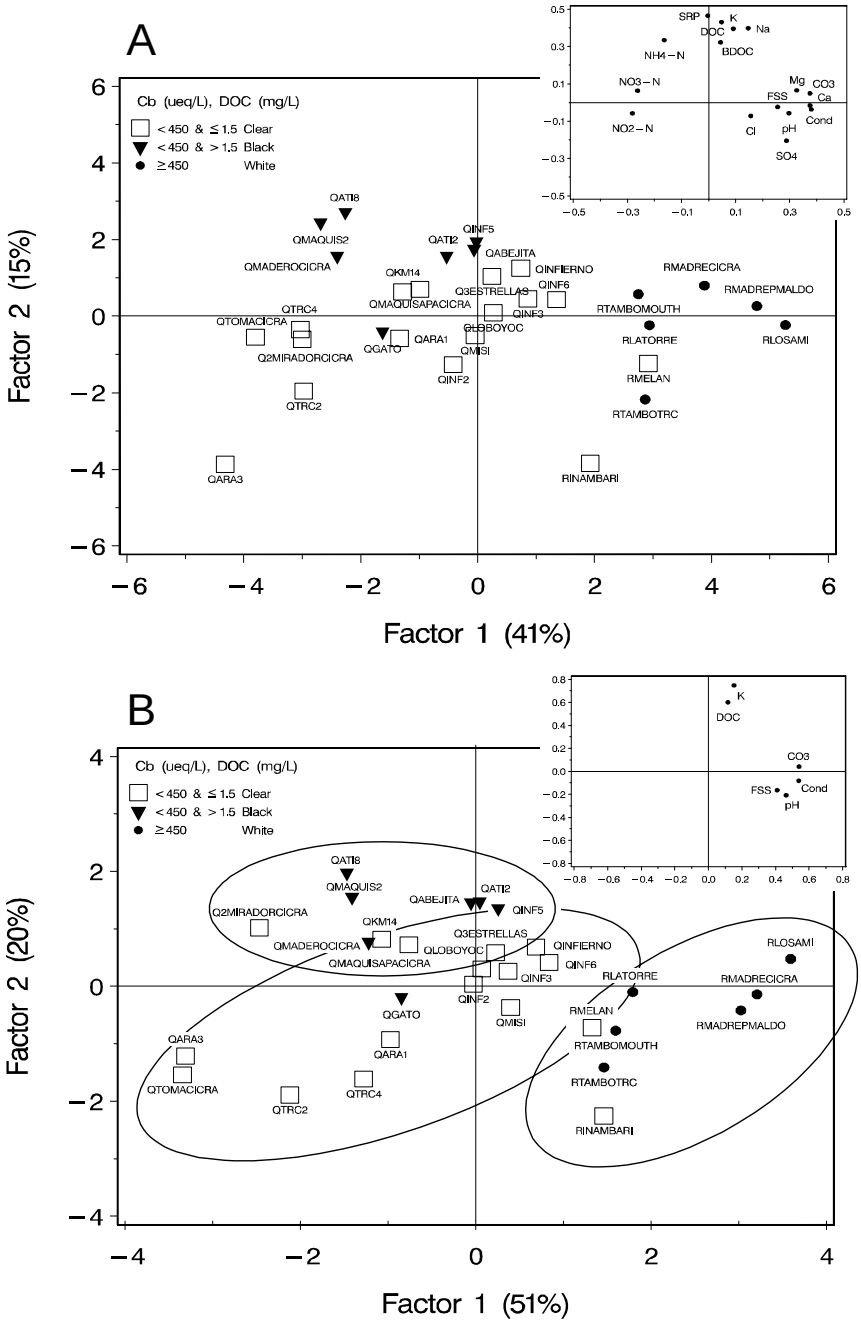

Figure 3.2. Site scores for the first 2 factors from a PCA run using all ion chemistry, nutrients, DOC/BDOC, FSS, alkalinity, conductivity and pH (A) and a PCA run using a reduced set of chemistry variables including Conductivity, pH, alkalinity, FSS, K+, and DOC. Variance explained by each factor is included in the axis headings. The inset provides the factor loadings (i.e. the input variables with the relative influence of each in separating the stream sites). Site symbols provide ranges in base cations (Cb) and DOC concentrations. Site QLAJOYA was not included in these PCAs because an intial analysis showed this site to be a significant outlier (approximate plotting position in plot A would have been: factor 1 = 5.9, factor 2 = 4.3).

large rivers sampled (identified by the leading 'R' in the site id) were separate from the other sites and for the most part could be classified as white waters. A notable exception is RINAMBARI which had an extremely low Na^+ concentration relative to the other cations measured at this site as well as relative to the Na^+ measured at all other sites (Fig. 3.1A).

Unlike the whitewater-designated sites, much less separation occurred between clear and black-water sites. True blackwater streams, in addition to having low ionic strength and relative high DOC, should also exhibit relatively low pH (Sioli 1975). The lowest pH measured during the August 2006 visit was 5.05 at site QTOMACICRA; the corresponding DOC concentration though was only 0.59 mg/L, which makes QTOMACICRA a clearwater stream. A majority of the sites (74%) had pH values > 6. The PCA result (Fig. 3.2B) reflects this discrepancy between pH and the black-water designation. Here, pH loads along the first axis (i.e. Factor 1) with measures of ionic strength and not with DOC, which separates sites along the second axis. Other factors, such as human landscape alteration (i.e. deforestation, urbanization), may be impacting stream chemistry at these sites beyond any geophysical controls. Further work is needed to both properly identify and assess the relative extent of those factors influencing stream chemistry in these streams.

Relationship between the stable nitrogen isotope composition ($\delta^{15}N$) of nitrate and nitrate concentration relative to the predominant watershed condition of the sampling sites.

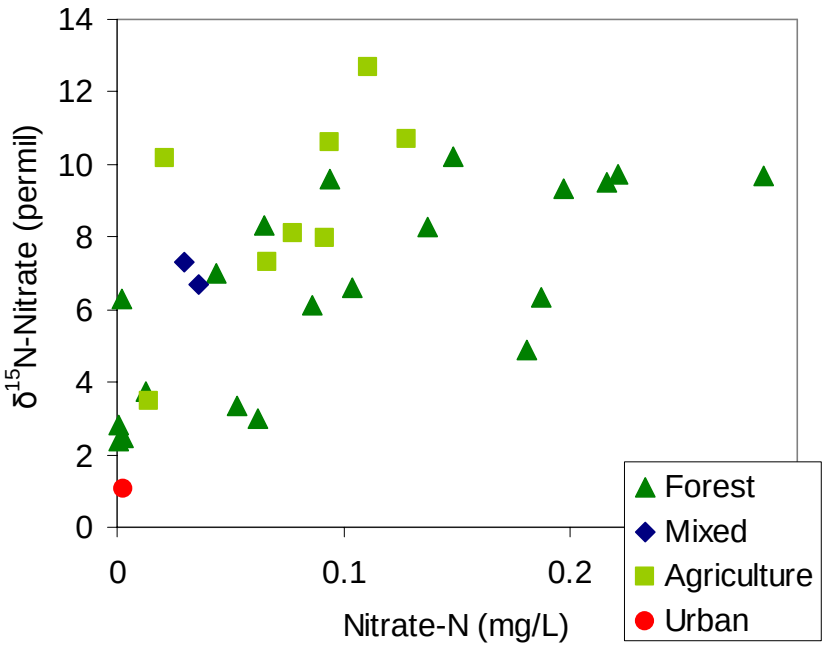

Figure 3.3. Stable nitrogen isotope composition ($\delta^{15}N$) of nitrate as a function of nitrate concentration.

Nitrate is the form of nitrogen most commonly associated with fertilizer, sewage and manure pollution in North America, leading to widespread eutrophication, or the overgrowth of algae. As

noted earlier, nitrate was found in relatively low concentrations throughout the study area. However, some trends are noteworthy. Nitrate was the only major ion (along with nitrite) that was negatively correlated to alkalinity, conductivity and pH ($r = -0.52, -0.45$ & -0.51, $p = 0.003$, 0.01 & 0.003 respectively). Furthermore, nitrate concentrations were positively correlated to the stable nitrogen isotope composition ($\delta^{15}N$) of nitrate (Fig. 3.3, $r = +0.58$, $p = 0.0007$), with the average of agricultural streams (8.9±2.8‰) higher than forested streams (6.5±2.8‰). Because $\delta^{15}N$ is generally enriched in manure and sewage over natural soils (Kendall 1998), the observed patterns may suggest that livestock are the cause of increased nutrient loads to streams, however slightly. At QLAJOYA, the exceptionally low nitrate concentration and depleted $\delta^{15}N$ is consistent with nitrification of ammonium ($[NH_4\text{-}N] = 1.2$ mg/L) followed by very rapid and nearly complete denitrification occurring in the oxygen poor and DOC rich sediments and waters (DO = 0.09 mg/L; DOC = 4.7 mg C/L) (Kendall 1998).

Fine and coarse particulate (top) plus dissolved and biodegradable (bottom) organic carbon measured at the sampling sites

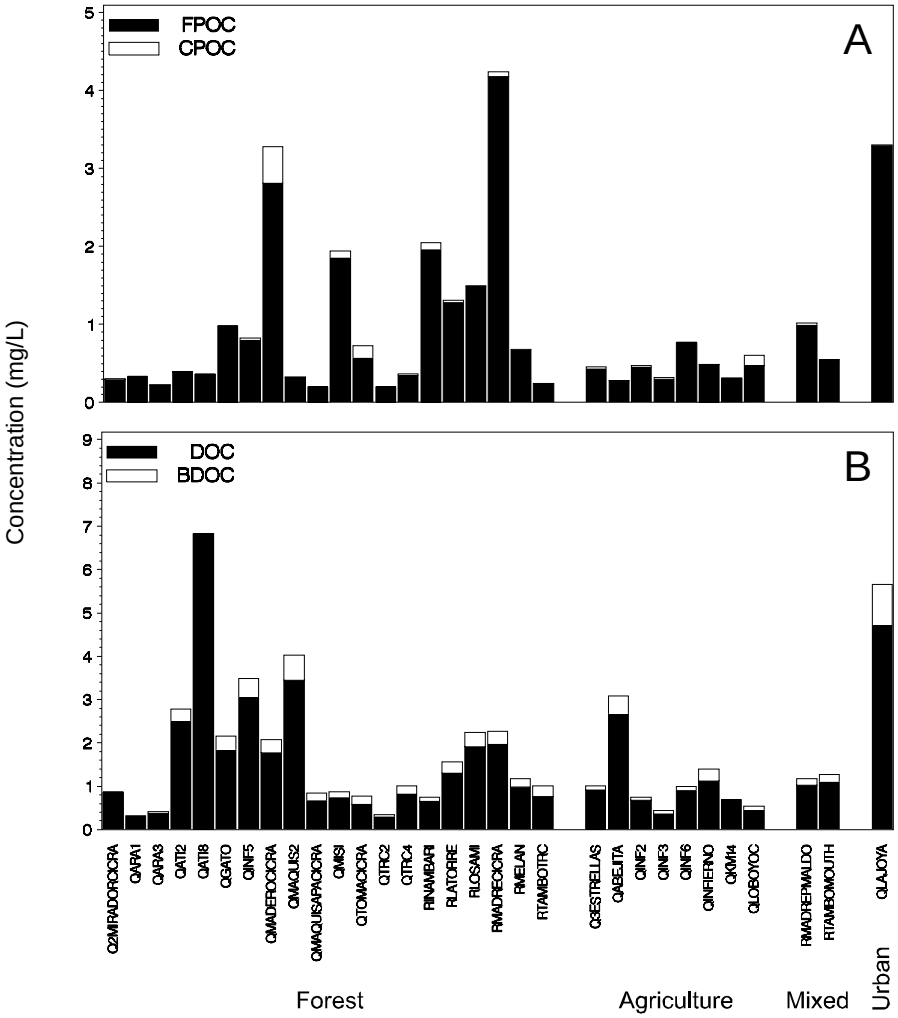

Figure 3.4. DOC/BDOC (A) and organic carbon from fine suspended solids (FPOC) and coarse suspended solids (CSS) (B) concentrations measured in August 2006 at 31 stream sites in the Madre de Dios River basin.

Dissolved organic carbon concentrations in the streams separated out along gradients that included high concentrations in blackwater streams (mean 3.31 mg C/L; range 1.77 to 7.94 mg C/L), intermediate concentrations in whitewater rivers (mean 1.82 mg C/L; range 0.76 to 1.91 mg C/L) and low concentrations in clearwater streams (mean 0.67 mg C/L; range 0.29 to 0.91 mg C/L) (Fig. 3.4). DOC concentrations within the range of ~ 1 to 9 mg C/L have been reported for other headwater streams (Johnson et al. 2006, Saunders et al. 2006) and lowland rivers (Castillo et al. 2004) within the Amazon. The very low concentrations (< 0.5 mg C/L) observed in 5 of the streams, while similar to those observed in dry tropical streams of Costa Rica (Newbold et al. 1995), are not known to have been reported for the Amazon. One highly polluted site, QLAJOYA, had an elevated DOC concentration (4.71 mg C/L) that could result from sewage or other organic inputs. BDOC concentrations followed the same pattern with mean concentrations declining from blackwaters (0.46 mg C/L) to whitewaters (0.35 mg C/L) to clearwaters (0.11 mg C/L) (Fig. 3.4). Both DOC and BDOC were negatively correlated to dissolved oxygen ($r = -0.47$ & -0.58, $p = 0.007$ & 0.0006 respectively) and positively correlated to ammonium ($r = 0.38$ & 0.59, $p = 0.03$ & 0.0005 respectively), suggesting that dissolved organic matter utilization by bacteria impacts water chemistry for other organisms. Stable carbon isotope ratios of DOC ranged from -26.0‰ to -32.2‰ with a median of -30.1‰, suggesting that the primary source of DOC are leaves of trees and other terrestrial plants using the C3 photosynthetic pathway. The most enriched $\delta^{13}C$ were observed in moderate-sized rivers draining the Andes (-26.0‰ and -27.6‰ for RTAMBOTRC and RINAMBARI respectively), as would be expected given known isotopic enrichment in plants of about 1‰ per 1000 m elevation gain (Körner et al. 1988, Körner et al. 1991). There was no obvious pattern of enrichment $\delta^{13}C$ in streams draining pastures , as one might expect if C-4 grasses were replacing trees as a dominant source of DOC, and as was discernable in streams and rivers of the southern Brazilian Amazon (Bernardes et al. 2004). However, detailed GIS datasets of landuse would be required to evaluate this question quantitatively for the study. $\delta^{13}C$ of DOC showed a weak positive correlation with dissolved oxygen ($r = 0.36$, $p = 0.04$) and weak negative correlations to DOC and BDOC ($r = -0.33$ & -0.33, $p = 0.07$ & 0.07 respectively), suggesting if anything, that pasture grasses did not contribute significantly to stream DOC or BDOC loads.

Particulate organic carbon concentrations (POC) were dominated by fine particles at all sites (Fig. 3.4) and concentrations did not fall out along the same gradients as described for DOC. One blackwater stream, QMADEROCICRA had over 3 mg/L of POC, while the rest of the blackwaters had concentrations of less than 1 mg C/L. One whitewater river RMADRECICRA had a POC concentration of over 4 mg C/L, while all other whitewaters, with the exception of the polluted QLAJOYA were less than 1.5 mg C/L. Two of the clearwaters, RINAMBARI and QMISI had POC concentrations that were close to 2 mg C/L, while all other clearwaters had POC concentrations that were less than 0.8 mg C/L. The “quality” or composition of the POC, on the other hand, was a strong function of fine suspended sediment concentration, and therefore separated whitewaters from clear and blackwaters. As seen in essentially all major rivers (Meybeck 1982, Devol and Hedges 2001), the weight percent carbon of fine suspended sediments (FSS) decreased asymptotically to about 1-2% with increasing FSS concentration (Fig. 3.5A), indicating that it is mostly mineral-bound (Mayorga and Aufdenkampe 2002, Aufdenkampe et al. In Press). Where FSS concentrations were < 50 mg/L, fine particulate organic carbon (FPOC) increased to %OC values as great at 12% (Fig. 3.5A). Carbon to nitrogen

Relationship between the percent organic carbon (top) and C/N ratio (bottom) in fine and coarse suspended sediments relative to fine suspended sediment concentrations.

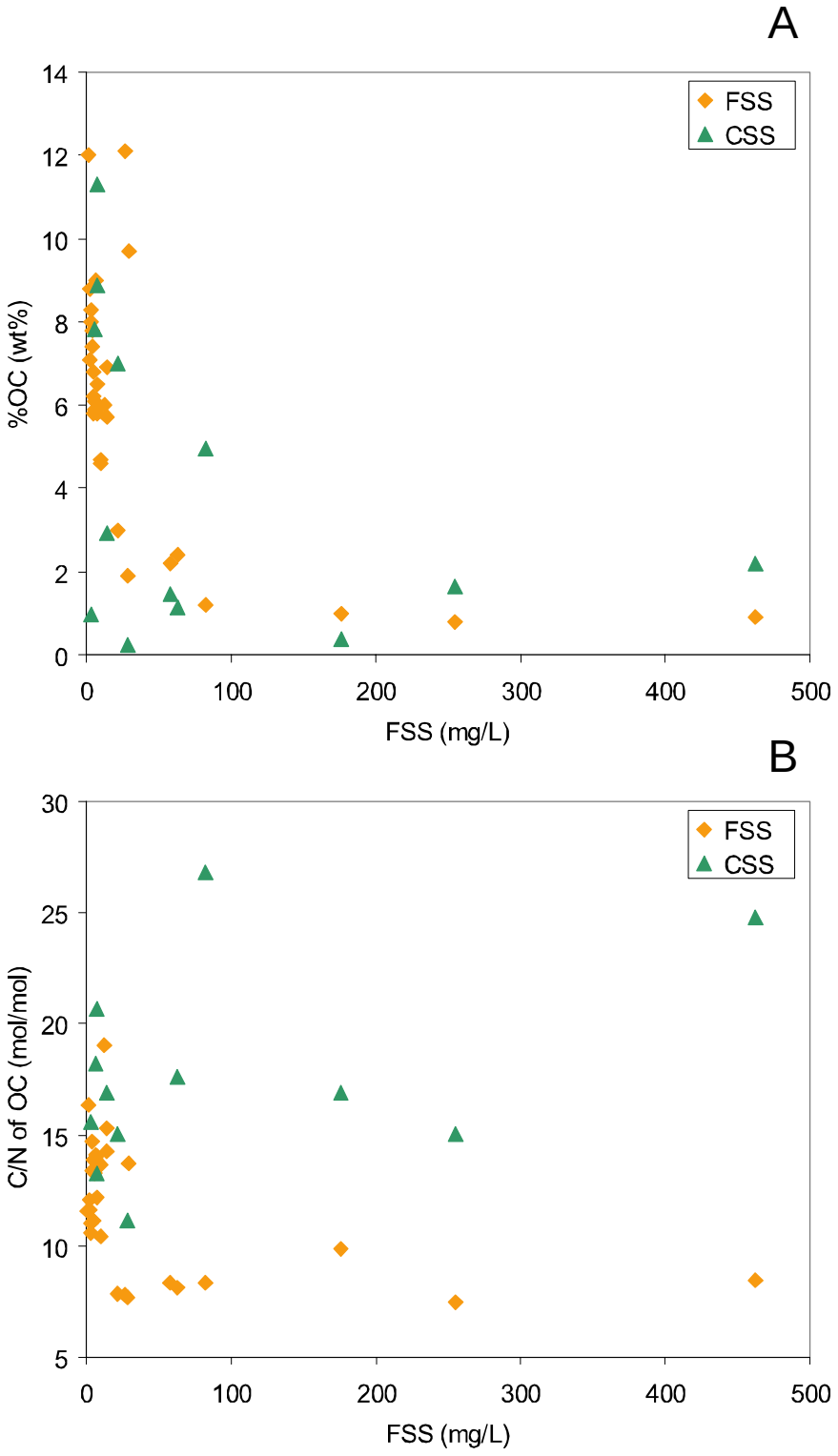

Figure 3.5. (A) Weight percent organic carbon (%OC) of fine suspended sediments (FSS) and coarse suspended sediments (CSS) as a function of FSS concentration. (B) Molar carbon to nitrogen ratio of FSS and CSS as a function of FSS concentration.

Relationship of stable C isotope composition (del ^{13C}) of dissolved, fine particulate, and coarse particulate organic C with fine suspended sediment concentration.

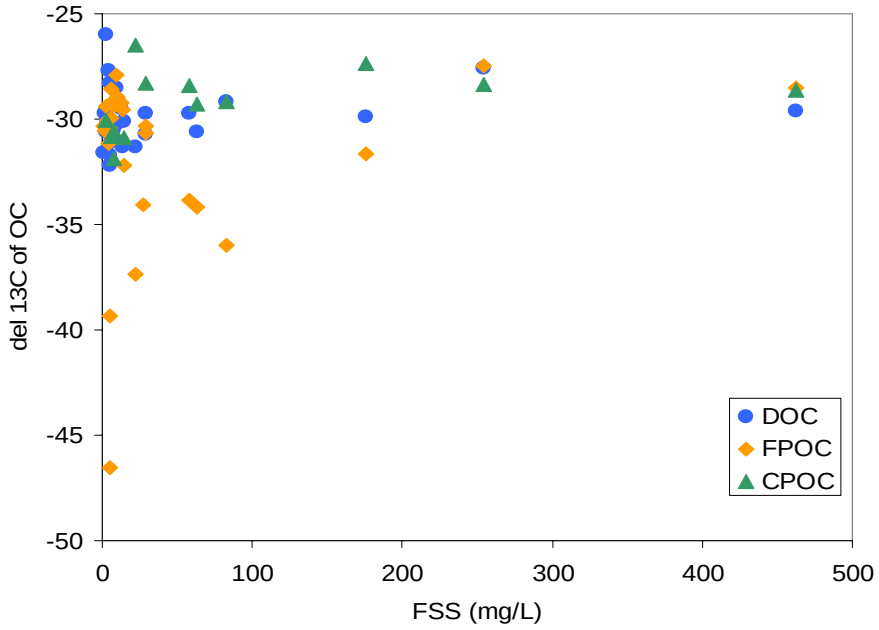

Figure 3.6. Stable carbon isotope composition (del ^{13C}) as a function of FSS concentration for the three studied organic size fractions.

Relationship between the stable N isotope composition ($\delta^{15}N$) of nitrate with the stable N isotope composition ($\delta^{15}N$) of fine particulate organic matter (FPOM) relative to the predominant watershed condition of the study sites.

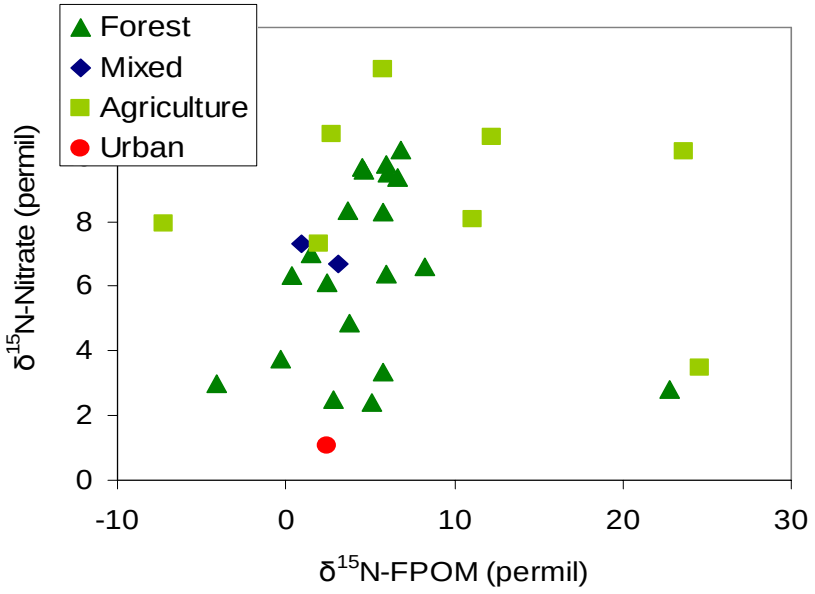

Figure 3.7. Stable nitrogen isotope composition ($\delta^{15}N$) of nitrate as a function of the stable nitrogen isotope composition ($\delta^{15}N$) of fine particulate organic matter (FPOM).

ratios of FPOM were also an asymptotic function of FSS concentrations, at 5-10 where FSS > 50 mg/L and 10-20 where FSS < 50 mg/L (Fig. 3.5B). Stable carbon isotopes of particles ranged from -27 to -32‰ for most sites, but 7 sites exhibited $\delta^{13}C$ values from -33.8 to -46.5‰ (Fig. 3.6) which can be best explained by algae growing on DIC that is depleted relative to the atmosphere due to organic carbon respiration (Mayorga and Aufdenkampe 2002). These latter sites with depleted $\delta^{13}C$ all had relatively low FSS concentrations and no canopy shading, and therefore sufficient light for algal growth. These elemental and isotopic patterns further confirm that FPOC in high sediment whitewaters is tightly mineral-bound and of likely soil origin, whereas FPOC from other stream/river types has a substantial component from leaves and possibly algae in those sites where there is sufficient light (Hedges et al. 1986, Hedges et al. 1994, Aufdenkampe et al. 2001). QKM14 is an example of a site where human disturbance has enabled algae to bloom ($\delta^{13}C$-FSS = -46.6), both due to increased nitrate concentrations (0.13 mg/L, third highest in study) likely due to human or sewage ($\delta^{15}N$-nitrate = 10.7‰) and also due to the ponding of stream water for recreational purposes (longer residence time with little shade). Last, the $\delta^{15}N$ of FPON is also sensitive to isotopic enrichment from inputs from manure and sewage and offers a complementary view of potential human impact to stream nutrient cycles (Fig. 3.7).

In summary, the biogeochemistry of streams and rivers in the study region are largely determined by geology (erosion rates, soil types) which sets inorganic ion and suspended sediment concentrations. These initial signatures are further modified by both natural biological processes and by human impacts to the landscape. The purpose of these data is to provide a description and baseline of the chemical habitat at each site and for interpreting the biological studies of aquatic ecosystem structure (Appendices 5 and 6) and function (Appendices 7 and 8) by defining the aquatic environment in which organisms live. In addition, however, the biogeochemical studies have illuminated a number of potential human impacts to these systems, such as: (1) clearly indicating that QLAJOYA is so highly impacted that its biogeochemistry falls outside the range of natural variability in the region; (2) suggesting that manure and sewage inputs may be enhancing nutrient loads in most sites with substantial pasture; (3) showing that although algal growth is generally not naturally important in the region due to shading by sediment or by forest canopy, removal of either by humans (ponding or cutting) will allow algae to bloom.

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Appendix 4. Molecular Tracers of Contamination

Anthony K. Aufdenkampe and Charles L.Dow

Executive Summary

- A total of 31 organic compounds that are tracers of human contamination were analyzed in 7 streams. The molecular tracers represented 6 classes of compounds (polycyclic aromatic hydrocarbons (PAH), pesticides, polychlorinated biphenyls (PCB),fecal steroids, caffeine, and fragrances).A tracer compound does not itself need to be toxic or directly contribute to water quality degradation. Rather, it only needs to enable discrimination between different sources of pollution therefore acting as a proxy for contaminant(s) originating from those same sources.
- PAHs, derived from combustion-generated soot and from petroleum, had levels in all streams that were uniformly low and below all EPA water quality toxicity criteria. For perspective, a recent study by the Stroud Center of the NY state Catskill Mountain region showed that 54 of 180 streams exceeded these same EPA water quality guidance values.
- Toxic levels of at least one pesticide or PCB were detected at 3 sites.
  - The urban stream, QLAJOYA had elevated concentrations of every pesticide and PCBs measured.
  - QINF3, a stream draining a banana plantation, contained exceptionally high concentrations of the insecticide Chlorpyrifos and the fungicide Metalayxl.
  - Two “pristine”, protected streams contained noteworthy pesticide concentrations: QTRC4 had Chlorpyrifos concentrations that were higher than at the highly impacted urban stream QLAJOYA and above chronic toxicity guidelines for aquatic life, and QATI8 contained the second highest concentrations measured in this study of the herbicide Atrazine (albeit still lower than levels considered toxic to aquatic life).
- Fecal steroid concentrations at the urban QLAJOYA approached levels associated with dangerous pathogen risk, and steroid ratios indicated a strong human component to the contamination. The pasture-dominated site QABEJITA had steroid signatures suggesting cattle as the primary fecal source.

Introduction

Degradation of water quality can occur from a variety of point and non-point sources originating from both anthropogenic and natural factors. Sources include human sewage, livestock manure, agricultural runoff, road and urban runoff, industrial effluent, mining activities, atmospheric deposition, and even wildlife. The range of contaminants includes excessive nutrient loading, heavy metals, pesticides, other toxic organic compounds, and pathogens. These contaminants have substantially negative impacts both to ecological integrity – as measured by aquatic biodiversity (Appendices 5-6), ecosystem function (Appendices 7-8), and ecosystem services – and to drinking water resources. Therefore, in order for policy-makers and managers to best maintain the quality of water resources, targeted efforts to reduce or eliminate primary contamination sources first require the accurate identification and quantification of all contaminant sources that contribute to the degradation of water quality.

The use of molecular tracers to identify sources of contaminants is a technique that qualitatively links chemical fingerprints unique to these sources with contaminants of concern (Leeming and Nichols 1996, Standley et al. 2000, Kolpin et al. 2002, Yunker et al. 2002, Buerge et al. 2003, Aufdenkampe et al. 2006). These tracer compounds do not themselves need to be toxic or directly contribute to water quality degradation, but rather they only need to enable discrimination between different sources and therefore act as proxies for contaminants originating from those same sources. For example, a recent and increasingly used proxy to detect potential sewage contamination is the fecal steroid coprostanol. While not considered toxic to humans or aquatic life at any measured environmental concentration, coprostanol is much more abundant in human feces than that of any other animal (Leeming et al. 1996). Therefore, high aquatic concentrations of coprostanol are a strong indicator of human sewage and or septic contamination (Leeming and Nichols 1996, Aufdenkampe et al. 2006).

A suite of 31 organic compounds were selected to act as robust proxies for a variety of contamination sources (Table 3.1). These compounds include twelve polycyclic aromatic hydrocarbons (PAH), four pesticides, three polychlorinated biphenyls (PCB), one fragrance material (FM), caffeine (CAF) and ten fecal steroids (FS).

Polycyclic aromatic hydrocarbons are found in raw and refined petroleum and coal products and are also formed during the combustion of vegetation, wood, waste, coal and petroleum. Thus PAHs have both natural and anthropogenic sources. The compounds that were quantified here were fluorene (FLU), phenanthrene (PHE), anthracene (ANT), 2-methyl phenanthrene (2MP), 1-methyl phenanthrene (1MP), fluoranthene (FLR), pyrene (PYR), benzo(a)anthracene (BAA), chrysene (CHR), benzo(b)fluoranthene (BBF), benzo(k)fluoranthene (BKF), and benzo(a)pyrene (BAP). These last five PAHs exhibit high toxicity to both humans and aquatic organisms (USEPA 2004).

Pesticides are synthetic compounds used by humans for their insecticidal, herbicidal or fungicidal properties. They are toxic by design. Here, 2 insecticides (Chlorpyrifos & DDT) were analyzed, one herbicide (Atrazine) and one fungicide (Metalaxyl) that were chosen based on their common use worldwide and in the tropics (http://www.epa.gov/oppbead1/pestsales/01pestsales/table_of_contents2001.html) and also on their amenability to analysis by the method used for the other tracers.

Polychlorinated biphenyls (PCB) are a group of 209 related compounds that were used extensively by the electrical industry as insulators and coolants until banned in the USA in 1977. PCBs include some of the most toxic substances listed under EPA National Recommended Water Quality Criteria, with 0.000064 µg/L of total PCBs (the sum of all 209 individual compounds) set as the maximum recommended concentration for the safe human consumption of fish from those waters (USEPA 2004). Because of this extreme toxicity, and also because of persistence in the environment and widespread worldwide use, PCBs are a valuable group of compounds when scanning for aquatic toxicity.

Fragrance materials are anthropogenic compounds used in a variety of consumer products such as soaps, detergents and lotions. Thus, fragrances enter the environment primarily through greywater sewage (Simonich et al. 2000). The compound that were quantified here is galaxolide (1,3,4,6,7,8-hexahydro-4,6,6,7,8,8-hexamethylcyclopenta[γ]-2-benzopyran, HHCB). HHCB is non-biodegradable, making it particularly suited for tracers studies (Simonich et al. 2002), and because of its unambiguous source can act as a proxy for other sewage related contaminants.

Caffeine is a natural compound that occurs in certain tropical plants, including tea and coffee, and is added to numerous food products and pharmaceuticals. In temperate climates, the primary source of caffeine to watersheds is via the urine of those who consume caffeine-containing products (Buerge et al. 2003).

Table 4.1. Molecular tracer compounds, reporting levels for the method used in this study and water quality criteria listed by NY State and US EPA.

Analyte	Abbreviation	Laboratory Reporting Level (µg/L)	75% Confidence No False Positives (µg/L)	95% Confidence No False Positives (µg/L)	NY ambient water quality guidance values (ug/L)[a]				EPA: Human health for consumption of (ug/L)[b]	
					H(WS)[c]	H(FC)[c]	A(C)[c]	A(A)[c]	Water + Organism	Organism Only
PAH										
fluorene	FLU	0.00046	0.0006	0.0023	50		1	5	1100	5300
phenanthrene	PHE	0.00068	0.0029	0.0067	50		5	45		
anthracene	ANT	0.00033	0.001	0.0033	50		3.8	35	8300	40000
2-methyl phenanthrene	2MP	0.0013	0.0011	0.0035						
1-methyl phenanthrene	1MP	0.00090	0.0005	0.0022						
fluoranthene	FLR	0.00042	0.0028	0.0099	50				130	140
pyrene	PYR	0.00045	0.00081	0.0078	50		5	42	830	4000
benz(a)anthracene	BAA	0.00068	0.00064	0.0012	0.002		0.03	0.23	0.0038	0.018
chrysene	CHR	0.00033	0.00073	0.0011	0.002				0.0038	0.018
benzo(b)fluoranthene	BBF	0.0003	0.00072	0.014	0.002				0.0038	0.018
benzo(k)fluoranthene	BKF	0.00084	0.00048	0.0015	0.002				0.0038	0.018
benzo(a)pyrene	BAP	0.00014	0.00055	0.0025	0.002	0.0012			0.0038	0.018
Pesticides										
Metalaxyl (Fungicide)	Metalaxyl	n.d.	n.d.	n.d.						
Atrazine (Herbicide)	Atrazine	n.d.	n.d.	n.d.						
Chlorpyrifos (Insecticide)	Chlorpyrifos	n.d.	n.d.	n.d.						
4,4'-DDT (Insecticide)	DDT	n.d.	n.d.	n.d.					0.00022	0.00022
PCB										
2,3',4,4',5-Pentachlorobiphenyl	PCB-118	n.d.	n.d.	n.d.					0.000064	0.000064
2,2',3,4,4',5'-Hexachlorobiphenyl	PCB-138	n.d.	n.d.	n.d.					0.000064	0.000064
2,2',4,4',5,5'-Hexachlorobiphenyl	PCB-153	n.d.	n.d.	n.d.					0.000064	0.000064
Fragrances & Caffeine										
galaxolide	HHCB	0.0037	0.0047	0.012						
caffeine	CAF	0.0023	0.0023	0.016						
Steroids										
coprostanol (5β-cholestan-3β-ol)	bCOP	0.000087	0.0016	0.022	0.3[d]	0.3[d]	0.3[d]	0.3[d]		
epi -coprostanol (5 β -cholestan-3α-ol)	EPI	0.00037	0.0026	0.026						
cholesterol (cholest-5-en-3β-ol)	CHOL	0.010	0.024	0.034						
cholestanol (5α-cholestan-3β-ol)	aCOP	0.0017	0.0027	0.032						
cholestanone (5α-cholestan-3-one)	aONE	0.00085	0.00074	0.011						
coprostanone (5β-cholestan-3-one)	bONE	0.0085	0.0031	0.038						
ethyl-cholesterol (24-ethyl-cholest-5-en-3β-ol)	eCHO	0.016	0.048	0.41						
ethyl-cholestanol (24-ethyl-5α-cholestan-3β-ol)	SNOL	0.016	0.008	0.015						
ethyl-coprostanol (24-ethyl-5β-cholestan-3β-ol)	eCOP	0.00047	0.00003	0.00024						
ethyl-*epi* -coprostanol (24-ethyl-5β-cholestan-3α-ol)	eEPI	0.0017	0.00003	0.00044						

a - NYSDEC. 1998. Division of Water Technical and Operational Guidance Series (1.1.1) - Ambient water quality standards and guidance values and groundwater effluent limitations. NY State Department of Environmental Conservation, NY.
b - USEPA. 2002. National recommended water quality criteria: 2002. EPA-822-R-02-047, United States Environmental Protection Agency, Washington, DC.
c - H(WS) = source of drinking water (water supply); H(FC) = human consumption of fish; A(C) = fish propagation - aquatic life (chronic); A(A) = fish survival - aquatic life (acute)
d - Based on a limit of 200 fecal coliforms per 100 mL and the upper 95% confidence limit for the relationship between fecal coliforms and coprostanol as given by Leeming and Nichols (1996)

Fecal steroids are natural compounds that are produced in the intestines of birds and mammals. Ratios of certain steroids to others allow for the discrimination between human fecal material

and that of other animals (Leeming et al. 1996). The steroids quantified for this study were coprostanol (bCOP), *epi*coprostanol (EPI), cholesterol (CHOL), cholestanol (aCOP), coprostanone (bONE), cholestanone (aONE), ethyl-coprostanol (eCOP), ethyl-*epi*coprostanol (eEPI), ethyl-cholesterol (eCHO), and ethylcholestanol (SNOL).

Methods

Field measurements and sampling

Molecular tracer samples were collected at seven sites by the biogeochemistry team as they conducted a wider survey of other parameters throughout the region (Table 2.1 in Appendix 2, Appendix 3). Samples for tracer analyses were directly collected from stream and river waters into two 2.6 L in pre-cleaned glass jugs (5.2 L total). All glass sampling equipment and sample jugs were precleaned of organic contaminants baking in a kiln at 480^{o}C for 4 hours. Metal and Teflon sampling equipment were cleaned with solvent rinses, as was any field equipment that needed to be reused between sites. After collection, water samples were stored in a cool and dark place, transported in coolers as excess baggage back to the USA, and extracted within 7 days.

Sample processing and analysis

Molecular tracers were extracted from all samples by liquid-solid extraction onto an Empore™ disk, using protocols previously developed (Aufdenkampe et al. 2006), which were based on EPA approved alternate test method 608 ATM 3M0222 and EPA Method 3535.

In brief, sample water was filtered through a glass fiber filter stacked on top of an Empore™ C-18 disk. Particulate tracer compounds were extracted from the filter by sonic extraction and dissolved tracers were eluted from the Empore disk with solvents. Surrogate recovery standards – perdeuterated phenanthrene (PHE-D10), perdeuterated chrysene (CHR-D12), perdeuterated perylene (PER-D12), perdeuterated caffeine (CAF-D9) and perdeuterated cholesterol (CHO-D6) – were added to the surface of both the filter and the disk, after they were separated but prior to extraction. Dissolved and particulate extracts were combined then back-extracted in a separatory funnel with an aqueous salt solution to remove impurities, mixed with anhydrous sodium sulfate to remove moisture, rotoevaporated, and transferred to auto-injector vials. Samples were gently dried under a stream of nitrogen, redissolved in 15 µL pyridine and spiked with 5 µL internal standard solution (25-ng/µL in each of p-terphenyl-d14 and 5α-cholestane in pyridine). In order to analyze fecal sterols, which contain alcohol groups, samples were then derivatized by purging sealed vials with N_2, adding 15 µL of BSTFA (N,*O*-bis(trimethylsilyl)trifluoroacetamide) with 1% TMCS (Trimethylchlorosilane), and heating to 70°C for 30 minutes in an aluminum heating block. These derivitized sample extracts were analyzed for each of the molecular tracers compounds by capillary gas chromatography – mass spectrometry (GC/MS) in selective ion monitoring (SIM) mode, using a J&W DB1701 column (30 m, 0.25 mm ID, 250 µm coating) on an Agilent 6890 series GC interfaced with a 5973n series MSD.

Quantification

Molecular tracer data were quantified with an automated data quantification system. In brief, after confirmation by the analyst, compound peak areas for standards and samples are exported from the Agilent GC-MS "ChemStation" chromatography software directly into a central server. This raw data is then manipulated within the server with SAS-based scripts to produce the final data. Thus, decisions – regarding how to fit the calibration curve, when to drop outlying standards, whether or not peak identity is adequately confirmed, etc. – were all made uniformly using the same objective criteria used in the previous studies (Aufdenkampe et al. 2006).

All data presented here are surrogate-corrected with the extraction recoveries measured within each sample for each surrogate standard, which were associated with tracer compounds as follows (see Table 3.1): perdeuterated phenanthrene (FLU, PHE, ANT, Carbaryl), perdeuterated chrysene (FLR, PYR, BAA, CHR, Atrazine, DDT, PCB118, PCB153, PCB138, HHCB, AHTN), perdeuterated perylene (BBF, BKF, BAP), perdeuterated caffeine (CAF) and perdeuterated cholesterol (fecal steroids). The average recovery of perdeuterated phenanthrene and perdeuterated chrysene is used for 2MP, 1MP Chlorothalonil, Metalaxyl, Chlorpyrifos. These associations between target and surrogate compounds were based on analysis of matrix spike data from Stroud's NY watershed project(Aufdenkampe et al. 2006).

Laboratory reporting levels (LRL) were assigned to each analyte (Table 4.1) using the definitions and methods of USGS Open File Report 99-193 (USGS 1999) and based on data from a previous project (Aufdenkampe et al. 2006) . In brief, the LRL is defined as the concentration above which there is 99% confidence that reporting a false negative will be avoided. In other words, if the ambient concentration is above the LRL, the laboratory is 99% confident to detect a concentration. The LRL is equivalent to 2 times the method detection limit (MDL) as defined by EPA in CFR Title 40 Part 136 Appendix B (USGS 1999). Also reported here are the 75% and 95% confidence limits for no false positives, as described by Aufdenkampe et al. (2006). For pesticides and PCBs, which were added to the method in 2006, insufficient data is available from which to calculate MDLs, LRLs or confidence limits for no false positives. However, from qualititative examination of chromatograms, these limits are suspected to be at or near minimum values measured here for DDT and all 3 PCBs.

Statistical analysis

A multivariate data analysis method, principal component analysis (PCA), was used to examine how the study sites grouped together based on molecular tracer concentrations and ratios. Seven fecal-steroid ratios were selected to maximize the separation of human versus non-human fecal-source materials collected in the Catskill mountain region of New York State (Aufdenkampe, unpublished data). The resulting factor loadings, or the proportion of variability explaining the separation of the various source materials by each given ratio, formed a predictive equation that could then be used to assess the potential fecal-contamination sources at other stream sites. This predictive equation was applied to the fecal-steroid ratios calculated for the Peru study sites to assess what potential fecal-contamination sources might be present in these streams and rivers. An important assumption is that the fecal-steroid signatures of human, large mammal, small mammal and bird source in the tropics are similar to that found in the temperate latitudes of the Catskills. However, because these source signatures are very similar between animals in

Australia versus the mid-Atlantic region of the USA, therefore this assumption is likely valid for this preliminary study.

A second set of PCAs were run using all tracer concentrations measured at the study sites. Concentration data were log_{10}-transformed after first adding 0.00003 to all values to avoid taking the log of zero. Two separate PCAs were run; the first included all sites with the second analysis excluding the QLAJOYA site.

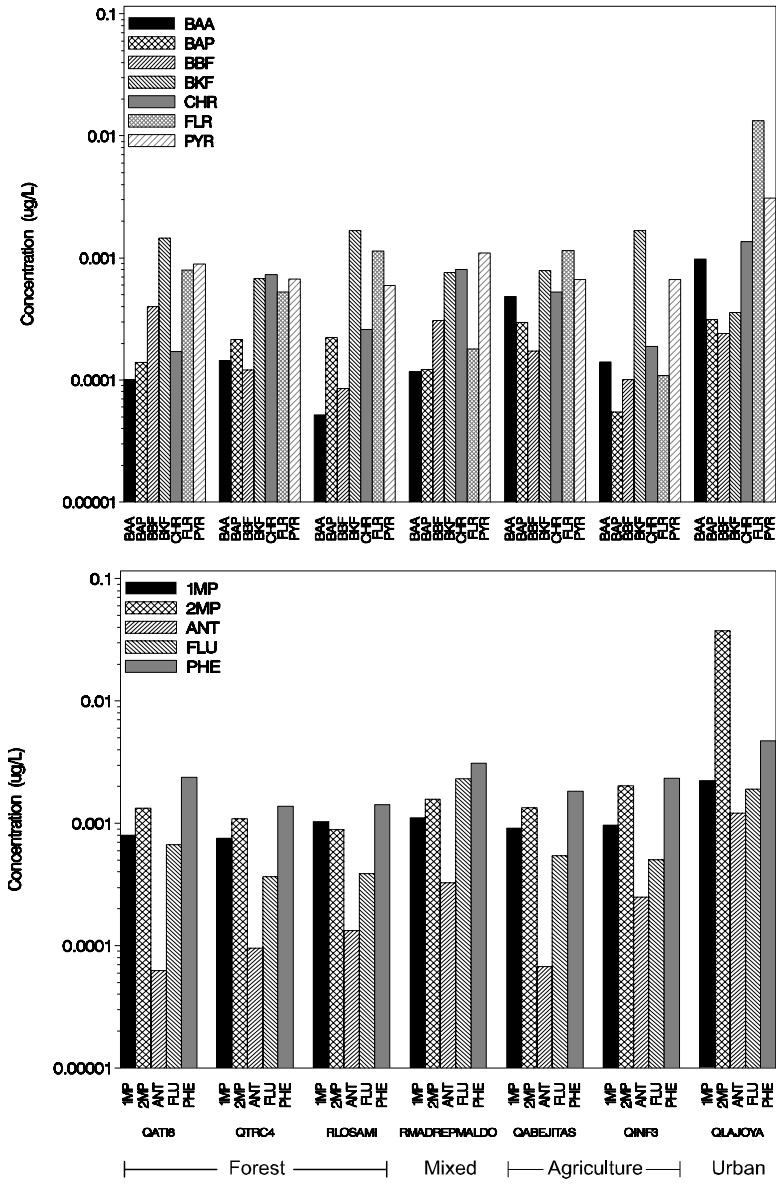

Figure 4.1. Concentrations of high (A) and low (B) molecular weight polycyclic aromatic hydrocarbons (PAH) at each of seven stream or river sites. Compound abbreviations and water quality criteria are given in Table 4.1.

Results and Discussion

The summed concentration of all 12 PAHs was nearly an order of magnitude higher in the urban stream, QLAJOYA (0.067 µg/L), than total PAHs at other sites, which ranged from 0.0067 to 0.012 µg/L (Fig. 4.1). However, even at QLAJOYA, concentrations were below those considered toxic to aquatic life or humans (Fig. 4.1, Table 4.1). For reference, concentrations of the five most toxic PAHs (BAA, BAP, BBF, BKF and CHR) were 2 to 10 times below guidance values set by NY State for drinking water supplies (NYSDEC 1998), and approximately half of streams surveyed in the Catskills (Aufdenkampe et al. 2006) had higher PAH concentrations than those observed in this study.

PAH ratios, which are useful in distinguishing petroleum from combustion sources (Dickhut et al. 2000, Yunker et al. 2002, Zakaria et al. 2002), suggested different PAH sources at different sites. Ratios of high molecular weight PAHs

(FLR, PYR, BAA, CHR, BBF, BKF, BAP) to low molecular weight PAHs (FLU, PHE, ANT, 2MP, 1MP) ($(H/L)_{PAH}$) > 0.5 are typical of soot or road-dust over petroleum sources (Yunker et al. 2002, Zakaria et al. 2002). High ratios of less stable to more stable isomers (i.e., ANT/(ANT+PHE) > 0.1 or FLR/(FLR+PYR) > 0.4) are typical of soot over petroleum and road-dust sources (Dickhut et al. 2000, Yunker et al. 2002). At QLAJOYA the three ratios ($(H/L)_{PAH}$) = 0.41, ANT/(ANT+PHE) = 0.26, FLR/(FLR+PYR) = 4.3) strongly suggest road-dust as the primary source. At this site, the stream intersects one of the most traveled roads in the region, and although unpaved, tire dust has a similar signature to that of asphalt. At the RMADREPMALDO, which had the second highest PAH concentrations, PAH ratios ($(H/L)_{PAH}$) = 0.40, ANT/(ANT+PHE) = 0.10, FLR/(FLR+PYR) = 0.16) all point to petroleum being the primary source. This sampling site was just outside of the largest river port in the region, so contamination from gasoline and engine oil is very likely. Elsewhere, signatures are less clear and sources probably mixed.

Distribution of Pesticide & PCB compounds relative to the predominant watershed condition for the study sites

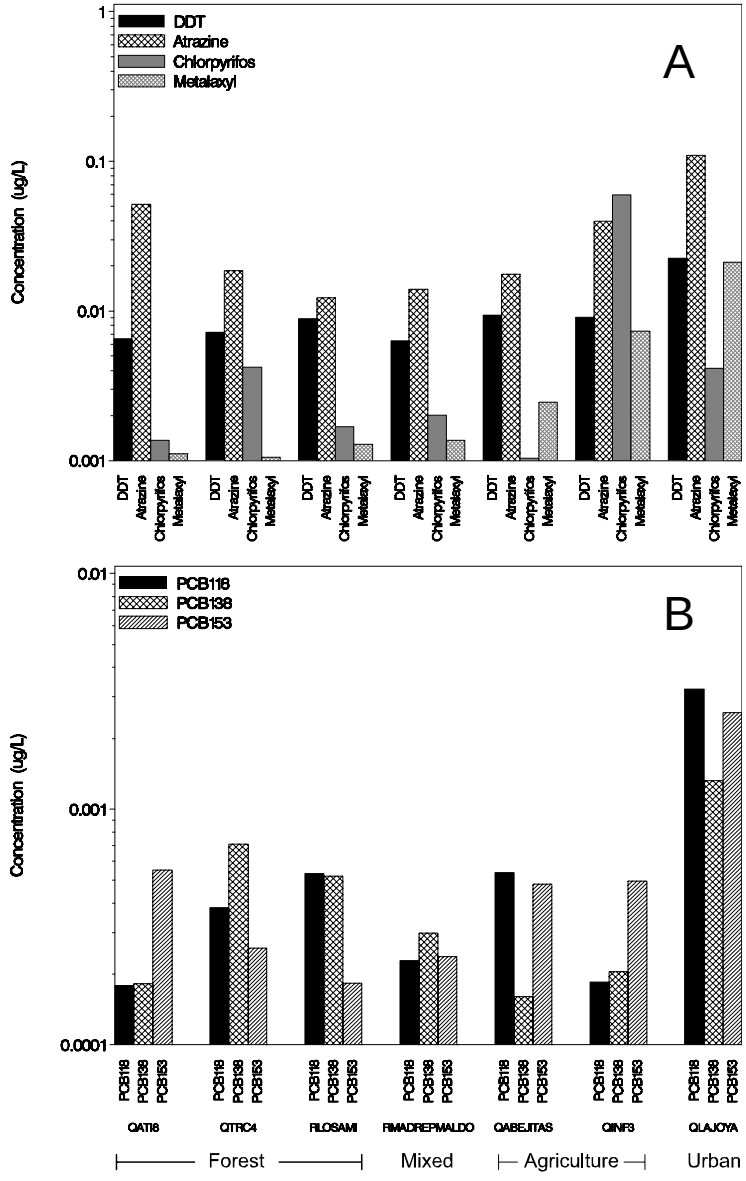

Figure 4.2. Concentrations of (A) pesticides and (B) polychlorinated biphenyls (PCB) at each of seven stream or river sites. Abbreviations and water quality criteria are given in Table 4.1.

In contrast to PAHs, toxic levels of at least one pesticide or PCB were detected at 3 sites (Fig. 4.2). Six of these seven assayed compounds were highest at the urban stream, QLAJOYA, with PCBs about an order of magnitude (factor of 10) higher than any of the other sites, and 2 orders of magnitude higher than EPA water quality criteria (Table 4.1). The insecticide chlorpyrifos was about three times higher than the water quality criteria set by Canada for chronic toxicity to aquatic

life (0.0035 µg/L) (http://www.pesticideinfo.org/Detail_Chemical.jsp?Rec_Id=PC33392), and the herbicide atrazine was higher at QLAJOYA than any other site, but still well below any water quality criteria (1.8 µg/L set by Canada for aquatic life; http://www.pesticideinfo.org/Detail_Chemical.jsp?Rec_Id=PC35042). QINF3, a stream draining a banana plantation, contained highest concentrations of the Chlorpyrifos measured in this study (0.060 µg/L, 20x higher than Canadian aquatic life criteria) and high concentrations of the fungicide Metalayxl. The aquatic macroinvertebrate community of this stream appears to be affected by these and potentially other toxics (Appendix 6). Two "pristine", protected streams contained noteworthy pesticide concentrations. QTRC4 had Chlorpyrifos concentrations slightly higher than at the urban QLAJOYA and above Canadian chronic toxicity guidelines for aquatic life, and QATI8 contained the seconded highest concentrations measured in this study of the herbicide Atrazine (although still 30x lower than levels considered toxic to aquatic life). Although preliminary results that warrant confirmation, these findings suggest that pesticides used during the routine grounds maintenance of tourism and conservation camps may find there way into nearby aquatic ecosystems.

In temperate freshwaters, the concentrations of caffeine and synthetic fragrances have been useful tracers of sewage contamination (Simonich et al. 2002, Artola-Garicano et al. 2003, Buerge et al. 2003, Phillips et al. 2005). QLAJOYA contained the highest concentration of the fragrance HHCB (0.027 µg/L, Fig. 4.3), which was similar to streams in the Catskills region of NY that received the high loads from sewage treatment plants (Aufdenkampe et al. 2006). Concentrations at other sites were in the range where >95% confidence that they are not false positives was not attained. These lower concentrations would be expected, given no reason to suspect municipal or domestic sewage at these sites. Caffeine on the other hand showed a pattern contrary to what is typically seen in temperate rivers and lakes (Fig. 4.3). Caffeine concentrations were highest at the most pristine sites and in the range only seen at contaminated sites in temperate latitudes (Aufdenkampe et al. 2006). These data suggest that sources from wild plants are more important than any human sources,

Distribution of caffeine and a synthetic fragrance relative to the predominant watershed condition for the study sites

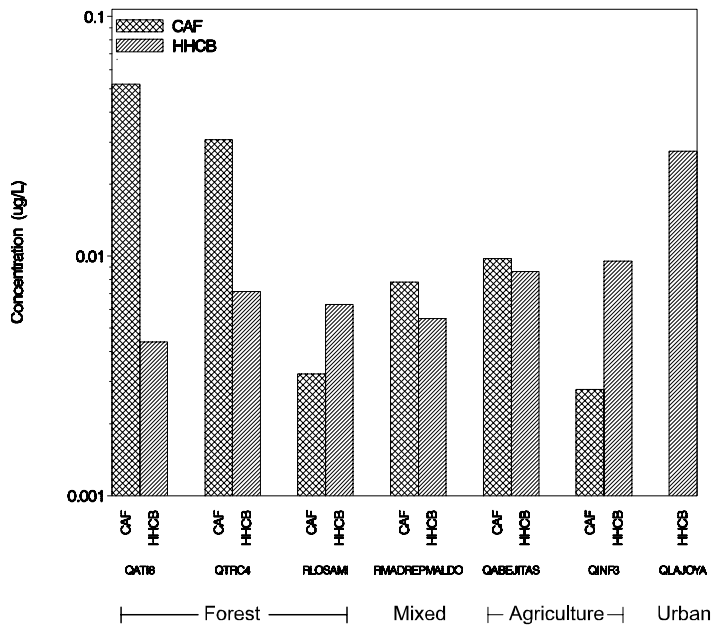

Figure 4.3. Concentrations of caffeine and the synthetic fragrance galaxolide (HHCB) at each of seven stream or river sites. Abbreviations and water quality criteria are given in Table 4.1.

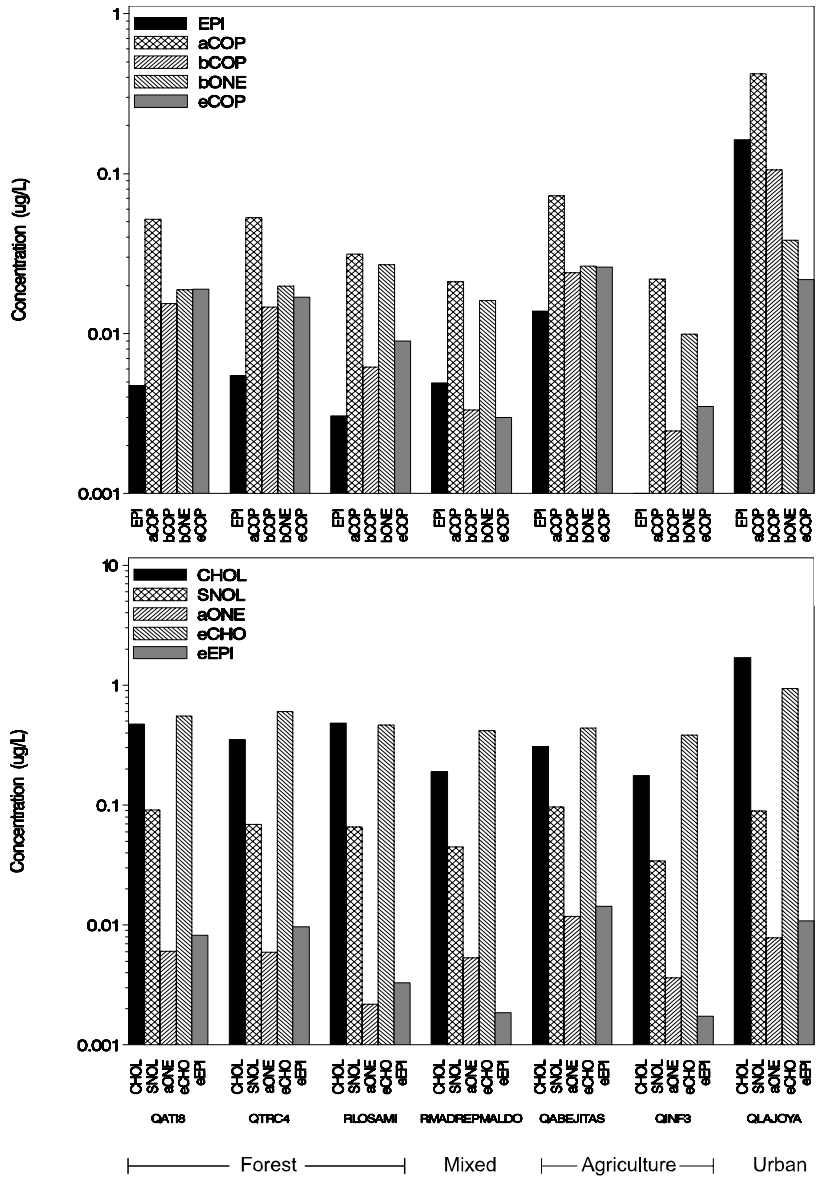

Figure 4.4. Concentrations of fecal steroids that are enriched in human sewage relative to fecal material from other animals (A), and common to all animals (B), at each of seven stream or river sites . Abbreviations and water quality criteria are given in Table 4.1.

and that caffeine has limited utility as a sewage tracer in the tropics.

Fecal steroid concentrations were highest at QLAJOYA, with the sum of all 10 compounds equal to 3.5 µg/L (Fig. 4.4). Total fecal steroid concentrations at other sites ranged from 0.63 µg/L at QINF3 to 1.2 µg/L at QATI8. These concentrations were near the median of that observed in Catskill streams (Aufdenkampe et al. 2006). Coprostanol – a steroid that predominates in human feces but is relatively less abundant in feces of other animals (Leeming et al. 1996) – was also highest at QLAJOYA.

A variety of ratios have been suggested as a means of distinguishing human fecal sources from others (Grimalt et al. 1990, O'Leary et al. 1999), but these ratios can at times offer ambiguous source separation and interpretation (Standley et al. 2000, Aufdenkampe et al. 2006). For this reason, a PCA-derived multimetric treatment of selected ratio data has recently been developed to clearly distinguish humans from other sources (Fig. 4.5). This multimetric was

applied to data presented here to interpret sources to these sites, with the caveat that fecal steroid "endmember" signatures could potentially be different in the tropics compared to the US Mid-Atlantic region. Calculation of this metric for sites studied here suggest that fecal steroids found at QLAJOYA do indeed have a large human source, but also a reasonably substantial source

Potential sources of fecal contamination at the study sites relative to source determinations from a previous study.

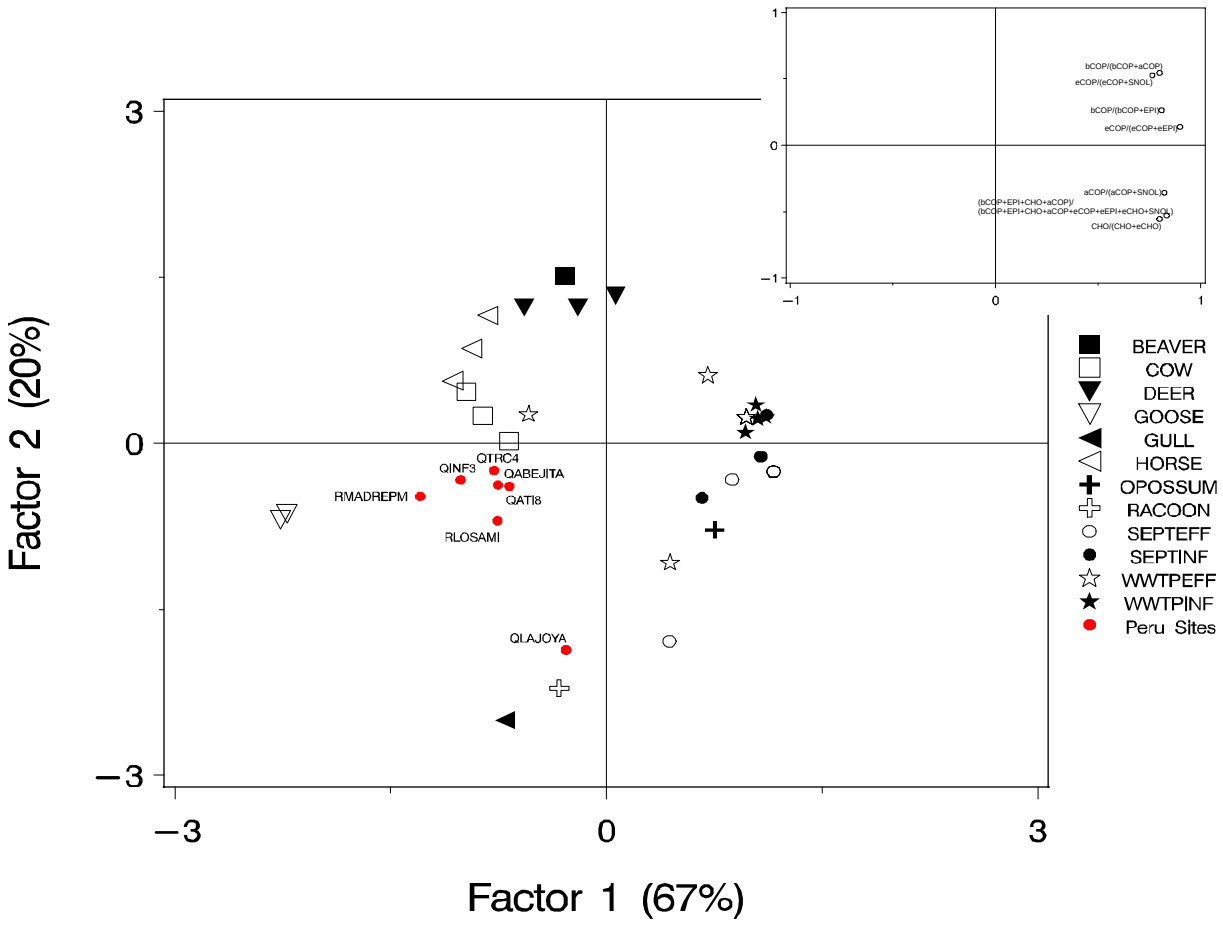

Figure 4.5. Principal component analysis of fecal steroid ratios from a variety of potential sources to streams in the Catskill Mountains of NY (Aufdenkampe et al., unpublished data). Peruvian stream site scores are generated and plotted in a second step, in order to evaluate potential sources to fecal steroids in a sample. Variance explained by each factor is included in the axis headings. The inset provides the factor loadings (i.e. the input variables with the relative influence of each in separating the stream sites).

from birds and or small mammals. All other sites cluster together where fecal sources are primarily from birds and large mammals. The cattle pasture site QABEJITA was located on the edge of this cluster nearest to cattle. PCAs based on the tracer concentration data showed that all tracer compounds seemed to increase or decrease together (Fig. 4.6) implying that that human impacts of one type

Grouping of sampling sites based on all tracer concentrations.

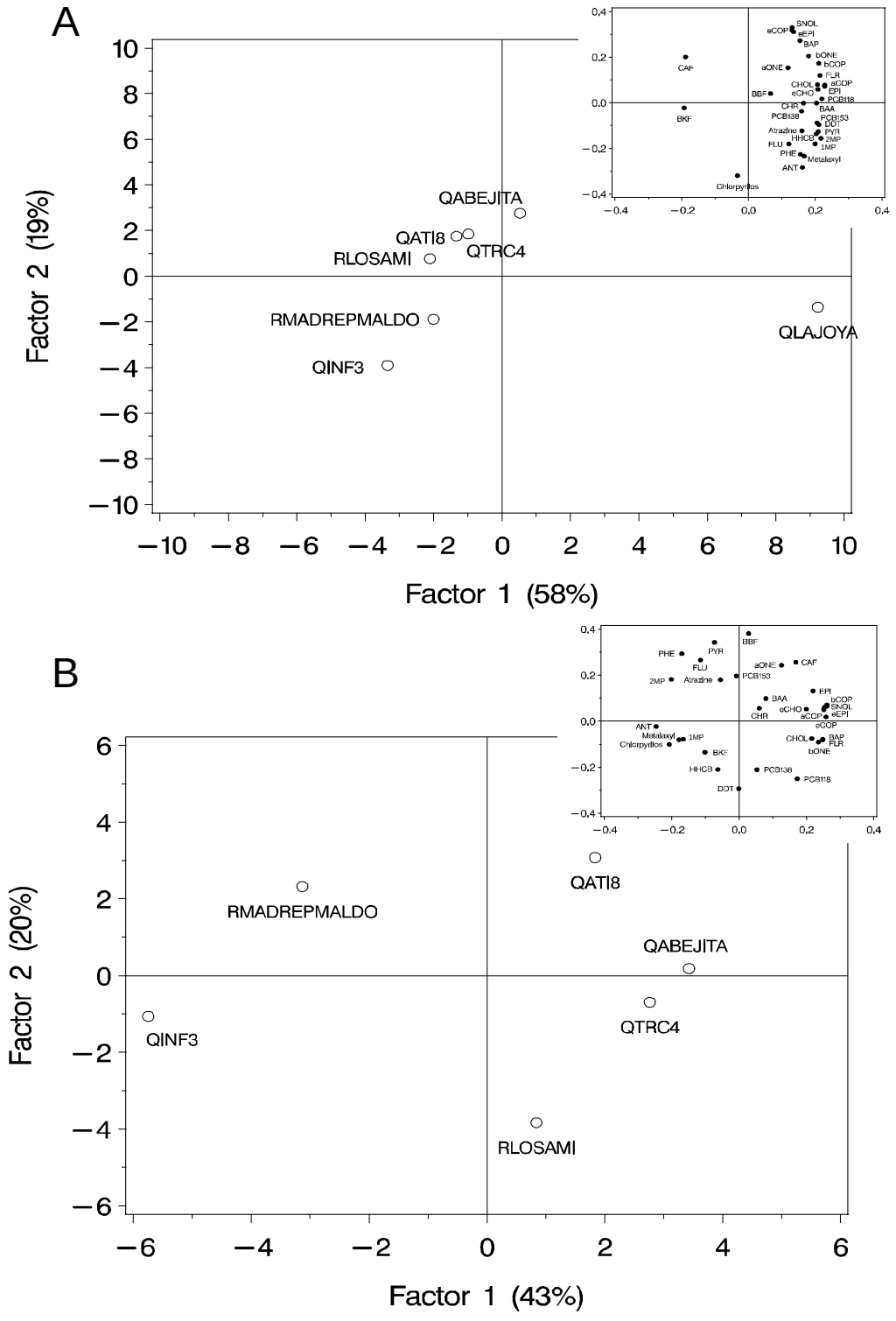

Figure 4.6. First two factors from PCAs run using all sites (A) and all sites excluding QLAJOYA (B) based on all tracer concentrations as input data. Variance explained by each factor is included in the axis headings. The inset provides the factor loadings (i.e. the input variables with the relative influence of each in separating the stream sites).

(e.g. fecal contamination) are associated to impacts from another (e.g. pesticides). However, this result may be entirely due to a single site, QLAJOYA, which clearly stands alone relative to the other sites (Fig. 4.6A). Therefore, a second PCA was run excluding QLAJOYA (Fig. 4.6B) in order to assess whether the site separation observed in the first PCA holds when this significant site outlier is removed. A different pattern does emerge among the tracer data in how these values separate the sites when excluding QLAJOYA. Along the first axis (Factor 1 in Fig 4.5B), steroid concentrations increase in one direction, while concentrations for 3 of the 4 pesticides increasing in the opposite direction. This factor separates the sites QINF3 and RMADREPMALDO from the other 4 sampling sites included in this analysis. Given that the watersheds for both QINF3 and QABEJITA are assumed to be predominantly agriculture, this PCA result shown in Fig. 4.5B suggests that types of agriculture have different impacts to water quaility: pasture (QABEJITA) as indicated by the increasing fecal steroid concentrations potentially from cattle versus some banana cropland/plantation (QINF3) that relies on relatively higher insecticide use.

Summary

The use of molecular tracers to discern among specific types of contamination sources has shown to be a quite effective stream water-quality assessment tool. The results of this study, representing the first known use of such tracers in the Amazon River basin, has provided very encouraging evidence that these compounds can be effectively used to distinguish among stream and river contamination sources as shown elsewhere. Caution must be exercised though in interpreting the results given the synoptic nature of the study design and the limited number of streams and rivers sampled for this study. For example, in the Catskill region of NY State, tracer concentrations showed appreciable temporal variability within a site (Aufdenkampe et al. 2006). Increased stream sampling of these compounds, both temporally and spatially, as well as sampling of source material in the region would greatly aid in solidifying the usefulness of tracers in assessing the sources of contamination to streams and rivers across the Amazon and in other tropical regions.

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Appendix 5. Microbial Diversity/ Ecology

Louis A. Kaplan, Karen M. Hogan, Paul M. Berube, and David A. Stahl

Executive Summary

- The initial survey of microbial communities in streambed sediments from the Peruvian Amazon indicates that the ammonia oxidizer community is primarily composed of Archaea and not Bacteria.

  (i) Definitive identification of ammonia oxidizing archaea will require cloning and sequencing, activities beyond this initial scope of work.

  (ii) The failure to detect bacterial ammonia oxidizing bacteria could be related to the low densities of ammonia oxidizing bacteria relative to the ammonia oxidizing archaea in the streambed, as has been shown in soils and the oceans.

- The survey revealed distinct bacterial community compositions in pristine streams that clustered according to dissolved organic carbon concentrations into blackwater and clearwater streams.

- Communities in the human impacted streams of each stream type shifted away from the compositions observed in the pristine streams.

Introduction

Bacteria and archaea represent the most widely distributed and abundant groups of organisms on Earth (Fuhrman et al. 1993, Ovreas et al. 1997, Venter et al. 2004). Their central role in the decomposition of organic matter (Axmanova et al. 2006, Judd et al. 2006) and cycling of nutrients (Santoro et al. 2006) and other elements (Teske et al. 1996) has important implications for global and regional biogeochemical cycles. Despite the importance of bacteria and archaea, and recent efforts to apply molecular techniques to elucidate community composition (Methe et al. 1998, Muylaert et al. 2002) community composition is just beginning to be described in many habitats, along with questions of diversity, activity, and the factors that influence community structure. This is particularly true in rivers (Crump et al. 1999, Brummer et al. 2003) and stream ecosystems (Eisenmann et al. 1999, Hullar et al. 2006).

The microbiology of streambed sediments of small streams within the Amazon basin, is not known to have been explored. So the work within Peru reported here offers an initial survey of these communities. This study focused on two main objectives. First, to exploit the ability of molecular methods to provide information about the presence of specific enzymatic pathways and perform a broad polymerase chain reaction (PCR)-based survey for ammonia-oxidizing bacteria and archaea in streambed sediments. This was stimulated, in part, by the importance of the nitrogen cycle to tropical stream function, as well as the recent discovery of an autotrophic ammonia-oxidizing archaeon (Könneke et al. 2005) and the finding that archaea predominate ammonia-oxidizing procaryotes in soils (Leininger et al. 2006) and the oceans (Wuchter et al.

2006). Second, to use molecular fingerprints of the microbial communities to provide information about how similar or unique the communities are in different streams, the diversity of the communities, and whether anthropogenic disturbances produce significant changes in microbial community composition.

Methods

Streambed sediments at 14 of these sites were analyzed for molecular fingerprints of the bacterial communities and for the presence of bacterial and archaeal genes for ammonia oxidation. Streambed sediments were collected in triplicate with a coring device. The top 3mm of the cores were transferred into a Whirl-Pak bag to form a composite sample, and the sealed bag was placed into a cooler. Within ≤ 12h the sediment samples for molecular microbiological analyses were subsampled into vials and the vials were sealed and then flash frozen in a cryoshipper that had been charged with liquid nitrogen.

Isolation of sediment DNA. Genomic DNA was isolated from approximately 0.5 g of sediment using the MoBio Ultraclean™ Soil DNA Isolation Kit. The extraction method used by this kit employs mechanical lysis of cells in a sodium dodecyl sulfate (SDS) solution. An optional 10 min incubation at 70°C following SDS addition was used to aid lysis performed in the Stahl lab since they have found it difficult to achieve lysis of the ammonia-oxidizing archaeon, *Candidatus* Nitrosopumilus maritimus. Following cell lysis, humic acids and proteins were selectively precipitated and the DNA was bound to a silica membrane in the presence of a high salt concentration. DNA was eluted with 75 µl of 10 mM Tris-HCl pH=8 in the Stahl lab, while 50 µl was used in the Kaplan lab, and all eluants were stored at -20°C.

PCR for Ammonia Oxidizer Survey. Target sequences were amplified from the sediment genomic DNA using primers that target the 16S rRNA gene and the *amoA* gene from both Bacteria and Archaea (Table 5.1). For Bacterial 16S primers (27f and 907r), 1 µl of diluted genomic DNA (10^{-1}) was used in a 20 µl PCR (35 cycles) at an annealing temperature of 52°C. For all other primers, 1 µl of undiluted genomic DNA was used at an annealing temperature of 55°C. Controls were Nitrosomonas europaea genomic DNA and 16S rRNA gene and *amoA* gene clones from *Candidatus* Nitrosopumilus maritimus. PCR products were visualized on a 1% agarose gel using ethidium bromide staining.

Table 5.1. Primers used in this survey.

Primer	Sequence	Target
27f	AGAGTTTGATCMTGGCTCAG	Bacteria 16S
907r	CCGTCAATTCMTTTRAGTTT	
1492r	ACGCCTACCTTGTTACGACTT	
Arch21F	TTCCGGTTGATCCYGCCGGA	Archaeal 16S
Arch958R	YCCGGCGTTGAMTCCAATT	
amoA-1F	GGGGTTTCTACTGGTGGT	Bacterial amoA
amoA-2R	CCCCTCKGSAAAGCCTTCTTC	
Francis_amoA_F2	STAATGGTCTGGYTWAGACG	Archaeal amoA
Francis_amoA_R	GCGGCCATCCATCTGTATGT	

Note: The Francis_amoA_F2 primer is a degenerate primer based on the published primer in (Francis *et al*. 2005. *PNAS* 102:14683-14688)

Gel showing presence of Bacteria and Archaea, the presence of Archaeal amoA genes and the absence of Bacterial amoA genes.

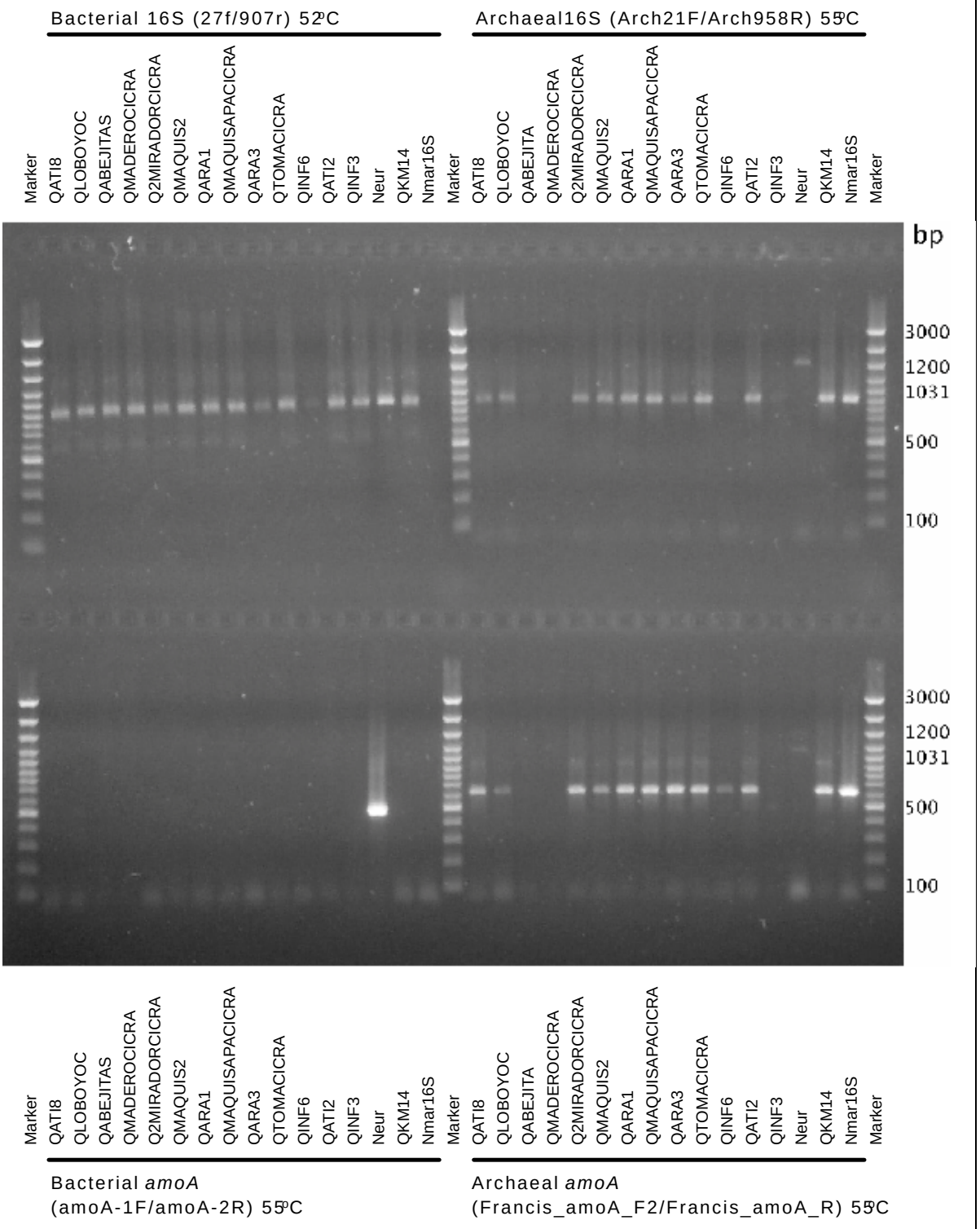

Figure 5.1. PCR amplicons from Peruvian small stream sediment samples.

Terminal Restriction Fragment Length Polymorphism (TRFLP). 16S rRNA gene sequences were amplified by PCR using universal bacterial primers (27f and 1492r in Table 5.1). Briefly, 50µL PCR reactions (5 replicate reactions/sample) contained approximately 25ng genomic DNA, 0.7µM universal bacterial primers, 25 µg bovine albumin serum, 2.5U *Taq* polymerase, 125µM each deoxyribonucleotides, and 1X standard *Taq* buffer (New England Biolabs, Ipswich, MA). PCR conditions were as follows: initial denaturation at 94°C for 1 min, 25 cycles of denaturation at 94°C for 30 sec, annealing at 55°C for 30 sec, extension at 72°C for 1 min and a final extension at 72°C for 5 min. Replicate PCR reactions were combined and purified and eluted with 20µL 10mM Tris-HCl (pH=8) using the Qiagen MinElute PCR Purification Kit. Approximately 250ng of purified DNA was digested using tetrameric restriction endonucleases *MspI* and *RsaI* at 37°C for 1 hour. Following digestion, salts and other impurities were removed from digested products and products analyzed using an ABI 3100 capillary sequencer (Applied Biosystems, Inc. Foster City, CA).

For each TRFLP profile, individual peaks were assigned a fragment size against a standard (GeneScan -500 ROX Size Standard) and the integrated peak area used to calculate total peak area. Peaks within 1bp of one another were manually binned and binned peak areas summed. Peaks <30bp or >500bp and peaks less than 0.5% of the total peak area were eliminated from further analysis. The relative percent area of each peak was calculated and used for statistical analysis. Nonmetric Multidimensional Scaling (NMS) was used to compare TRFLP profiles among different streams after Sorensen's distance was calculated. The square root of the relative percent area of each peak was arcsine transformed to reduce skew in the data set. A final plot was generated using the 2nd- and 3rd-Axis NMS scores of each TRFLP profile.

Results and Discussion

This rapid PCR based survey for ammonia-oxidizing archaea indicates that the ammonia oxidizer community in the sediments of Peruvian small streams is primarily composed of Archaea and not Bacteria (Fig. 5.1). PCR products were successfully obtained using Bacterial 16S primers, but not for primers that target Bacterial amoA sequences. It is not unusual, however, to have insufficient numbers of bacterial nitrifiers to amplify the amoA gene from environmental samples. There are few studies that have made direct comparisons in a single environment, but in general the archaeal variant appears to be more widely distributed in soils (Leininger et al. 2006), the ocean (Wuchter et al. 2006), and estuarine sediments (Beman and Francis 2006). In contrast, PCR products were obtained in assays targeting both Archaeal 16S rRNA genes and Archaeal amoA genes. This result indicates the likelihood, but not certainty, of finding ammonia-oxidizing archaea in these systems. It has been found that the Archaeal amoA primers used in this survey may also target cyanobacteria sequences (personal communication, José de la Torre). When coupled with the observation of high concentrations of ammonia in pristine streams (Appendix 3), the results suggest that Archaea are important in the nitrogen cycle of these streams. These results warrant confirmation by cloning and sequencing the PCR products followed by comparison to a database of Archaeal *amoA* sequences.

The TRFLP community fingerprints revealed a clustering of communities based on the DOC concentrations of the streams (see Appendix 3). The communities from blackwater streams and clearwater streams separated along axis 2 (Fig. 5.2). Dissolved organic carbon has been implicated in structuring microbial communities of bacteria suspended in stream water (Judd et al. 2006), the interstitial water of streambeds (Axmanova et al. 2006), and bacteria colonizing rock surfaces in streambeds (Hullar et al. 2005) but not for bacteria attached to streambed sediments. Additionally, while only 3 impacted streams were sampled for the bacterial community structure, the community from an impacted stream blackwater stream separated from the communities in the two impacted clearwater streams, and all the communities from impacted sites were shifted from the communities in pristine streams.

Dissolved organic carbon concentrations appear to play an important role in structuring communities of bacteria in streambed sediments.

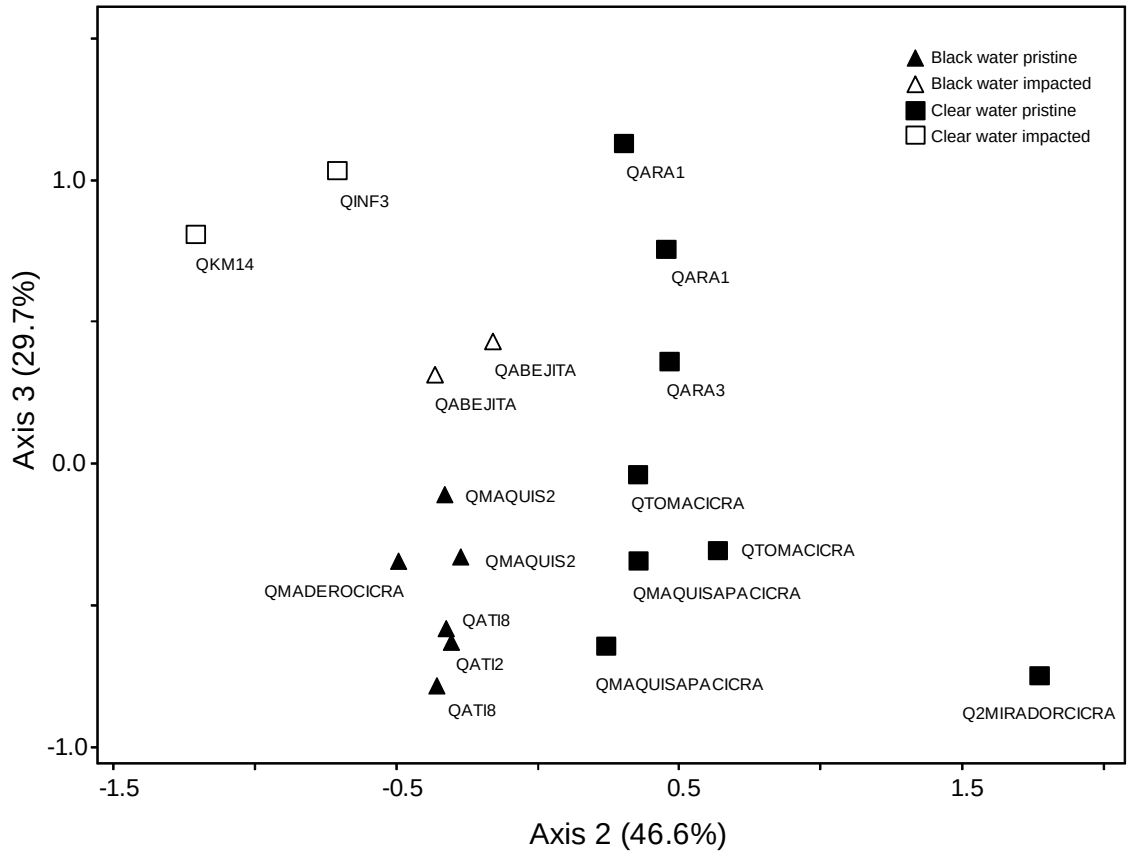

Figure 5.2 NMS analysis showing the separation of bacterial communities from pristine blackwater and clearwater streams (solid symbols) and the shift in communities from impacted streams (open symbols).

The TRFLP data were also used to assess community richness, defined as the total number of species within a stream. Our analysis is based on the assumptions that each TRFLP peak represents a single operational taxonomic unit (OTU) and that an OTU is synonymous with a species. Species richness ranged from 25 at Q2MIRADORCICRA to 47 at QARA1 and

QABEJITA. There were no significant differences due to stream type (blackwater versus clearwater) or pristine versus impacted (ANOVA, $\alpha = 0.05$). These values for species richness are likely underestimates of true richness, because of violations in the assumption that each peak represents a single OTU, and because rare organisms present in low population densities were not detected. However, these values are consistent with data collected from other stream or river systems (Sekiguchi et al. 2002, Hullar et al. 2006, Winter et al. 2007).

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Appendix 6. Macroinvertebrate Diversity/Ecology

John K. Jackson & R. Wills Flowers

Executive Summary

•The Andes Amazon region supports a highly diverse aquatic insect fauna based on hand collections at 23 study sites

•For the 12 study sites that were sampled most intensively for macroinvertebrates; 204 macroinvertebrate taxa (mostly genera) were collected, with a significant percentage representing species new to science

•50% of the taxa were found only at one or two sites, suggesting significant differences from site to site in species composition

•The number of taxa in macroinvertebrate groups sensitive to pollution (e.g., EPT, where EPT represents the insect orders Ephemeroptera or mayflies, Plecoptera or stoneflies, and Trichoptera or caddisflies) ranged in the study from 0 (highly polluted stream in the town of Puerto Maldonado) to 29 (a pristine stream in a conservation area)

•Lowest EPT values were for a highly polluted stream (QLAJOYA) in the town of Puerto Maldonado and on a banana plantation outside of Puerto Maldonado (QINF3). Highest EPT values were from 4 streams draining well-forested watersheds, three of which were part of conservation areas.

•These results indicate that macroinvertebrate groups sensitive to pollution (e.g., EPT) can be used to gauge levels of stream disturbance in the Andes Amazon region similar to how they are used in north temperate regions.

Introduction

This study element used naturally occurring benthic (i.e., bottom-dwelling) macroinvertebrates (insects and some common non-insects such as aquatic worm, flatworms, leaches, shrimps, crabs, and molluscs) to describe stream and watershed health based on the biological integrity of the macroinvertebrate fauna (i.e., what species are there and their relative and true abundances). Aquatic organisms have provided water quality assessment programs with valuable insight for more than 100 years (Cairns and Pratt 1993), and benthic macroinvertebrates are the most common group of aquatic organisms included in these programs (Hellawell 1986). Benthic macroinvertebrates are a cost-effective, commonly used, and widely accepted tool in water quality monitoring programs for a number of reasons (Weber 1973, Rosenberg and Resh 1993). First, most river and stream ecosystems have relatively diverse macroinvertebrate assemblages (100-200 species in temperate streams), with species from several different orders [e.g., Ephemeroptera (mayflies), Trichoptera (caddisflies), Coleoptera (beetles), Diptera (true flies)]. Each species is to some degree unique; as a result, each potentially possesses different tolerances to changes in environmental conditions. Thus, together, the aquatic macroinvertebrates are a

sensitive measure of environmental change and stress. Second, their limited mobility and relatively long life spans (a few months to a year or more) make the presence or conspicuous absence of macroinvertebrate species at a site a meaningful record of environmental quality during the recent past, including short-term infrequent events that might be missed by periodic water sampling or avoided by more mobile/migratory fish. Third, aquatic macroinvertebrates are an important link in the food web, functioning as primary consumers (herbivores and detritivores) of plant and microbial matter that are then available to secondary consumers such as fish. Fourth, their abundance lends itself to statistical analysis, which can play an integral role in water quality assessment programs. Finally, aquatic macroinvertebrates have proven to be effective tools for communicating water and watershed issues to students, decision makers, and the public.

The use of macroinvertebrates as a biomonitoring tool is well accepted throughout the world. Protocols for macroinvertebrate monitoring in all 50 states in the US as well as most other industralized countries (e.g., European Union, Australia, New Zealand, Japan). These countries have benefited from more than a 100 years of taxonomic and ecological research on temperate stream ecology and pollution responses in temperate streams. However, standard protocols are unavailable for most countries in Latin America, Africa, and Asia. The challenges there are many, primarily because basic taxonomic and ecological knowledge for freshwater macroinvertbrates remain very limited. This in turn has made it difficult to develop a basic understanding of pollution responses for the faunas in the streams of those countries. Peru and its neighbors in the Andes Amazon are no exception to this current situation.

Methods

A dual approach was used to inventory the macroinvertebrate fauna at each site: intensive hand collections as well as leaf pack samplers. Both are well know methods that have been used in a wide variety of streams throughout the world. This combined approach helped insure that a better sample of macroinvertebrates was obtained at each site relative to reliance on a single approach. The dates sampled are indicated on Table 6.1.

The strategy for the hand collections was to collect macroinvertebrates from all possible microhabitats within each site. This includes areas along the margin of the stream that had slow currents, shallow riffles/runs with moderate to fast velocities, shallow pools, roots masses, and leaf and wood debris. Overall, any recognizable microhabitat was examined intensively and the fauna present collected. Benthic macroinvertebrates were collected from each station by sampling benthic habitats with a kick net, by scrubbing rocks and wood with a brush in a bucket of water and collecting the material in a 125-µm mesh sieve, by hand-picking specimens from natural substrates (rocks, leaves, wood, etc.), and by sieving smaller sediments through a 4.76-mm, 1.47-mm, and 125-µm mesh sieves. Collected material was examined in an enamel pan, and all specimens were preserved in 90% ETOH immediately. Field work was performed primarily by W. Flowers, D. H. Funk and B. W. Sweeney working concurrently, with occasional assistance by K. Travers, J. Blaine and others. The duration of collecting was several man-hours per site. In general, if after a few hours of collecting in a microhabitat, no new recognizable species were found, it was assumed that the majority of species from the station had been collected. This was the standard protocol used during the present survey, as well as during previous surveys

conducted at sites throughout the world. This is an effective approach because: (1) all potential habitats are sampled (i.e., shallow riffles, pools, edges) and the data characterizes the entire aquatic insect assemblage, and (2) it includes a wide variety of species that differ in their tolerance to various types of environmental change.

Table 6.1. Details on the timing of sample collection in 2006

Site	Date of hand collections & leaf bags placed in stream	Date leaf bags collected from stream	Number of leaf bags examined
Q2MIRADORCICRA	24AUG06	29SEP06	2
QMAQUISAPACICRA	23AUG06	29SEP06	2
QABEJITA	20AUG06	26SEP06	3
QATI8	17AUG06	14SEP06	5
QARA1	26AUG06	02OCT06	2
QINF2	18AUG06	15SEP06	2
QINF3	19AUG06	25SEP06	2
QINF5	19AUG06	25SEP06	2
QINFIERNO	18AUG06	15SEP06	2
QKM14	21AUG06	26SEP06	2
QLAJOYA	19AUG06	na	0
QTRC4	28AUG06	04OCT06	5

Table 6.2. Sites where hand collections were made, but leaf pack samplers were destroyed by floods or stolen, or were not installed (RTAMBOTRC, RMADRECICRA, QGATO). Data from these sites were not included in the analyses below because of the missing leaf pack component.

QMADEROCICRA	QARA3
QMAQUIS2	QTRC2
QTOMASCICRA	
QATI2	RMADRECICRA
QLOBOYOC	QGATO
Q3ESTRELLAS	RTAMBOTRC

At the same time as the hand collections, leaf pack samplers were placed in each site. Leaf pack samplers were preconstructed by placing 8 leaflets (5 g) of fresh palmiche (*Geonoma* sp., probably *Geonoma deversa* (Arecaceae)) in a plastic mesh bag. Bags were deployed in moderate current, tied with 30-lb fishing line (monofilament) to logs or trees in the stream or on the bank.

Leaf packs were retrieved after 4-5 weeks in the stream and preserved with 95% ETOH. Leaf packs were lost at a number of sites; the impact of these losses was most significant at QLAJOYA where no leave packs were recovered. The macroinvertebrate specimens were brought to the Stroud Water Research Center (the leaf pack samplers and chironomid midges from hand collections) or Florida A&M University (all non-chironomid midges in hand collections) and identified to the lowest practical taxonomic unit. We were often not able to achieve species level because of the size of specimen, the condition of the specimen, and in

many cases the absence of available keys for undescribed species or larvae that have not been associated with a described adult.

The macroinvertebrate data were summarized with four biometrics. EPT Richness is the total number of EPT taxa (i.e, mayflies, stoneflies, caddisflies) found at a site. All of these are considered sensitive to moderately sensitive to pollution and habitat disturbance. Total Richness is the total number of macroinvertebrate taxa, insect and non-insects. This includes pollution-sensitive and pollution-tolerant taxa. Chironomid Richness is the total number of chironomid midges found at a site. The Chironomidae are a very diverse group of insects that includes both pollution-sensitive and pollution-tolerant taxa. Rare Taxa was the number of macroinvertebrate taxa found at only 1 of the 12 study sites.

Results and Discussion

A total of 204 macroinvertebrate taxa was collected across the 12 sites (Table 6.3). All major taxonomic groups except Plecoptera (stoneflies), Megaloptera (dobson and alder flies), and aquatic Lepidoptera (butterflies and moths) were represented by numerous genera/species. Plecoptera, Megaloptera, and aquatic Lepidoptera are generally not specious in tropical and subtropical streams and rivers. Richness (i.e., the number of taxa identified) was greatest for Diptera (two-winged flies, 71), and most of these (62) were chironomid midges. This was followed by Coleoptera (beetles, 27 taxa), Odonata (dragonflies and damselflies, 25), Trichoptera (caddisflies, 24), and Ephemeroptera (mayflies, 23).

Of the 204 macroinvertebrate taxa, 69 taxa were found at only 1 of 12 sites and 33 additional taxa were found at only 2 of 12 sites (Table 6.3, Appendix 6.1). This represents exactly half of the aquatic macroinvertebrate fauna collected in the region, found at only 1 or 2 sites. This pattern reflects the overall rarity of a significant portion of the regional fauna as well as natural differences among sites (e.g., TRC vs. CICRA), anthropogenic differences among sites (e.g., QLAJOYA vs. QINF5). Similar patterns have been observed for benthic macroinvertebrate faunas in North Temperate streams (e.g., Arscott et al. 2006). Differences in sampling effort (i.e., the number of sites) among portions of the region presumably contributed to the pattern: sampling more sites increases the total number or rare taxa among the sites but decreases the likelihood of a taxon being found at only one site. For example, 12 rare taxa were found at a site sampled for the TRC area. Two sites sampled in the CICRA area contained a total of 14 rare taxa (5 at one site and 9 at the other). Nine sites were sampled in and around Puerto Maldanado and found 43 rare taxa across those sites, but only 1 to 8 rare taxa per site.

The results indicate that the Andes Amazon region supports a highly diverse aquatic insect fauna. For example, 23 genera of mayflies (Ephemeroptera) were found at the 12 primary sites, and 34 genera across all 23 sites in the study area. For comparison, Dominguez et al. (2002) list only 44 genera known from **the entire Amazon Basin**. Of the mayflies collected in the Andes Amazon as part of this study, several represent substantial extensions of the ranges previously know for these genera. In the Leptophlebiidae, *Hylister* was collected in several streams yet it was previously known from just one species in the Atlantic Forest area of Brazil. Also found were the leptophlebiid genera *Paramaka* and *Tikuna* in the Andes Amazon region yet they were previously known only from streams north of the main channel of the Amazon. In the Baetidae,

the collection of *Waltzoyphius* at several sites in the Andes Amazon is noteworthy because this genus has previously been reported only from the Guyana and Brazilian Shields (French Guiana and Brazil–Paraguay). Unfortunately, data on general distributions of other aquatic insect groups in the Amazon Basin are fragmentary, making similar comparisons currently impossible.

The aquatic macroinvertebrate fauna in the streams of the Andes Amazon was strikingly different relative to what has been seen over many years in the streams of Panamá and Costa Rica (primarily on the Osa peninsula near Panamá and in the Área de Conservación Guanacaste near Nicaragua), For example, the dragonfly families Libellulidae and Gomphidae represented 75% of the dragonflies and damselflies (Odonata) collected in the Andes Amazon as part of this study while damselflies made up only a small portion. In contrast, damselflies (families Calopterygidae and Coenagrionidae) made up over 50% the odonate fauna in Costa Rica. Morover, the mayfly (Ephemeroptera) family Leptophlebiidae was overwhelmingly dominant in the Andes Amazon, comprising almost 75% of all the mayflies found, while the mayflies in Costa Rica and Panamá were generally evenly divided between the families Baetidae, Leptohyphidae, and Leptophlebiidae (Fig. 5.1). The abundance of Leptophlebiidae is as interesting as scarcity of the family Leptohyphidae in the Andes Amazon because the principal type of stream in the forest there is slow with a muddy bottom and lots of submerged leaves and wood. In Central America, such streams are ideal habitat for Leptohyphidae. Why Leptohyphidae should be scarce in what should be its preferred habitat in Peru is unknown.

Additional sampling and taxonomic studies would be expected to result in more discoveries such as those illustrated above, especially if much information is unrecognized because many (e.g. > 50%) of the species in the region are still unknown to science. In addition, for those stream species that have been named, most larvae (i.e., the life stage used understand ecological function and to diagnose stream health) have not been associated with the adult stages that are principally used in initial species recognition. Others have estimated that only about 2% of Neotropical caddisfly species have associated larvae stages. It is fair to assume that this is representative to all aquatic insects in the Andes Amazon.

Macroinvertebrate Trends Across All Sites

Macroinvertebrate assemblages were characterized at the different sites using two approaches: biometrics that present numeric descriptions various aspects of community structure, and multivariate analyses that make synthesize similarities and differences in species present and their relative abundance. EPT Richness is one of the most commonly used biometric used to describe stream macroinvertebrate community structure and to assess stream degradation; mayflies, stoneflies, and caddisflies are all considered sensitive or moderately sensitive to water pollution and habitat degradation (Resh and Jackson 1993). EPT Richness ranged from 29 taxa at QTRC4 to 0 taxa at QLAJOYA, with a variety of values in between (Fig. 6.2). This indicates there exists a significant range of conditions in the Andes Amazon region, from pristine areas that support a wide variety of pollution-sensitive taxa to impacted areas that have lost most if not all of their pollution-sensitive taxa and thus support primarily pollution-tolerant taxa.

Table 6.3. Number of taxa collected at each sites and across sites for individual orders (or families in the case of chironomid midges) as well as groups of orders. The taxa list for each site can be found in Appendix 6.1.

	Q2MIRADOR CICRA	QMAQUISAPA CICRA	QABEJITAS	QATI8	QARA1	QINF2
Plecoptera	1	1	0	1	1	2
Odonata	6	9	10	14	9	9
Ephemeroptera	5	13	9	10	6	9
Hemiptera	4	5	9	6	1	1
Trichoptera	6	8	4	12	10	7
Megaloptera	1	1	1	1	2	1
Leptidoptera	0	0	0	0	1	0
Diptera	33	29	27	28	21	12
Coleoptera	6	6	2	9	9	5
Non-Insect	5	5	5	4	5	5
Total Richness	67	77	67	85	65	51
Chironomid Richness	29	27	24	25	16	8
EPT Richness	12	22	13	23	17	18
Rare Taxa	9	5	3	8	2	6

	QINF3	QINF5	QINFIERNO	QKM14	QLAJOYA	QTRC4	All sites
Plecoptera	0	1	1	1	0	3	3
Odonata	5	7	5	11	2	13	25
Ephemeroptera	5	12	5	9	0	8	23
Hemiptera	2	4	2	2	5	3	15
Trichoptera	1	9	10	9	0	18	24
Megaloptera	1	3	1	0	0	2	3
Leptidoptera	0	0	0	0	0	1	1
Diptera	13	24	14	27	0	33	71
Coleoptera	1	8	8	5	3	13	27
Non-Insect	6	3	3	5	3	5	12
Total Richness	34	71	49	69	13	99	204
Chironomid Richness	11	20	10	23	0	27	62
EPT Richness	6	22	16	19	0	29	50
Rare Taxa	3	5	8	1	7	12	69

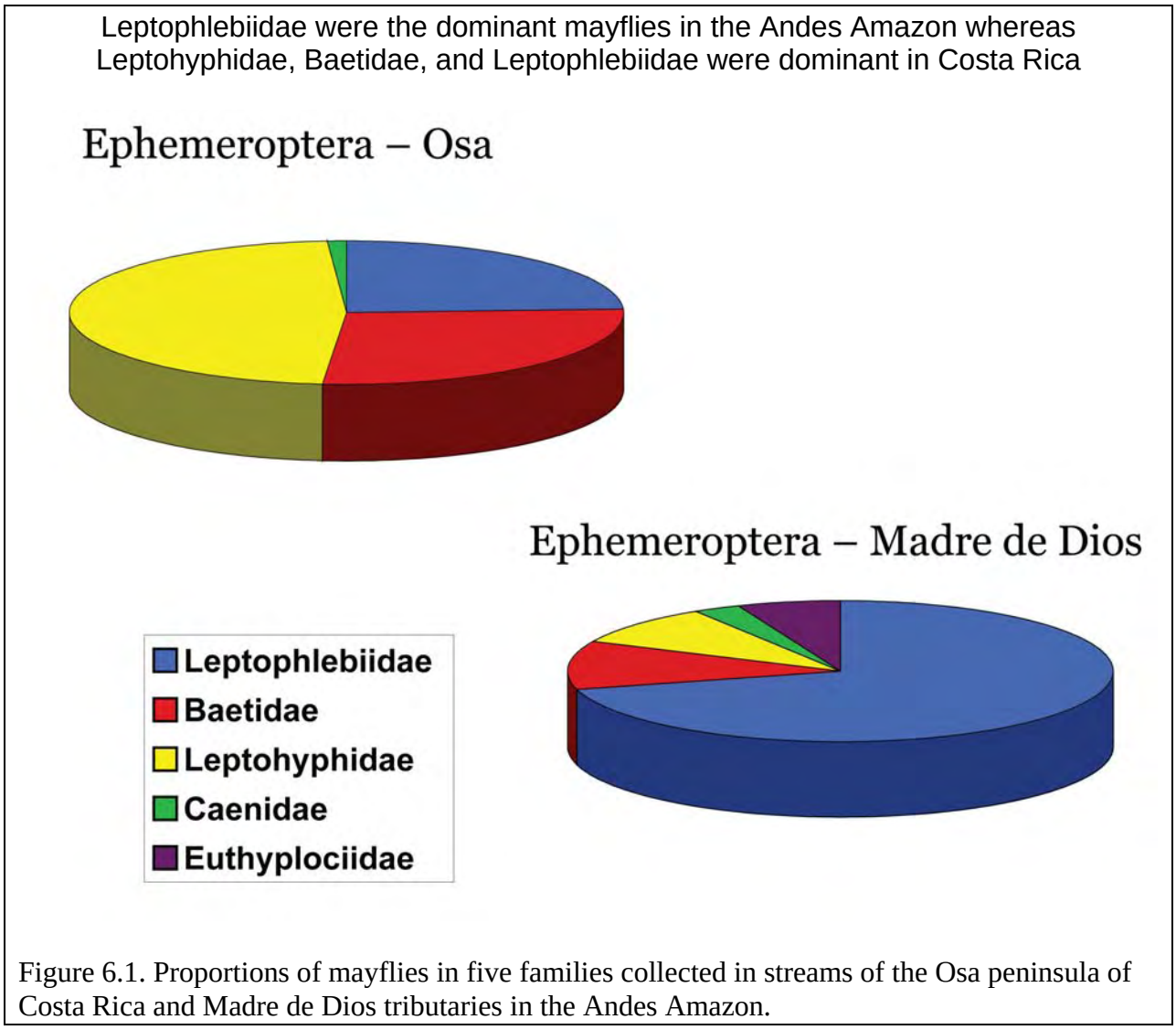

Figure 6.1. Proportions of mayflies in five families collected in streams of the Osa peninsula of Costa Rica and Madre de Dios tributaries in the Andes Amazon.

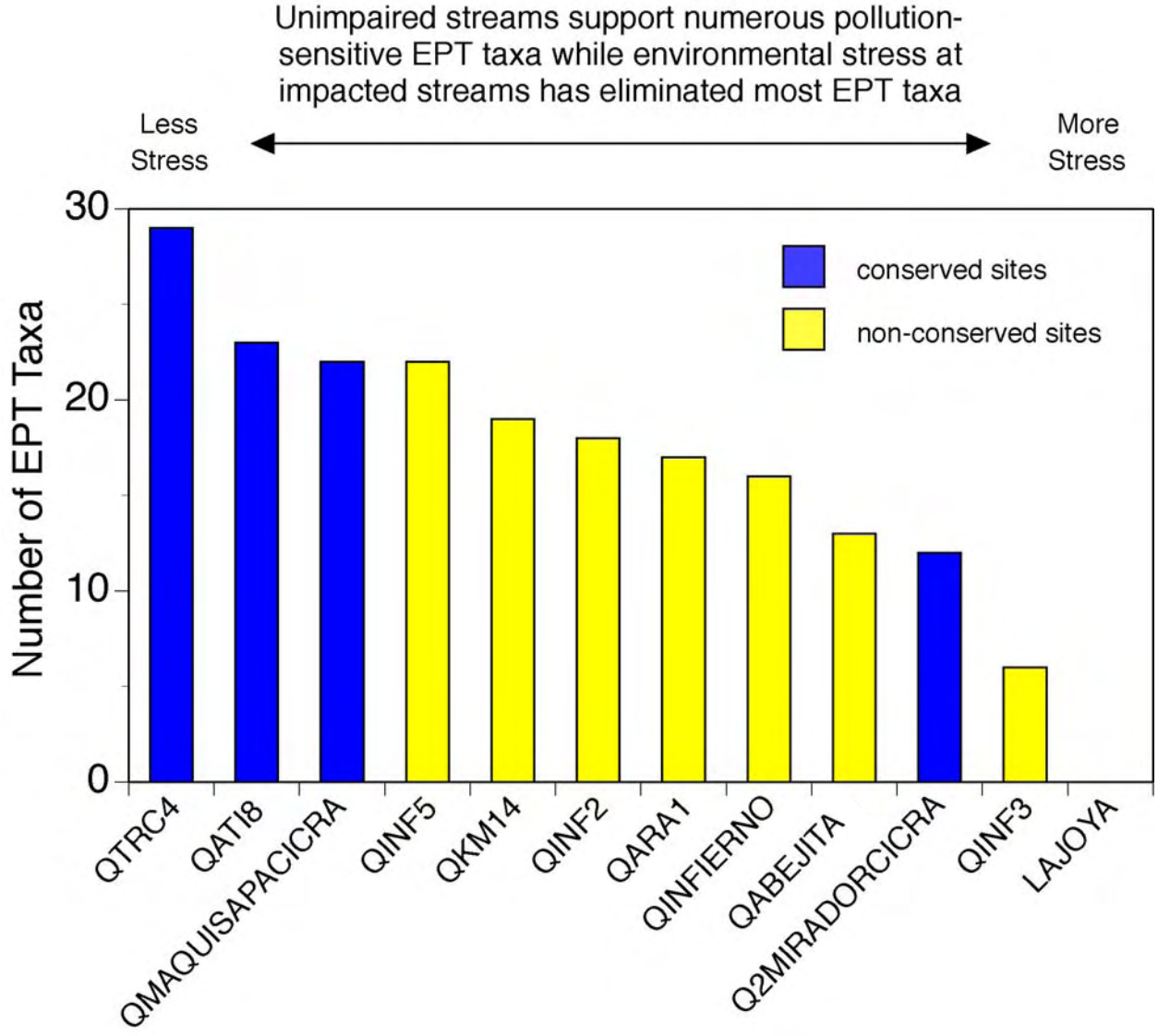

Figure 6.2 Number of EPT taxa (i.e., EPT Richness) collected at each site from both hand collections and leaf pack samplers. Sites are arranged in descending order based on EPT Richness. EPT Richness generally decreases as environmental stress increases

Total Richness and EPT Richness have often been observed to be correlated, and this was generally true among the sites in this study (Fig. 6.3). Both Total Richness and EPT Richness are generally highest at undisturbed streams, and gradually decline as environmental stress increases. Even though environmental stress at QLAJOYA was great enough that no EPT taxa were found, the site was not devoid of aquatic macroinvertebrates – several pollution-tolerant taxa (e.g., snails and air breathing Hemiptera) were collected. The general relationship among sites observed for EPT Richness is also apparent when Total Richness per site is plotted (Fig. 6.4). Two data points, representing QABEJITAS and Q2MIRADORCICRA, are apparent in both

Figs. 6.3 and 6.4 in that Total Richness appears somewhat high relative to EPT Richness observed. Conversely, two data points, representing QINF2 and QINFIERNO, are apparent in both Figs. 6.3 and 6.4 in that Total Richness appears somewhat low relative to EPT Richness. This is because chironomid midge taxa collected in the leaf packs were either more (QABEJITAS and Q2MIRADORCICRA) or less (QINF2 and QINFIERNO) numerous at these sites relative to the trend across the other sites. While some of this is assumed to be natural spatial and temporal variation in EPT and Total Richness, there is no evidence that any of these sites, including the protected Q2MIRADORCICRA, would support macroinvertebrate assemblages similar to those observed at the better sites.

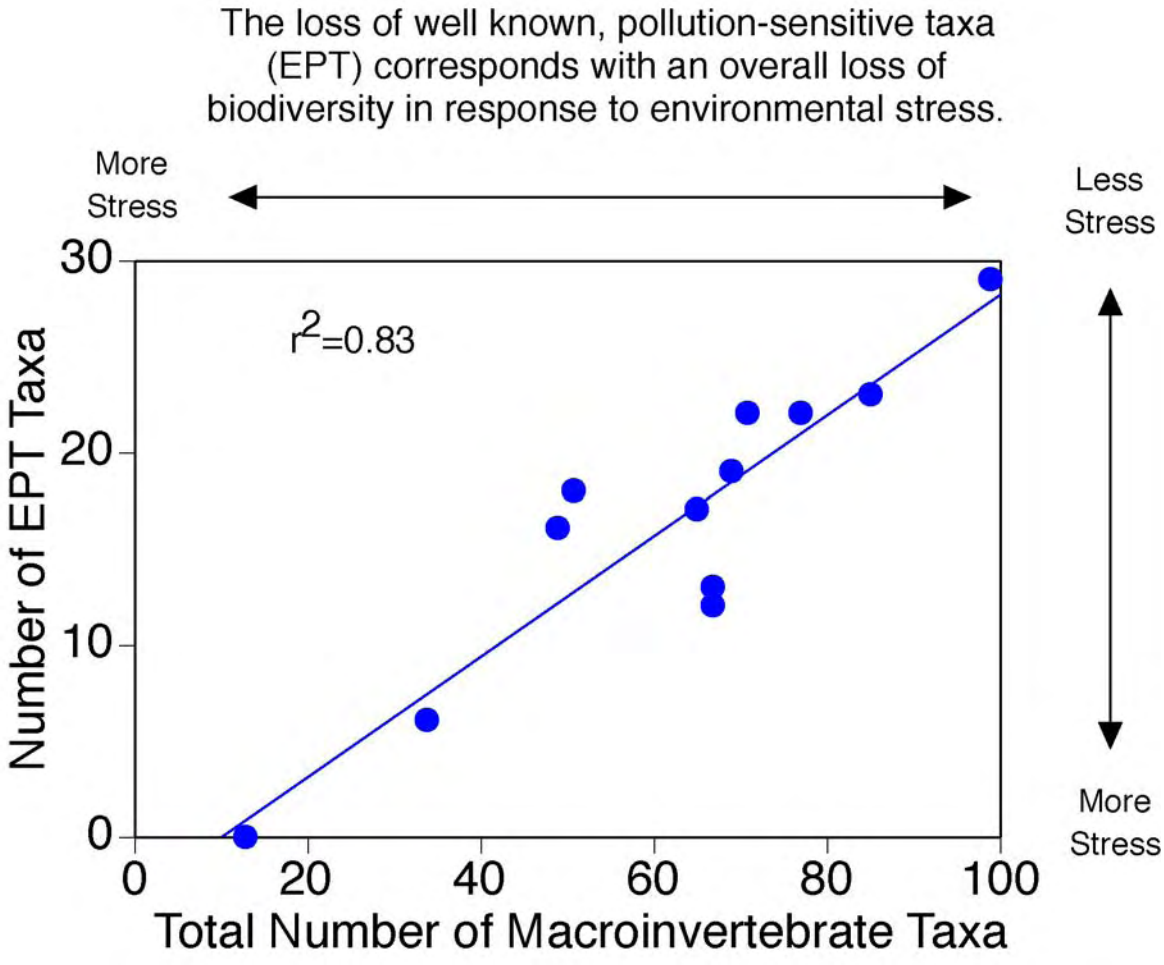

Figure 6.3 Correlation between Number of EPT taxa (i.e., EPT Richness) and the total number of macroinvertebrate taxa (i.e., Total Richness) collected at each site from both hand collections (without chironomids) and leaf pack samplers

Conditions at Protected Sites in the Andes Amazon

Three protected sites, QTRC4, QATI8, and QMAQUISAPACICRA, had the highest EPT Richness and Total Richness, and among the highest Chironomid Richness. Each supported a

number of unique taxa not found at the other sites. These observations, relative to data from the other sites, support the more pristine nature of these watersheds and the utility of aquatic macroinvertebrates for identifying high quality sites.

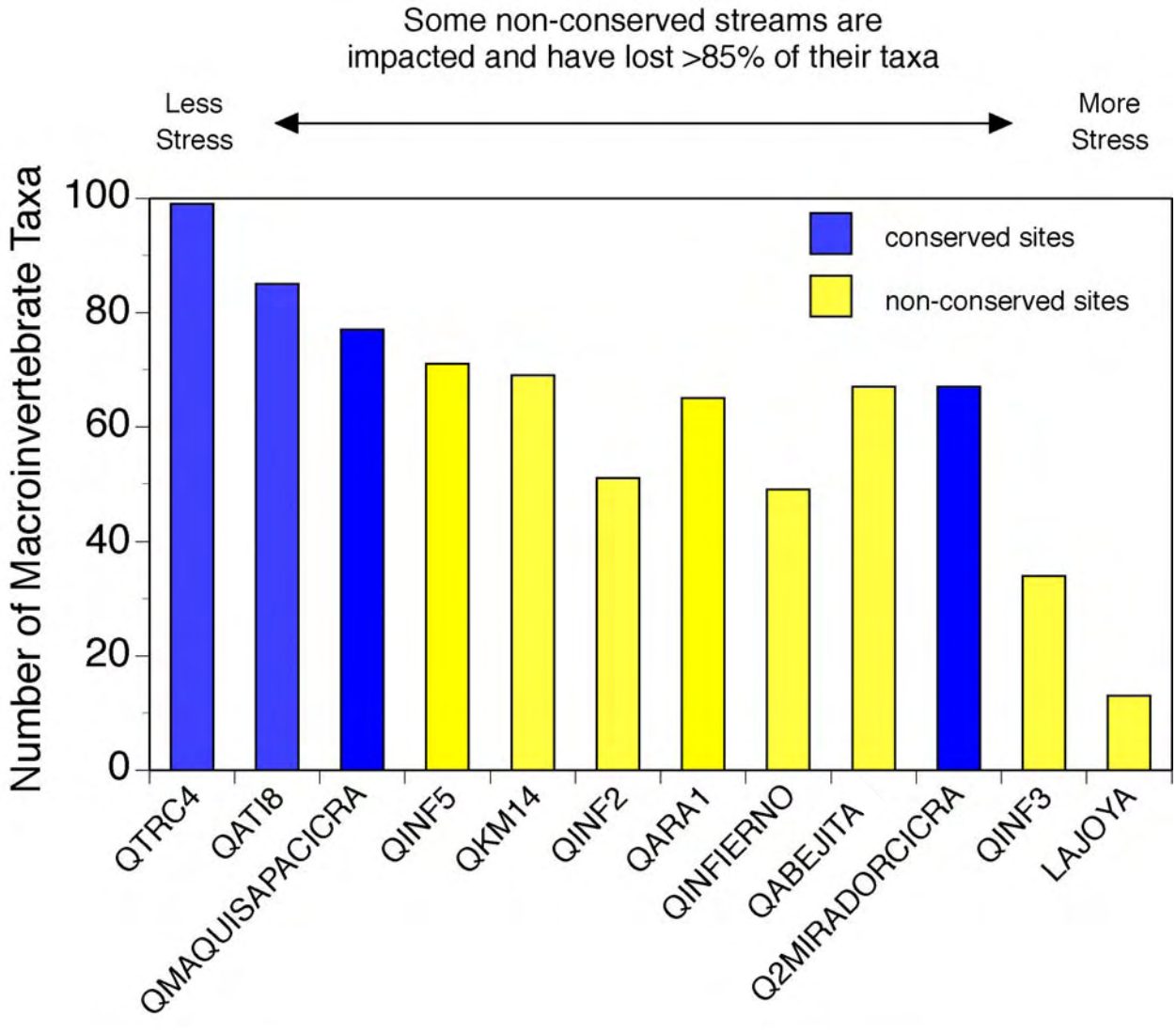

Figure 6.4 Total number of macroinvertebrate taxa (i.e., Total Richness) collected at each site from both hand collections (without chironomids) and leaf pack samplers. Sites are arranged in descending order based on EPT Richness. Total Richness generally decreases as environmental stress increases.

One protected site, Q2MIRADORCICRA, seems somewhat degraded based on the number of pollution-sensitive mayflies, stoneflies, and caddisflies collected (Fig. 6.2). A loss of 45-59% of EPT Richness (i.e., EPT Richness = 12 vs 22-29) would generally be interpreted as evidence of degradation. However, Q2MIRADORCICRA supported the most diverse group of chironomids of all the sites sampled, and was comparable the other three protected sites. This apparent contradiction, low EPT Richness with high Chironomid Richness, could reflect a sampling artifact as well as anthropogenic stresses that are currently unknown. Evidence from other study

elements suggests that Q2MIRADORCICRA is a somewhat different type of stream. Conductivity and pH was low, DOC and K were high (see Appendix 3). The bacterial assemblage at Q2MIRADORCICRA differed from all other sites, which also suggests that environmental conditions as that site was also affecting the bacteria.

Evidence of Stream Degradation Among Non-protected Sites in the Andes Amazon

Eight sites were sampled that were outside the protected areas. Based on their macroinvertebrates, they ranged in quality from comparable to the three pristine, protected sites to severely impaired. QINF5 was the highest quality of the non-protected sites sampled. EPT Richness was high (22 taxa), which was comparable to the three pristine, protected sites. This is a small watershed that remains well forested; there is little or no human activity in the watershed (except near the upper boundary). It is clearly a very high quality watershed, even though it is near the Trans-Oceanic Highway.

Based on other studies of unimpacted and impacted sites at a variety of locations in North and Central America, a range of biometric values are known to describe pristine conditions. This range can represent a temporal phenomenon as well as a spatial phenomenon related to underlying differences (e.g., soils, forest type, channel morphology). Pristine conditions consider this natural variation, with impacts defined as conditions that fall outside of the natural variation observed in a region. The significant loss of EPT Richness and Total Richness at three sites (QLAJOYA, QINF3, QABEJITA) is sufficient to suggest all four of these sites as exhibiting significant impairment associated with anthropogenic activity. Both QINF3 and QLAJOYA exhibit taxa losses (e.g., 73-79 and 100% of EPT, respectively) likely associated with severely impaired conditions. QLAJOYA drained the most developed watershed sampled, and had very high concentrations for most analytes measured relative to the other sites sampled. No other site had a chemical signature similar to that of QLAJOYA. The loss of EPT and other taxa at QLAJOYA presumably reflects a toxic combination of pollutants and conditions resulting from the pollutants (e.g., low dissolved oxygen) that come from a number of upstream sources. QINF3 drained an agricultural (bananas) watershed, and did not have unusually high or low concentrations of basic analytes such as anions, cations, and nutrients. However, the chemical tracers analyses found pesticide concentrations (i.e., the insecticide Chlorpyrifos and the fungicide Metalayxl, Appendix 4) were the highest among all sites sampled. It would appear that these pesticides (or others that were applied with them but not measured here) may be at toxic levels, reducing the number of EPT and other macroinvertebrate taxa collected at QINF3. QABEJITA drained an agricultural (livestock) watershed and, with a 41-55% loss of EPT Richness, might be considered moderately impaired. Like Q2MIRADORCICRA, the Chironomid Richness was somewhat high. The presence of several EPT taxa and numerous chironomid taxa suggest that the environmental stress affecting the macroinvertebrates was less than was observed at QLAJOYA and QINF3. As noted above, one protected site, Q2MIRADORCICRA, exhibited EPT and Total Richness that were similar to QABEJITA, and might be considered moderately impaired. This site exhibited somewhat unusual chemical characteristics, and supported a unique microbial assemblage. It is possible that this in a natural phenomenon, but the undisturbed and protected nature of the area suggests that this watershed may have had some legacy land use issues (e.g., past mining activity) that are not immediately visible but are still affecting the stream.

Finally, four sites (QKM14, QINF2, QARA1, QINFIERNO) fell between the more high quality sites (QTRC4, QATI8, QMAQUISAPACICRA, QINF5) and the clearly impacted (QLAJOYA, QINF3, QABEJATA, Q2MIRADORCICRA) sites. None of these sites would be considered to be highly developed or to have intensive agriculture. Three of the sites (QKM14, QINF2, QINFIERNO) had significant deforestation in the upper watershed. This appeared most intense for QINFIERNO, which has a large area of intensive land use associated the Trans-Oceanic Highway. Both QKM14 and QINF2 had deforestation and agricultural activities upstream of the sampling site, but not as intense as was observed at QINFIERNO. EPT and Total Richness at QARA1 were lower than was expected given the QARA1 watershed appears to be relatively undisturbed and is an important source of drinking water. It is possible that this stream is somewhat unusual (e.g., it drains and flows across some recent floodplains of the R. Tambopata) or if there remain some negative effects from past land use as at Q2MIRADORCICRA. Thus, classifying these less impacted sites is presently difficult with the available data because it is difficult to determine how much the lower richness values reflect the anthropogenic activities in these watersheds and how much is natural variation that has not yet been well qualified in this region.

In summary, the above discoveries are remarkable and numerous, illustrating how much remains to be discovered concerning species names, their habits and ecological role, and their response to environmental stress. In the mean time, these discoveries do not suggest that the taxonomic and ecological information needed to manage the waters and watersheds of the Andes Amazon is beyond what currently is in hand. Rather, aquatic macroinvertebrate faunas appear relatively finite compared with the terrestrial invertebrate faunas that have attracted so much attention for their almost unimaginable biodiversity. In addition, the pollution responses observed here for aquatic macroinvertebrates of the Andes Amazon (see above) are consistent with those observed and interpreted based on the efforts of 1000s of workers over many decades at North Temperate sites. No doubt some differences between Neotropical and North Temperate responses will eventually be found, but the basic framework appears sufficient to begin to use aquatic macroinvertebrates to identify sites that are relatively pristine as well as those exhibiting significant environmental impact. Therefore, in the current study, four sites can be classified as pristine/unimpaired, four sites as moderately/severely impaired, and four sites as in between (i.e., slight impairment?). This in-stream perspective is currently missing in watershed management and conservation decisions concerning the waters and watersheds of the Andes Amazon. This information is sufficient to identify extreme environmental stress, and to guide management and conservation efforts to avoid or remediate extreme environmental impacts. Additional information is needed to identify and understand less dramatic impacts, and integrate these relationships into management and conservation efforts.

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Appendix 6.1. Presence of taxa indicated with an * for each of 12 sites collected in August 2006. Site order is the same as in Table 6.1.

	Q2MIRADO RCICRA	QMAQUISA PACICRA	QABEJITA	QATI8	QARA1	QINF2	QINF3	QINF5	QINFIERNO	QKM14	QLAJOYA	QTRC4
PLECOPTERA												
Perlidae: Anacroneuria	*	*		*	*	*		*	*	*		*
Perlidae: Inconeuria				*								*
Perlidae: Kemphyia												*
Perlidae: undet					*	*			*			*
EPHEMEROPTERA												*
Baetidae: Americabaetis	*	*	*	*	*	*	*	*		*		*
Baetidae: Callibaetis			*	*			*					
Baetidae: Cloeodes		*	*			*						
Baetidae: Paracloeodes		*										
Baetidae: Waltzoyphius	*	*	*		*			*		*		
Baetidae: undet			*	*		*	*	*	*	*		*
Caenidae: Brasilocaenis	*		*	*	*							
Caenidae: Caenis												*
Caenidae: undet	*	*	*									*
Euthyplociidae: Campylocia	*	*			*			*		*		*
Leptohyphidae.: Anmanahyphes		*		*				*				
Leptohyphidae.: Leptohyphes									*			*
Leptohyphidae.: Tricorythodes		*						*		*		
Leptohyphidae: undet		*			*	*			*	*		*
Leptophlebiidae: Thraulodes						*						*
Leptophlebiidae: Ecuaphlebia		*	*	*	*			*		*		*
Leptophlebiidae: Hagenulopsis		*						*				*
Leptophlebiidae: Farrodes		*	*	*		*		*	*	*		
Leptophlebiidae: Miroculis	*	*	*	*		*	*	*		*		*
Leptophlebiidae: Terpides		*						*				
Leptophlebiidae: Ulmeritoides				*			*	*		*		
Leptophlebiidae: Tikuna bilineata				*			*					
Leptophlebiidae: Fittkaulus			*							*		
Leptophlebiidae: Hylister				*		*			*			
Leptophlebiidae: Hydrosmilodon						*						
Leptophlebiidae: undet	*		*		*	*			*	*		*
Polymitarciidae: Campsurus						*		*	*			
ODONATA												
ZYGOPTERA: undet				*		*						
Calopterygidae: Hetaerina	*		*	*		*						*
Calopterygidae: undet												*
Dicteriadidae: Heliocharis amazona		*	*	*	*					*		*
Coenagrionidae: Argia	*	*	*	*	*	*	*	*		*		*
Coenagrionidae: Leptobasis			*									
Coenagrionidae: Telebasis		*	*	*				*		*		

	Q2MIRADO RCICRA	QMAQUISA PACICRA	QABEJITA	QATI8	QARA1	QINF2	QINF3	QINF5	QINFIERNO	QKM14	QLAJOYA	QTRC4
Coenagrionidae: undet		*								*		
ANISOPTERA: undet					*	*				*		*
Libellulidae: Brechymorhoga						*			*	*		*
Libellulidae: Brechymesia				*								
Libellulidae: Elasmothemis				*	*				*			*
Libellulidae: Macrothemis ?	*	*	*	*	*	*	*	*	*	*		*
Libellulidae: Orthemis ?											*	
Libellulidae: Perithemis ?	*		*	*	*			*		*	*	*
Libellulidae: Dythemis						*						
Libellulidae: Aeschnosoma pr. forcipula			*	*								*
Libellulidae: undet.							*					*
Libellulidae: Genus A Peru						*						*
Megapodagrionidae: Heteragrion	*	*		*	*	*		*		*		
Megapodagrionidae: undet	*					*						
Perilestidae:								*				
Polythoridae		*								*		*
Protoneuridae			*							*		
Gomphidae: Ariogomphus		*	*	*		*	*	*		*		*
Gomphidae: Erpetogomphus												*
Gomphidae: Epigomphus ?		*		*	*					*		
Gomphidae: Desmogomphus ?					*				*			
Gomphidae: Phyllogomphoides									*			
Gomphidae: Phyllocycia		*		*	*	*	*					
Gomphidae: Progomphus	*			*			*					*
Gomphidae: Undet								*				
MEGALOPTERA												
Corydalidae: Corydalus	*	*	*		*	*		*	*			*
Corydalidae: Chloronia				*	*			*				*
Sialidae: Sialis							*	*				
LEPIDOPTERA												
Pyralidae:					*							*
HEMIPTERA												
Belostomatidae			*	*						*	*	
Tenagobia	*											
Corixidae: undet.			*	*			*	*				
Gerridae: undet			*	*		*		*			*	
Veliidae: Rhagovelia	*	*	*			*	*		*			
Veliidae: Stridulivelia			*									
Veliidae: Microvelia	*											
Veliidae: Undet.							*					
Naucoridae: Ambrysus	*	*	*	*	*			*	*			*
Naucoridae: Cryphocricos		*										*
Nepidae: Curicita												

	Q2MIRADO RCICRA	QMAQUISA PACICRA	QABEJITA	QATI8	QARA1	QINF2	QINF3	QINF5	QINFIERNO	QKM14	QLAJOYA	QTRC4
Nepidae: Ranatra			*	*						*	*	
Notonectidae		*	*					*				
Pleidae: Neoplea		*										*
Pleidae: undet		*	*									*
Hydrometridae											*	
Mesoveliidae: Mesoveloidea				*								
Hebridae: undet.											*	
COLEOPTERA: undet									*			
Dryopidae: Dryops	*											
Dryopidae: Pelonomus		*		*	*	*						*
Dryopidae: undet.						*	*					
Dytiscidae: Potamonectes				*								
Dytiscidae: undet.				*	*	*		*	*		*	
Elmidae: Cylloepus	*									*		
Elmidae: Gyrelmis				*	*							
Elmidae: Dubiraphia	*											
Elmidae: Heterelmis	*	*	*	*	*			*	*	*		*
Elmidae: Hintonelmis									*			
Elmidae: Macrelmis A									*			
Elmidae: Microcylloepus										*		
Elmidae: Neoelmis	*				*							
Elmidae: Phanocerus									*			*
Elmidae: Stegoelmis								*				*
Elmidae: Xenelmis												*
Elmidae: Genus A Peru		*		*				*	*	*		
Elmidae: Genus B Peru				*				*	*			*
Elmidae: Genus C Peru		*										*
Elmidae: Genus D Peru				*	*							*
Elmidae: Genus E Peru								*				
Gyrinidae: Gyretes			*									
Gyrinidae: Gyrinus					*							
Gyrinidae: larva undet.			*									
Hydrophilidae: Derallus												*
Hydrophilidae: Tropisternus											*	
Hydrophilidae: unidet.				*		*		*			*	
Hydraenidae: undet					*							*
Noteridae		*										
Psephenidae: Undet												*
Ptilodactylidae: Anchytarsus		*			*	*		*	*	*		*
Scirtidae: Undet	*			*		*					*	*
TRICHOPTERA												
Calamoceratidae: Phylloicus	*	*	*	*	*			*	*	*		*
Calamoceratidae:Banyallarga								*				

	Q2MIRADO RCICRA	QMAQUISA PACICRA	QABEJITA	QATI8	QARA1	QINF2	QINF3	QINF5	QINFIERNO	QKM14	QLAJOYA	QTRC4
Glossosomatidae								*				*
Glossosomatidae: Protoptilinae				*								
Helicopsychidae: Helicopsyche				*		*			*	*		*
Helicopsychidae: undet		*										
Hydropsychidae:Blepharopus				*	*			*		*		*
Hydropsychidae: Leptonema	*			*		*			*	*		*
Hydropsychidae: Leptonema sp. 1 Peru	*											*
Hydropsychidae: Smicridea	*	*		*	*	*		*	*	*		*
Hydropsychidae: Smicridea sp.1 Peru						*						
Hydropsychidae: Macronema	*	*		*	*			*	*			*
Hydropsychidae: undet					*	*			*	*		*
Hydroptilidae: Ochrotrichia									*			*
Hydroptilidae: Oxyethira						*						
Hydroptilidae: Neotrichia			*	*	*	*		*	*	*		*
Hydroptilidae: Metrichia/Ochrotrichia						*						
Hydroptilidae: undet				*		*			*	*		*
Leptoceridae: Brachysetodes						*			*	*		*
Leptoceridae: Triplectides		*			*			*		*		*
Leptoceridae: Oecetis		*	*	*	*			*		*		*
Leptoceridae: Hudsoneuria												*
Leptoceridae: Nectopsyche									*			
Leptoceridae: undet		*		*		*		*	*	*		*
Odontoceridae: Marilia	*				*							*
Odontoceridae: unid	*											*
Philopotamidae: Chimarra									*			
Philopotamidae: Wormaldia	*	*		*								*
Polycentropodidae: Cyrnellus												*
Polycentropodidae: Polycentropus		*	*	*	*		*					*
Polycentropodidae: Polyplectropus		*			*							*
Polycentropodidae: unid	*		*	*								*
Xiphocentronidae				*								
DIPTERA												
Ceratopogonidae: Undet	*	*	*	*	*	*	*	*		*		*
Ceratopogonidae: Forcipomyia					*				*			*
Chironomidae: Ablabesymia	*	*		*				*				
Chironomidae: Chironomus							*					
Chironomidae: Clinotanypus	*											
Chironomidae: Corynoneura	*	*	*	*	*				*	*		*
Chironomidae: Cricotopus spp.	*		*		*	*			*			
Chironomidae: Labrundinia			*									
Chironomidae: Larsia	*		*	*		*	*	*		*		*
Chironomidae: Microtendipes		*										*

	Q2MIRADO RCICRA	QMAQUISA PACICRA	QABEJITA	QATI8	QARA1	QINF2	QINF3	QINF5	QINFIERNO	QKM14	QLAJOYA	QTRC4
Chironomidae: Nanocladius	*	*	*	*	*			*	*	*		*
Chironomidae: Nilothauma			*	*						*		
Chironomidae: Orthocladius									*			
Chironomidae: Parachironomus	*	*	*	*				*		*		*
Chironomidae: Parametriocnemus						*				*		*
Chironomidae: Pentaneura P1										*		*
Chironomidae: Pentaneura P2	*	*	*		*							*
Chironomidae: Pentaneura	*	*	*	*				*				
Chironomidae: Phaenopsectra							*					
Chironomidae:Polypedilum illinoense grp		*	*		*		*	*	*	*		*
Chironomidae: Polypedilum halterale grp	*	*	*	*						*		
Chironomidae: Polypedilum nr aviceps				*		*						
Chironomidae: Polypedilum nr flavum								*				
Chironomidae: Polypedilum spp..		*		*								
Chironomidae: Rherotanytarusu	*	*			*	*	*	*	*	*		*
Chironomidae: Stempellina	*	*	*									*
Chironomidae: Stenochironomus					*							*
Chironomidae: Tanytarsus P1	*	*	*	*						*		*
Chironomidae: Tanytarsus P2	*	*	*	*	*	*		*		*		*
Chironomidae: Tanytarsus P3	*	*	*	*		*	*	*				*
Chironomidae: Tanytarsus P4			*	*								*
Chironomidae: Tanytarsus P5	*											*
Chironomidae: Tanytarsus P6	*	*	*		*		*	*		*		*
Chironomidae: Tanytarsus P7		*		*								*
Chironomidae: Tanytarsus P8	*	*	*				*	*		*		*
Chironomidae: Tanytarsus P9			*	*				*				*
Chironomidae: Tanytarsus P10		*			*							*
Chironomidae: Tanytarsus P11	*		*	*			*	*		*		
Chironomidae: Tanytarsus P12		*	*							*		
Chironomidae: Tanytarsus P13		*	*									
Chironomidae: Tanytarsus P14	*	*	*				*			*		
Chironomidae: Tanytarsus P15	*	*								*		
Chironomidae: Tanytarsus P16		*						*				
Chironomidae: Tanytarsus P18								*	*			
Chironomidae: Tanytarsus P20								*		*		
Chironomidae: Tanytarsus P21					*							
Chironomidae: Tanytarsus	*	*	*	*	*			*		*		*
Chironomidae: Thienemanniella			*		*	*			*	*		*

	Q2MIRADO RCICRA	QMAQUISA PACICRA	QABEJITA	QATI8	QARA1	QINF2	QINF3	QINF5	QINFIERNO	QKM14	QLAJOYA	QTRC4
Chironomidae: Harnischia		*										
Chironomidae: Harnischia complex genus P	*											
Chironomidae: Lauterborniella	*	*	*	*				*		*		*
Chironomidae: Tanypodinae	*		*				*	*		*		*
Chironomidae: Stempellinella	*			*	*			*		*		
Chironomidae: Hyporhygma									*			
Chironomidae: Limnophyes												*
Chironomidae: Constempellina		*		*								*
Chironomidae: Xestochironomus				*								
Chironomidae: Djalmabatista	*			*								
Chironomidae: BEARDIUS	*	*	*	*	*		*	*		*		
Chironomidae: Chironominae genus A Peru			*	*	*							*
Chironomidae: Nilothauma B				*								
Chironomidae: nr Polypedilum (PERU)	*											
Chironomidae: nr Hyporhygma	*											
Chironomidae: nr Fissimentum	*											
Chironomidae: Chiornominae genus B Peru		*										
Chironomidae: nr Georthocladius								*				
Chironomidae: nr Paratendipes									*			
Chironomidae: Lopescladius	*			*								
Culicidae: undet.						*						
Empididae: Hemerodromia					*	*			*			*
Empididae: undet.	*				*				*	*		*
Psychodidae: Pericoma/Telmatoscopus												*
Psychodidae: undet					*							
Simuliidae: Simulium					*							*
Simuliidae: undet	*		*	*	*	*		*	*	*		*
Tipulidae: Tipula												*
Tipulidae: Antocha												*
Tipulidae: Limnophila				*								
Tipulidae: undet	*	*	*		*		*	*	*	*		*
Tabanidae: undet								*				
DECAPODA												
Grapsidae	*	*				*				*		
Palaemonidae		*			*	*	*	*		*		*
ACARI	*	*	*	*	*	*	*	*	*	*		*
MOLLUSCA												
Planorbidae											*	
Physidae											*	
Viviparidae											*	

	Q2MIRADO RCICRA	QMAQUISA PACICRA	QABEJITA	QATI8	QARA1	QINF2	QINF3	QINF5	QINFIERNO	QKM14	QLAJOYA	QTRC4
Ancylidae							*					
OLIGOCHAETA	*	*	*	*	*	*	*	*	*	*		*
HIRUDINEA	*		*				*					*
NEMATOMORPHA: GORDIOIDEA				*								
TURBELLARIA			*		*	*				*		*
NEMATODA	*	*	*	*	*		*		*			

Appendix 7. Nutrient Processing

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Executive Summary

- Nutrient processing was assessed through measurements of the uptake of phosphate, ammonium, and two carbohydrates (glucose and arabinose) in three small streams draining undisturbed forest, second-growth forest, and pastureland with a riparian forest, respectively.
- Ammonium uptake in all three streams was slower than in most undisturbed temperate streams. That is, ammonium moved farther downstream (spiraling length was longer) before being used by algae and microbes than it would in a temperate stream of comparable size.
- The rate of ammonium uptake was nearly identical in all three streams, despite differences in land use and streambed characteristics.
- Phosphorus uptake in the two forest streams was too slow to measure reliably, and much slower than in most undisturbed temperate streams.
- The rate of phosphorus uptake in the stream draining a pasture was very high, among the highest reported worldwide. Some or most of this "uptake" may actually be chemical co-precipitation rather than biological uptake.
- Carbohydrate (glucose and arabinose) uptake was in the range found in temperate streams, but higher in the pasture stream than in the forest streams.
- Results (low cycling rates, higher phosphate uptake in the pasture) are consistent with the only other measurements reported from the Amazon basin, but they are not consistent with the paradigm (from temperate streams) that disturbance increases spiraling length.
- The very high phosphorus uptake and low phosphorus concentration in the pasture stream, whether biotic or abiotic, raise the concern that deforestation may reduce supplies of this nutrient to downstream ecosystems.

Introduction

The rates at which streams use and cycle nutrients are of interest both because nutrient cycling is ecosystem service (Palmer et al. 2004)—a process that directly or indirectly supplies human needs such as food production or water purification—and because such rates provide a sensitive measure of human impact on an ecosystem, relative to what its condition or function would be in the absence of human activity (Odum 1985, Newbold et al. 2006). In streams and rivers, nutrients move downstream as they cycle, and the combined processes are termed *spiraling* (Webster and Patten 1979). Nutrient spiraling in streams is typically quantified in terms of "uptake length," which is the average distance that a biologically available nutrient atom moves downstream before being used biologically, or in terms of "uptake velocity," which is a measure that of the rate that biologically available nutrient is removed from the water column and sequestered on the streambed. Recent years have seen the development of a large database of measurements of nutrient spiraling, in temperate streams (Ensign and Doyle 2006), together with growing evidence that spiraling can provide a sensitive indicator of human impact (e.g., Butturini

and Sabater 1998, Meyer et al. 2005, Newbold et al. 2006). However, very few measurements have been made in tropical streams (e.g., Merriam et al. 2002, Neill et al. 2006).

In this study, the uptake of phosphorus, ammonium, and two forms of dissolved organic matter (glucose and arabinose) were quantified in lowland streams in the Amazon River Basin (Peru). The streams were similar in size but differed in land use history. One stream, a tributary to the Tambopata, designated QTRC4 (see Table 2.1 and Fig. 2.1 of Appendix 2 for site locations), has had no known human impact of significance. A second stream, a tributary to the Madre de Dios River designated QATI8, drains a region of secondary growth forest allowed to grow after the abandonment of agriculture several decades prior to the study. The third stream, QABEJITA, a tributary to the Madre de Dios River designated QABEJITA, drains pastureland although the riparian zone of the study reach was forested and livestock access was restricted. The streambed of QTRC4 consisted primarily of cobbles and pebbles, whereas the beds of the other two streams were mostly silt and sand.

Methods

Stream solute additions

Solute uptake length (S_w) for NH_4^+, PO_4^{3-}, glucose, and arabinose were measured in ~100-m reaches of each of three streams in August 2006 under baseflow conditions, using metered additions of small quantities of the respective nutrients (Newbold et al. 1981, Webster and Ehrman 1996). Each addition involved simultaneous injections of a conservative tracer (sodium bromide), PO_4^{3-}, $^{15}NH_4^+$, glucose, and arabinose, over a period of 90 to 105 minutes at rates designed to achieve concentration elevations in the stream of 10 µg/L PO_4^{3-}-P, 0.5 µg/L NH_4^+-N, 14 µg/L glucose-C, and 12 µg/L arabinose-C. The amount of $^{15}NH_4^+$ added was designed to attain a ^{15}N enrichment in the stream of 5000-1000‰. One day before additions, time-of-travel was estimated with rhodamine WT and channel width and depth were measured at 20 transects throughout the reach. Streamflow was estimated from 10-15 velocity and depth measurements across a transect using a Marsh-McBirney Flo-Mate model 2000 portable flow meter.

Immediately before solute addition, replicate water samples for ambient concentrations were taken at each of 5 downstream sampling stations, spaced throughout the reach. Five subsequent water samples were taken from each station within the period of plateau concentration, or in the period of maximal concentrations, for assay of $^{15}NH_4^+$, soluble reactive P (SRP), glucose, and arabinose, in addition to the conservative tracer. Supplementary samples for total dissolved P (TDP), total dissolved N (TDN), and NO_3^-, in addition to NH_4^+ and SRP, were taken from just upstream of the injection site and at the station above the lowermost station before, during, and after each addition. Samples for N and P assays were field-filtered using 0.45-µm Fisherbrand cellulose nitrate membrane filters. Samples for glucose and arabinose assays were sterile-filtered using Millipore 0.22-µm Millex GP filters. All samples were chilled and frozen as soon as possible. However, some samples (particularly those from QATI8) remained unchilled for as much as 4 days and unfrozen for as long as 12 days.

Uptake length, S_w for a given analyte was estimated from the concentration elevations, $\Delta C(x,t) = C(x,t) - C(x,t_0)$, where $C(x,t)$ and $C(x,t_0)$ are the concentrations of the analyte (SRP, $^{15}NH_4^+$,

glucose, or arabinose) measured at a distance x (m) downstream of the injection point at time t (after the beginning of the injection), and time t_0 (immediately before the injection), respectively. The ratio, r_c, is the concentration elevation relative to that of the Br^- (the conservative tracer), $r_c = \Delta C/\Delta[Br^-]$, and was calculated to adjust for longitudinal dilution and dispersion. The longitudinal loss rate, k_l, of the solute was estimated by nonlinear regression from the relationship $r_c(x) = r_0 \exp(-k_l x)$, where r_0 is the concentration ratio elevation at $x = 0$. S_w for the respective solute was calculated as $S_w = 1/k_l$ (Newbold et al. 1981). S_w was converted to a mass-transfer coefficient, or uptake velocity (V_f), calculated as $V_f = k_l v_w d = v_w d/S_w$ (where v_w is the water velocity) to express uptake rates in a form that is independent of the size (depth and velocity) of the stream. Conservative tracer (Br^-) data were analyzed with a 1-dimensional advection–dispersion model that includes a transient storage component (OTIS-P, Runkel et al. 1998) to describe stream flow characteristics, including: flow (Q), cross-sectional area (A, from which $v_w = Q/A$, and $d = A/w$), cross-sectional transient storage area (A_s), longitudinal dispersion coefficient (?), and transient storage exchange coefficient (α). The transient storage represents short-term detention of the stream water in lateral zones, such as backwaters or eddies, or within the sediments where the downstream velocity is negligible (Bencala and Walters 1983). In this study, the size of the transient storage zone is reported as the ratio A_s/A, and the rate of transfer into the transient storage zone is reported both as a hydraulic uptake length ($S_{hyd} = v_w/\alpha$), and as a hydraulic exchange velocity ($v_{hyd} = \alpha d$) for dimensional consistency with nutrient uptake lengths and uptake velocities, respectively.

Analytical methods

The carbohydrates, glucose and arabinose, were analyzed by high performance liquid chromatography (HPLC) with pulsed amperometric detection (Dionex 500) (Cheng and Kaplan 2001). Br^- was analyzed by ion chromatography with conductivity detection (Dionex 500). SRP was determined by the ascorbic acid method (EPA method 365.1). TDP was determined as SRP after ammonium persulfate digestion (EPA method 365.5). NH_4^+ was determined by the phenate procedure (EPA method 350.1), and NO_3^- (including NO_2^-) by Cd reduction (EPA method 353.2). Total dissolved N (TDN) was determined as the sum of NO_3^--N plus soluble Kjeldahl N (SKN, semiautomated phenate block digestion followed by NH_4^+ assay).

$^{15}NH_4^+$ was assayed by first spiking a 50 mL sample from the field with 30 µg of natural-abundance NH_4^+-N (δ= -5.17‰) prepared from a laboratory standard. Approximately 15 mg of Linde Ionsiv W-85 Ion Exchanger was then added and the sample manually shaken every 5 to 10 minutes for greater than 1 hour to adsorb all NH_4^+. The sample was then filtered onto a pre-ashed Whatmann GF/C glass fiber filter (Fisher AP40) and dried at 50°C for >48 hours under a steady stream of N_2 gas. Whole dried filters were then placed in tin boats and assayed for $^{15}N/^{14}N$ by EA-IRMS (Costech ECS 4010 - ThermoFinnegan DeltaPlus XP). The concentration of $^{15}NH_4^+$ in the original stream sample was calculated from the final $^{15}N/^{14}N$ ratio by correcting for the spike addition and for fractionation effects as determined from spiked blanks.

Results

Physical and chemical characteristics

The three streams were similar in size in channel characteristics, with the flow of the largest stream (QTRC4) being slightly over twice that of the smallest (QABEJITA) (Table 7.1). Velocity was higher at QTRC4 than at the other two streams, reflecting a higher gradient. QTRC4 also had a wider channel and lower water depth than the other two streams. Transient storage was minor in QATI8 and QABEJITA. For both streams, the hydraulic uptake length was much longer than the reach length, which implies that most of the water entering the reach passed directly through without entering transient storage. Also, for both streams, the cross sectional area of the transient storage volume (A_s) was less than 15% of the advecting cross sectional area. In contrast, QTRC4 had a relatively short hydraulic uptake length (74 m), and transient storage was 67% as large as the advecting volume.

Table 7.1. Physical and chemical characteristics of study reaches

	Stream		
Parameter	QATI8	QABEJITA	QTRC4
Date of Nutrient Addition	18-Aug-06	22-Aug-06	27-Aug-06
Length (m)	100	100	94
Width (m)	2.3	1.4	4.4
Depth (m)	0.14	0.11	0.06
Velocity (m/s)	0.026	0.024	0.035
Stream flow (L/s)	8.4	3.9	9.2
Hydraulic uptake length (m)	1576	317	74
Hydraulic exchange velocity (mm/s)	0.002	0.008	0.03
Transient storage ratio, A_s/A	0.14	0.11	0.67
Mean daily water temperature (°C)	22.9	21.5	24
NH_4^+-N (mg/L)	0.018	0.025	0.012
oPO_4^{-3}-P (mg/L)	0.0066	0.0014	0.0068
Glucose (nm)	42	59	27
Arabinose (nm)	27	5.3	2.5
NO_3^--N (mg/L)	0.053	0.021	0.216
Conductivity (µS.cm-1)	11.6	26.3	8
Total alkalinity (mg/L as $CaCO_3$)	78	243	54
DOC (mg/L)	7.94	2.66	0.82
BDOC (mg/L)	0.84	0.42	0.20
% BDOC	10.6	15.8	23.8

The chemical characteristics of QABEJITA, which drained pasture land, differed from the two forest streams, having higher NH_4^+, conductivity, and alkalinity, but lower PO_4^{3-} and NO_3^-. One of the forest streams, QTRC4, had much higher NO_3^- and much lower DOC concentrations than the other two streams.

Uptake lengths and uptake velocities

Ammonium uptake length (S_w for NH_4^+) ranged from 55 m in QTRC4 to 92 m in AQATI8 (Table 7.2). Expressed as uptake velocity, V_f, which adjusts for differences in stream size, NH_4^+ uptake was nearly identical among all three streams at 0.040-0.041 mm/s (Table 7.3).

Significant PO_4^{3-} uptake was measurable only in QABEJITA where the uptake length was 11 m. The PO_4^{3-} uptake lengths of the other two streams were >300 m at QATI8 and >220 m at QTRC4. The V_f for PO_4^{3-} was 0.24 mm/s at QABEJITA but <0.012 mm/s at ATI8 and <0.009 mm/s at QTRC4.

The uptake lengths for both glucose and arabinose were less at QABEJITA than at the other two streams, while uptake velocities were correspondingly greater. Uptake of glucose was not significant at QATI8. However, the glucose data from QATI8 yielded very poor replication and the lower confidence limit of S_w was less than that of QTRC4, which could represent poor sample preservation (some or all of the samples from QATI8 were held for as long as 14-d before being fully frozen) rather than a truly low glucose uptake velocity.

Table 7.2. Uptake Lengths (m) estimated from whole-stream additions. Lower and upper 95% confidence limits of uptake length estimate are given in parentheses. Where uptake was not significant (P>0.05), the uptake length is reported as > the lower 95% confidence limit.

	Stream		
Nutrient	QATI8	QABEJITA	QTRC4
Ammonium	92 (78,111)	66 (60,73)	55 (48,66)
Phosphate	>300 (300,∞)	11 (9,14)	>220 (220,∞)
Glucose	>34 (34,∞)	25 (20,32)	56 (47,70)
Arabinose	138 (112,181)	64 (47,103)	211 (165,294)

Table 8.3. Uptake velocity (mm/s) estimated from whole-stream additions, computed from uptake length (Table 7.2), velocity, and depth (Table 7.1). Where significant (P>0.05) uptake was not measured, the estimate is reported as < the upper 95% confidence limit

	Stream		
Nutrient	QATI8	QABEJITA	QTRC4
Ammonium	0.041	0.040	0.040
Phosphate	<0.012	0.240	<0.009
Glucose	<0.107	0.108	0.039
Arabinose	0.027	0.041	0.010

Discussion

The NH_4^+ uptake velocity of near 0.04 mm/s found in all three streams was lower than typical of undisturbed temperate streams. Among 155 published measurements of NH_4^+ uptake velocity (Ensign and Doyle 2006), nearly all from temperate climates and most from relatively undisturbed streams, the median was 0.09 mm/s and the uptake velocities from this study ranked just above the lowest quartile (26%). However, it is more appropriate to limit the comparison to 5NNH4 tracer additions because the more common method of non-labeled NH4 addition under-estimates uptake velocity (Mulholland et al. 2002). Among 29 published estimates of uptake velocity from isotopic additions (Webster et al. 2003, Ensign and Doyle 2006), the median uptake velocity was 0.17 mm/s, and only 1 measurement was lower than the estimates of 0.04 mm/s from this study.

There have been very few estimates of NH_4^+ uptake velocity published from tropical streams. Three measurements using 15N additions have been reported from a stream in Puerto Rico (Merriam et al. 2002), ranging from 0.15 to 0.18 mm/s, or approximate 4-fold higher than those of this study. Neill et al. (2006) measured NH_4^+ uptake velocity in four streams (two in forest and two in pasture) in the Amazon basin (Rondonia, Brazil). They reported one uptake velocity of 0.06 mm/s, somewhat higher than reported here, from a forest stream, but uptake velocities of 0-0.02 mm/s in the other two streams. Although they used the enrichment method so that their results are probably lower than would have been obtained from an isotope addition, their results are generally consistent with ours, with uptake velocities lower than most reported from temperate regions.

One reason for the relatively low NH_4^+uptake velocity of this study may simply be that streamwater NH_4^+concentrations were relatively high. High concentrations saturate the uptake of NH_4^+ which in turn reduces the uptake velocity (Davis and Minshall 1999, Dodds et al. 2002, Newbold et al. 2006). The NH_4^+ concentrations in the study streams (12-25 µg/L) were substantially higher than the median of 3.5 µg/L among for the 29 published 15N-based NH4 uptake velocities (Ensign and Doyle). Ammonium concentrations in the Brazilian streams

studied by Neill et al. (2006) ranged from 56 to 97 µg/L, even higher than those of this study, suggesting that their low uptake velocities, like ours, are the result of high NH_4^+concentrations.

Phosphate uptake, in contrast to NH_4^+ uptake, varied greatly among streams. In the two forest streams PO_4^{3-} uptake was too low to estimate, whereas in the pasture stream (QABEJITA) the PO_4^{3-} uptake velocity of 0.24 mm/s was among the highest reported worldwide. Ensign and Doyle (2006) tabulated 196 measurements of PO_4^{3-} uptake velocity (all from temperate or arctic regions), reporting a median value of 0.04 mm/s. The uptake velocity measured at QABEJITA ranked 7th from the highest, placing it in the 96th percentile of reported values.

Neill et al. (2006) reported PO_4^{3-} uptake velocities of 0.0005 and 0.018 mm/s in the two Brazilian forest streams that they studied, both substantially lower than most of the reported temperate values and consistent with the very low (<0.012 mm/s) uptake observed in QATI8 and QTRC4 (both in forest). Also consistent with this study, Neill et al. 2006 observed that PO_4^{3-} uptake in their two pasture streams (0.083 and 0.028 mm/s) was higher than in forest streams, although still much lower than the uptake observed at QABEJITA.

Water column PO_4^{3-} concentrations do not provide an adequate explanation for the low PO_4^{3-} uptake velocities in the forested reaches of this study. Background dissolved PO_4^{3-}-P concentration was 7 µg/L for both streams, only slightly above the median of 5 µg/L for the temperate and arctic studies assembled by Ensign and Doyle (2006). On the other hand, the very low background water column PO_4^{3-}-P of 1.4 µg/L in the pasture stream (QABEJITA) was probably related to the very high PO_4^{3-} uptake. Causality is difficult to infer, however, because the high uptake may draw down the available phosphorus while, simultaneously, the rate of uptake increases because available phosphorus is in short supply. The relationship of PO_4^{3-} uptake to PO_4^{3-} concentration observed in Brazilian pasture streams studied by Neill et al. 2006 stands in sharp contrast to results presented here. Whereas the concentration of PO_4^{3-} was found to be unusually low in the pasture stream, Neill et al. (2006) found concentrations of PO_4^{3-}-P (25-56 µg/L) that were much higher than in typical undisturbed temperate streams, and higher than in the forest streams of the same study. Nonetheless, in both studies, PO_4^{3-} uptake was higher in pasture streams than in forest streams.

Uptake of carbohydrate (glucose and arabinose) was higher in the pasture stream than in the forest streams (although no estimate could be made for QATI8, one of the forest streams). Few measurements of carbohydrate uptake are available for comparison, but the uptake of glucose in QTRC4 (forest) of 0.039 mm/s was lower than the mean (0.055 mm/s) of 27 measurements reported by Newbold et al. 2006 from ten streams in varying land uses in southeastern New York, USA. Similarly, the uptake of arabinose QTRC4 of 0.027 mm/s was near the mean of 0.021 mm/s from the same study. The uptake of arabinose from QATI8 (also in forest), however, was near the lowest value (0.009 mm/s) reported from the New York streams. By contrast, both the glucose and arabinose uptake velocities from Q Abejita (pasture) were higher than all but one of 28 measurements of each respective carbohydrate. Thus, the uptake of carbohydrates roughly followed the pattern of PO_4^{3-} uptake—moderately low in the forest streams, but unusually high in the pasture stream.

The more rapid cycling of PO_4^{3-} and carbohydrates in QABEJITA--the pasture stream—may be driven by the higher rates of ecosystem metabolism, particularly ecosystem respiration, found in this stream (see Appendix 8—Metabolism). Previous studies have shown a link between nutrient uptake velocities and ecosystem metabolism (e.g., Hall and Tank 2003, Webster et al. 2003, Newbold et al. 2006). It remains unclear, however, why the uptake velocity of NH_4^+, in contrast to the other nutrients, showed no differences among streams. One possibility is that the influences of NH_4^+ concentration and metabolism on uptake velocity acted in opposite and therefore compensating directions. Among the three streams, QABEJITA had the highest concentration of NH_4^+ and the highest rates of ecosystem respiration and gross primary production, whereas QTRC4 had the lowest values for all three parameters. The depressing effect of high nutrient concentration on uptake velocity may have balanced the enhancing effect of high metabolism, and vice versa.

The higher nutrient uptake and metabolism of the pasture stream (QABEJITA), as well as differences in the nutrient status between the pasture and forest streams may all be related to conditions farther upstream of the reach studied in the pasture stream. These conjectures are based on a comparison of the Brazilian pasture streams studied by Neill et al. 2006 with QABEJITA. Unlike QABEJITA, which flowed through a riparian forest, the Brazilian pasture streams had no riparian forest but instead were bordered by wetland grass which contributed large quantities of organic matter that reduced dissolved oxygen concentrations to anoxic and near anoxic conditions. The anoxia, in turn, produced elevated concentrations of Fe+2 and PO4-3 in the stream water. In QABEJITA, upstream from the study reach, the canopy was open and the stream and/or its banks supported growths of macrophytes and/or grasses much as in the Brazilian streams. The suggestion is that conditions within the riparian-forested study reach at QABEJITA were influenced by upstream conditions that were similar to those of the Brazilian streams. Under this scenario, the elevated metabolism in this study reach may have been supported by the decomposition of organic matter transported downstream from the more open reach. However, the ecosystem respiration in the study reach was not so high as to maintain anoxia (see Appendix 8). Thus the dissolved Fe+2 and PO4-3 that may have been mobilized in the upstream open reach may have co-precipitated under the higher oxygen regime of the study reach. This co-precipitation could account for the extremely low PO4-3 concentration observed in the study reach and, possibly, for the floc observed on the streambed. Also, as suggested by Neill et al. (2006) the oxidation of the Fe+2 might account for some of the very high apparent uptake of PO4-3 from the water column and, as well, for some of the oxygen consumption that is tentatively ascribed to respiration. Of particular significance is the possibility that downstream co-precipitation of PO4-3 may remove more of the PO4-3 than is originally mobilized by upstream anoxia. If this scenario is correct, and emphasis is placed on the highly speculative nature of this scenario, it would suggest that open pastures may have major consequences to downstream availability of phosphorus.

This study supports evidence from temperate regions that spiraling—specifically the measurement of nutrient uptake—is a sensitive indicator of anthropogenic impacts on stream ecosystems. However, the form of the response—an increase in nutrient uptake or a shortening of the spiraling length—differs from the previously observed increases in spiraling length, possibly because interactions with abiotic processes are involved.

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Appendix 8. Ecosystem Metabolism

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Executive Summary

- Algal biomass and ecosystem metabolism were studied in two lowland streams (QATI8 and QABEJITA) with substrata primarily sand and silt, and one upland stream with higher gradient and substrata primarily pebble and cobble.
- Algal biomass (measured by chlorophyll concentration) in the lowland streams was very low, but the level in QTRC4 was similar to that in shaded temperate-region streams.
- Algal growth (P = primary productivity) in all streams was relatively low due to heavy shade and relatively low nutrient concentrations. Algal growth in QABEJITA was greater than in QATI8, perhaps because of agricultural land use in the QABEJITA watershed.
- Ecosystem respiration (R) was extremely high in QABEJITA, but rates in QATI8 and QTRC4 were in the range of temperate-region streams.
- The ratio of P/R in QATI8 is the lowest compared to reported values, but the ratios in the other two streams, while low, have been reported before.
- These streams are sustained by energy inputs from the watershed and an important component of this is leaf litter. Riparian zones should be maintained in native vegetation.

Introduction

Ecosystem metabolism measurements provide estimates of rates of primary productivity and ecosystem respiration. Primary productivity is the rate of addition of energy (as plant biomass) to an ecosystem by photosynthetic organisms. Ecosystem respiration is the loss of energy resulting from the oxidation of organic matter by organisms in the stream. The amounts of production and respiration and the balance between these processes provide clues as to how a stream ecosystem functions. For example, if respiration exceeds primary productivity, energy will have to be added to the ecosystem in the form of detritus from the watershed for the system to maintain itself. If the reverse occurs the ecosystem is generating sufficient energy (some of which may be stored, at least temporarily) to maintain itself. There are few measures of these functions in tropical streams. Functional attributes such as primary productivity and respiration are being evaluated as integral components of monitoring studies when attempting to assess ecosystem condition (Bunn 1995, Rapport et al. 1998, Young et al. 2004) and recent work in temperate streams highlights the value of their inclusion (Bunn et al. 1999, Young and Huryn 1999, Bunn and Davies 2000, Sweeney et al. 2004, Meyer et al. 2005, Bott et al. 2006).

Metabolism was compared in three streams, two of which were low-gradient lowland streams, one in good condition draining a forested watershed (QATI8) and the other impacted by agriculture in the watershed (QABEJITA). Streambed substrata were dominated by sand and silt in those streams. The third stream, QTRC4, differed from the lowland streams by having greater slope and substrata dominated by cobble and pebbles. It was included to allow comparison with studies of temperate zone streams with similar geomorphology.

Methods

Metabolism measurements

Each study reach was delimited by an upstream injection substation, an upstream sonde (dissolved O_2/temperature data logger) substation, and a downstream sonde substation. Conditions affecting reaeration were similar above the upstream and downstream sonde locations. Discharge, stream width and depth, water velocity and other site characteristics are reported in Table 8.1.

Ecosystem metabolism was determined from measures of dissolved O_2 change. Five sondes (YSI model 600XLM, Yellow Springs, Inc., Yellow Springs, OH), held in water-saturated towels, were calibrated according to the manufacturer's instructions. Sondes were then placed at a single location in the thalweg of the stream for a ~3h comparison before deployment for experimental measurements. Differences between sondes were compensated for when analyzing data. Sondes were deployed in duplicate to the upstream and downstream substations. Pairs of sondes were chosen on the basis of similarities of dissolved O_2 readings near the end of the comparison period and probe characteristics (e.g., sensor charge and voltage). The 5th sonde was retained for Quality Assurance/Quality Control (QA/QC). Dissolved O_2 concentrations and water temperature were measured and logged at 15-min intervals for a 2.5-d period. Daily QA/QC checks were made by comparing instantaneous readings of dissolved O_2, % saturation, temperature, specific conductance, and sensor charge of the deployed sondes to the readings on the QA/QC sonde using a YSI 650MDS meter.

Table 8.1. Physical and chemical characteristics of study reaches pertinent to metabolism measurments.

		Stream		
Parameter	n	QATI8	QABEJITA	QTRC4
Length (m)		90.2	90.3	80.5
Width (m)	20	2.3	1.4	4.4
Depth (m)		0.14	0.11	0.06
Velocity (m/s)		0.026	0.024	0.035
Discharge (m^3/s)		0.00837	0.00386	0.00924
% Propane lost during transit		27.66	69.26	46.6
Reaeration Coefficient (k) (d^{-1})		9.7	33.3	25.8
% Tree Canopy		93.2	87.7	91.5
mean Total Daily PAR (mol quanta$\cdot m^{-2}\cdot d^{-1}$)	2	0.44	1.09	2.12
mean Daily Temperature (°C)	2	22.89	21.49	24.04
mean Daily median dissolved O_2 saturation (%)	2	52.6	50.4	84.8
NH_4-N (mg/L)		0.013	0.026	0.005
NO_3-N (mg/L)		0.053	0.021	0.216
oPO_4-P (mg/L)		0.0066	0.0014	0.0068
Conductivity (µS.cm-1)		11.6	26.3	8
Total alkalinity (mg/L as $CaCO_3$)		78	243	54
DOC (mg/L)		7.94	2.66	0.82
BDOC (mg/L)		0.84	0.42	0.20
% BDOC		10.6	15.8	23.8

Photosynthetically active radiation (PAR) was measured by securing 2 LI 190SA Quantum sensors (LI-COR, Lincoln, NB) to stakes at the upstream and downstream substations. PAR was measured every 15 s, and 15-min integrals were logged on LI-COR 1400 data loggers. The tree canopy was photographed at 5 locations equally spaced along each study reach using a digital camera (Fujifilm S5100) equipped with a fisheye lens (Opteka 0.22x). The camera was positioned 0.67 m above the water surface at the center of the stream. Each photograph captured the canopy for a distance of ~25 m.

Reaeration coefficients were determined from measurement of propane evasion (after Marzolf et al. 1994, 1998, Young and Huryn 1998). Propane was bubbled into the stream at the injection site through a 1.5-m-long gas-diffuser tube (Aquatic Eco-Systems, Apopka, FL), and a Br^- conservative-tracer solution was injected simultaneously using a peristaltic pump. The injection site was far enough upstream to ensure mixing of sources and lateral dispersion at the uppermost sampling station. Samples were collected at 5 substations over the length of the study reach. Br^- was monitored over the entire injection at the 1st and 5th substations, and 5 propane and 5 Br^- samples were taken when concentrations were at a plateau. Propane and Br^-samples were collected at the remaining substations only during the plateau. Sampling times were set on the day before the experiment by timing the transit of a pulse of bromide (measured using conductivity) through the reach. Field blanks were collected at each substation before the start of the injection. Conservative-tracer samples were collected in 30-mL plastic bottles. Each propane sample was collected by filling 12-mL exetainer tube, exchanging 3 volumes of water through the tube using flexible tubing and a 60-mL syringe, collecting the last one, and closing the tube under water. Samples were refrigerated during storage.

In addition to the open-system metabolism measures which included both benthic and water-column activity, measurement of water-column metabolism was made separately. Ten BOD bottles (6 light and 4 dark) were filled with stream water. Initial dissolved O_2 concentration, temperature, and % saturation were measured using a YSI Model 58 dissolved O_2 meter and probe with stirrer for use with BOD bottles. The bottles were incubated in the stream for 4 to 6 h during which PAR was monitored. Following incubation, dissolved O_2 concentration, temperature, and % saturation were re-measured.

Substratum and biomass assessments

Transects (9 – 10 per reach) were set between upstream and downstream sondes, and up to 10 equidistant lateral points were designated along each transect. At each point, stream depth was measured, and predominant types of substrata and attached biomass (cover type) were assessed using a viewing bucket. Substratum categories followed those of Hynes (1970). Cover types were categorized by macroscopic appearance as: green algae, black cover (slime scraped from rocks that appeared black), floc (amorphous grayish flocculent material), leaf litter, bare, and silt.

Replicate (3–5) samples for periphyton chlorophyll *a* and organic matter analyses were collected for cover types that made up ≥10% of the encounters in the mapping effort. Soft substrata were sampled by inserting a plastic tube (11.25 cm id) into the streambed and suctioning the enclosed surface sediments with a meat baster. Samples of periphyton on rocks were scraped, brushed, and washed into a jar and leaves were treated similarly but less vigorously. The planar surface area of the upper rock surface or leaf was traced onto a piece of paper for area quantification

using image analysis techniques (see below). Samples were held on ice until return from the field. That evening, samples were centrifuged (3500 x *g*, 45 min.) and recovered pellets were frozen. If supernatant fluids appeared turbid, the fines were collected on GF/F filters that were then frozen. Samples were analyzed for chlorophyll *a* within 3 to 4 wk.

Laboratory analyses

Br^- was analyzed by ion chromatography (Dionex Model ISC-3000, Dionex, Sunnyvale, CA; see the Analytical Methods section in Appendix 7).

In preparation for propane analyses, 2 syringe needles were inserted through the septum of the exetainer tube, and 1.5 mL of water were displaced by injecting air into the tube to produce a head space. Tubes were shaken horizontally for 3 h at room temperature to equilibrate propane between the water and head space. Propane content was determined on 50-µL samples of head-space gas using capillary gas chromatography as detailed in Bott et al. (2006). Propane peaks at the farthest downstream substation ranged between 30 % and ~72% of the 1st substation values. The reaeration coefficient was computed using proportional loss over distance; absolute propane concentrations were not necessary.

Chlorophyll-containing pellets were thawed in the laboratory, and chlorophyll was extracted overnight in 90% acetone (made basic with 0.4 ml NH_4OH added to 4 L reagent) at –20°C. Following centrifugation (15 min, 10,000 x *g*, 4°C), absorbances of the supernatant fluids were determined spectrophotometrically at 665 nm and 750 nm (for turbidity) before and after acidification with 2 drops of 1 N HCl. Extractions were repeated on samples until chlorophyll *a* absorbance was either 10% of the value obtained from the 1st extraction or <0.1 absorbance units at 665 nm. Samples were iced and handled under low light. Concentrations were determined using the equations of Lorenzen (1967), which include correction for pheophytin. Following extraction, the pellets were dried at 60°C, weighed, ashed (500°C for 6 h), cooled, and reweighed for an analysis of organic matter content as ash-free dry mass, AFDM).

Rock and leaf outlines were digitized and planar surface area was determined using public domain Image J 1.37 software (US National Institutes of Health; http://rsbweb.nih.gov). Tree canopy photos were processed using Image-Pro Plus 5.0 software. Color photos were segmented to black and white images of sky and tree canopy. The proportion of total area accounted for by the canopy was determined using the Image J 1.37 software. The canopy values from the 5 photos were averaged to generate a mean % canopy cover for each stream.

Data analyses

Chlorophyll *a* concentrations were obtained for two cover types, floc and leaf litter, amounting to 70% and 82% of the total at QATI8 and QABEJITA, respectively. At QTRC4 concentrations were obtained for 93% of the cover types. Periphyton chlorophyll *a* concentrations were matched with the estimated percentage of total reach area consisting of that cover type to generate a weighted periphyton chlorophyll *a* concentration/m^2 (standing stock) for the reach. A total chlorophyll *a* standing stock at QTRC4 was generated by adding moss chlorophyll to periphyton chlorophyll. Organic matter data were handled similarly.

The loss of propane with downstream distance was determined by nonlinear regression of the

[propane/Br^-] ratio against downstream distance (SAS/STAT, version 9; SAS Institute, Cary, NC) using an exponential model (Wanninkhof et al. 1990). The dilution-corrected proportion of propane lost/m was multiplied by water velocity, 1.39 (to correct for molecular size, Rathbun et al. 1978), and 60 (s/min) to generate K_{O2} (1/min). Both water velocity through the reach and mean depth of the reach were derived from a computer model of Br^- concentrations using OTIS-P as described in Appendix 7.

O_2 data were analyzed using the 2-station (upstream–downstream) approach (Bott 2006, after Owens 1974). Reaeration coefficients were corrected to ambient temperatures based on Elmore and West (1961). The hourly rate of change of dissolved O_2 concentration (Odum 1956) corrected for reaeration was computed at each 15-min interval over a 24-h diel period. The average hourly rate of ecosystem respiration during darkness (PAR < 2 µmol quanta m^{-2} s^{-1}) was extrapolated to 24 h (ER_{24}). Gross primary productivity (GPP) was computed by adding photoperiod respiration to net O_2 change during the photoperiod. Net daily metabolism (NDM) was computed as the difference between GPP and ER_{24} (NDM = GPP – ER_{24}).

Mean O_2 change in dark bottles was added to the net O_2 change in each light bottle to yield an estimate of water-column GPP, the average of which was compared to whole-system metabolism for the corresponding time period.

A photosynthesis–irradiation (PI) curve was prepared for each day by regressing change in dissolved O_2 (PS) against average PAR (instantaneous light intensity, I) every 15 min during the period of increasing PAR intensity from sunrise to mid-day. A hyperbolic tangent function was fit to the data (Jassby and Platt 1976)

$$PS = \beta' + PS_{max} \times \tanh\left[\frac{\alpha \cdot PAR}{PS_{max}}\right]$$

where α is the initial slope of the regression, β' is analogous to community respiration (used to position the curve correctly in each analysis), and PS_{max} is the maximum rate of photosynthesis. A saturation intensity (I_S) was obtained from the relationship [PS_{max}/α]. GPP was normalized for total daily PAR to allow a ranking according to GPP/PAR.

Statistical analyses were done using $\log_{10}(x)$-transformed data with a constant added before transformation when needed. Differences between sites were determined from analyses of variance (ANOVA) followed by the Scheffe multiple range test (MRT) when ANOVAs were significant ($p \leq 0.05$). The Scheffe test was used because it is conservative and these data had minimal replication.

Results

Pertinent Site Characteristics

Reach lengths were almost identical in the two lowland streams at ~90 m, and 80 m at QTRC4 (Table 8.1). QABEJITA was the narrowest stream, but QATI8 was slightly deeper. QTRC4 was wide and shallow. Discharge at QABEJITA was about half that of the other two streams. Water velocity in the low-gradient lowland streams was approximately two-thirds of that in TRC4. The reaeration coefficient was lowest in QATI8 (~10 d^{-1}) and 2.5 – 3.5 times that in the other streams. All streams were densely shaded with canopy densities between 88 and 93%. PAR

levels were very low (≤1 mol quanta photons·m^{-2}·d^{-1}) in the lowland streams, and only twice that at QTRC4. Mean daily water temperature was greatest in QTRC4 (24 °C), but that value was only 1 – 2.5 °C greater than in the other streams. Mean O_2 saturation values were only between 50 and 53% in the lowland streams, but 85% in QTRC4. NH_4, conductivity and total alkalinity were higher in QABEJITA, but NO_3 and PO_4 concentrations were lowest there. DOC and BDOC concentrations were greatest in QATI8.

Benthic substrata and cover types

The predominant benthic substrata in QATI8 and QABEJITA were sand and clay (Fig. 8.1). The only hard substrata in those lowland streams were roots and wood, which amounted to <10% at either site. In contrast, the more heterogenous streambed at QTRC4 contained predominately pebble (38.6%), cobble (36.4%), and sand (10.3%) with lesser amounts of silt and clay (7.6%), boulder (6.5%) and roots (0.5%).

Lowland streams (QATI8, QABEJITA) were dominated by soft substrata whereas substrata in QTRC4 were more heterogeneous and dominated by cobbles and pebbles.

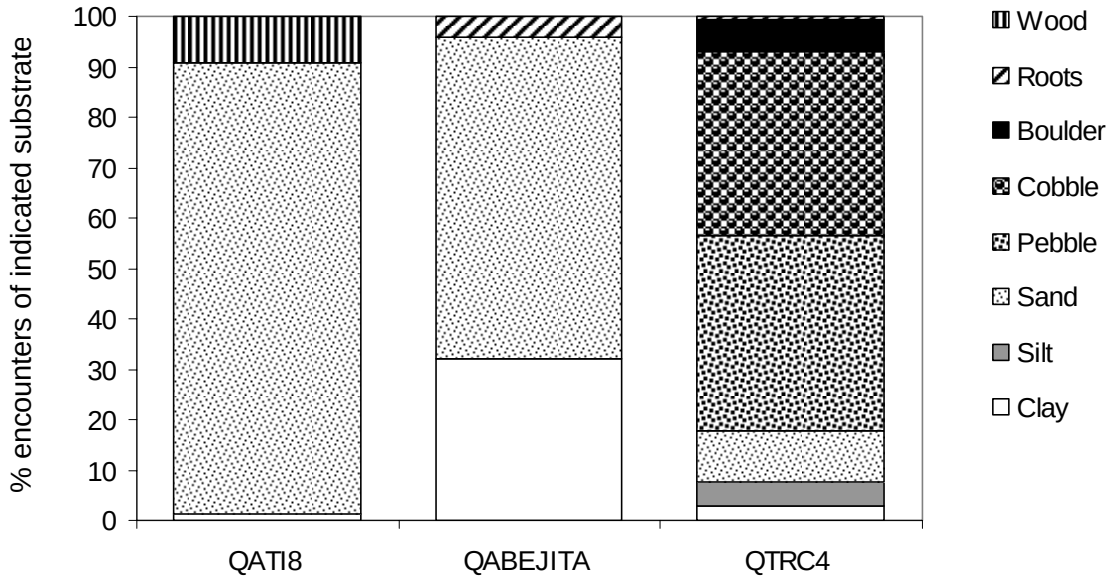

Fig. 8.1. Benthic substrata in each study stream.

Leaf litter accounted for from 36.8 to 40.7% of the cover types encountered in all of these streams. Another cover type frequently encountered in the lowland streams was a grayish-white amorphous flocculent material (termed “floc”), presumably organic in nature. While algal (1.4% green cover and 4.2 % black cover) and moss (11.8%) cover types were measurable in QTRC4, those covers were negligible in the two lowland streams. Rocks appearing bare (coated with a thin colorless biofilm) accounted for 28.5% of cover types in QTRC4.

Chlorophyll a and organic matter

Periphyton chlorophyll concentration was lower in QABEJITA (0.51 mg/m^2) than QATI8 (0.92 mg/m^2), and both of these lowland streams had lower periphyton chlorophyll than QTRC4 (4.49

Chlorophyll concentrations in QATI8 and QABEJITA were extraordinarily low. The concentration in QTRC4 has been encountered in other studies of temperate streams.

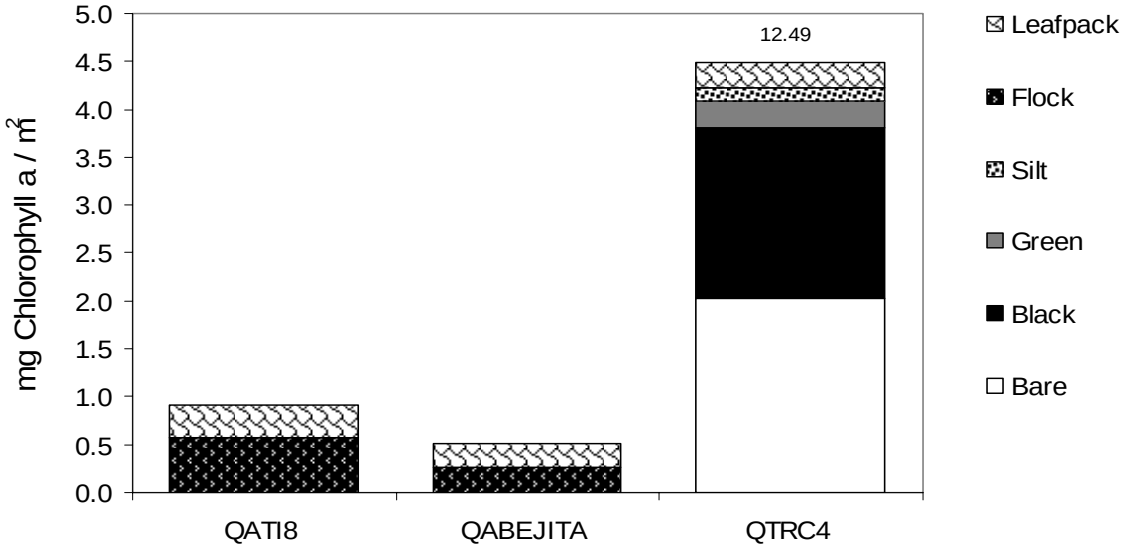

Fig. 8.2. Concentrations of periphyton chlorophyll *a* in each study stream. The number over the histogram for QTRC4 is the total chlorophyll including that present in mosses.

Particulate organic matter concentrations associated with periphyton were very high in QATI8 and QABEJITA while the concentration in QTRC4 has been encountered in other studies.

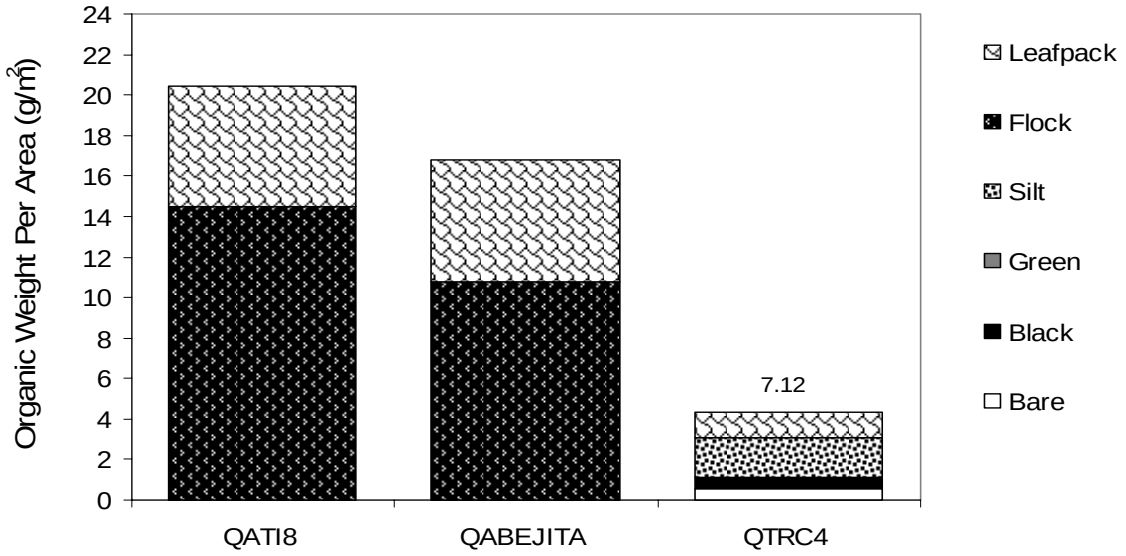

Fig. 8.3. Concentrations of periphyton organic mass in each study stream. The number over the histogram for QTRC4 is the total organic mass including that found in mosses.

mg/m^2; Fig. 8.2). When moss chlorophyll was included in the estimate for QTRC4, the total chlorophyll standing stock increased to 12.49 mg/m^2. Microscopic examination of cover types revealed a notable absence of algae in samples from the lowland streams.

In contrast to chlorophyll concentrations, there was more periphyton-associated organic matter in the two lowland streams (16.8 and 20.4 g/m^2 in QABEJITA and QATI8, respectively) than in QTRC4 (4.36 g/m^2 for periphyton and 7.12 g/m^2 including moss) (Fig. 8.3).

Algal growth (GPP) was relatively low in all streams but the value for QATI8 was the lowest measured relative to previous studies. Ecosystem respiration (ER) in QABEJITA was the highest measured relative to studies of similarly sized shaded streams. Net Daily Metabolism (NDM) reflects the high respiration rates. The P/R ratio (GPP/ER_{24}) in QATI8 and QABEJITA was lower than in similarly sized temperate streams, while QTRC4 had a ratio similar to some temperate streams. The ratio for QATI8 is the lowest measured to date.

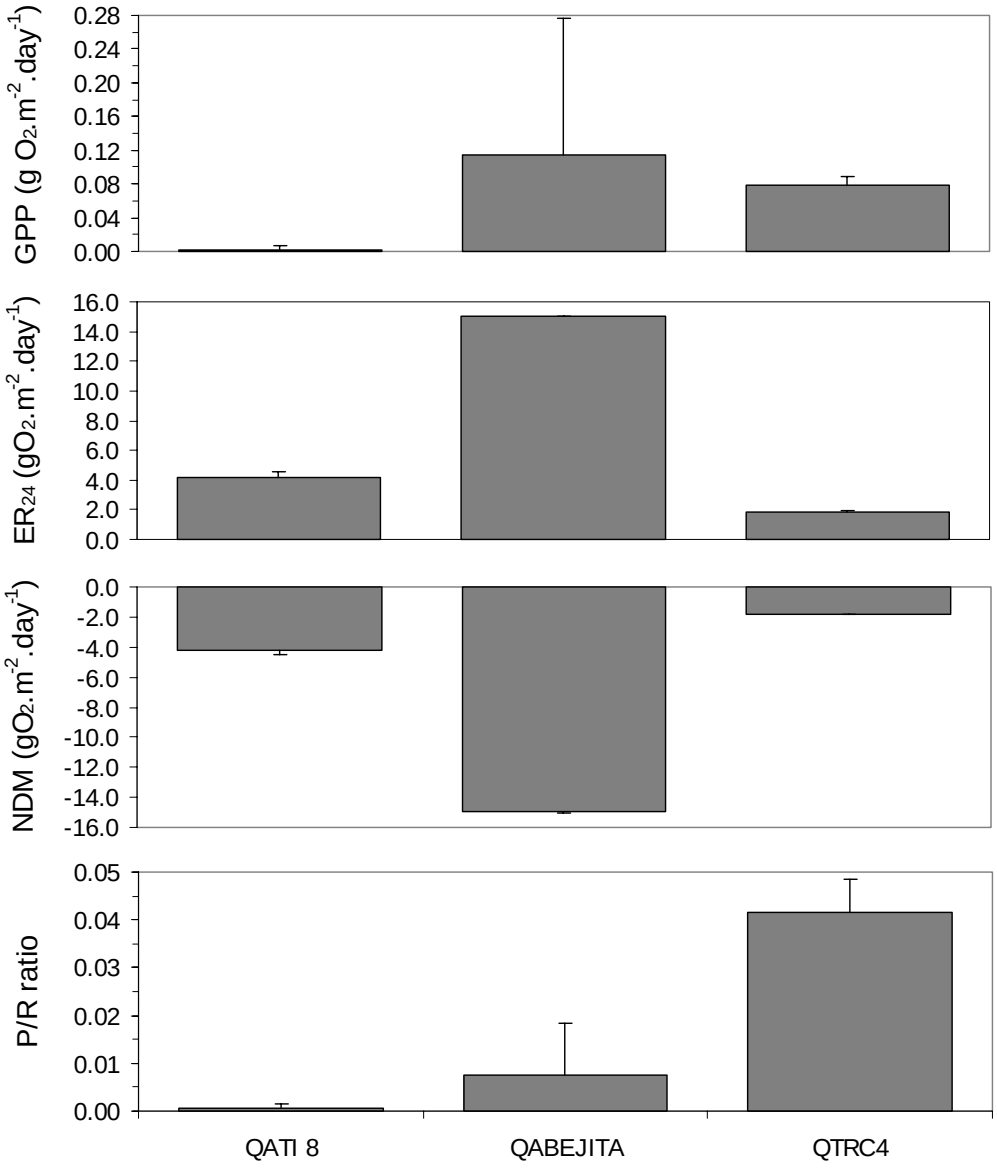

Fig. 8.4. Ecosystem metabolism in each study stream. GPP = Gross primary Productivity, ER_{24} = Ecosystem Respiration over 24 h, NDM = net daily metabolism, and P/R ratio = GPP/ER_{24}.

Metabolism

Gross primary productivity (GPP), defined as the total amount of energy fixed photosynthetically, averaged 0.003, 0.115 and 0.078 g $O_2 \cdot m^{-2} \cdot d^{-1}$ in QATI8, QABEJITA, and QTRC4 respectively (Fig. 8.4). On one day in both QATI8 and QABEJITA the estimate of GPP was negative and a zero was substituted. This anomaly arises because (1) ecosystem respiration is measured and used in the GPP computation (not primary producer respiration), and (2) changes in O_2 concentration were relatively small (0.5 – 1.5 mg/L over 24 h) relative to the accuracy of the probes (± 0.1 mg/L). Owing to the large standard deviation for QABEJITA, differences between streams were not statistically significant (ANOVA, $p>0.05$). It is accurate to conclude however, that GPP in these streams is low.

Saturation curves were obtained in PI analyses only for one day in QATI8 and QABEJITA, but for both days in QTRC4. Estimates of maximum net O_2 production (PS_{max}) either were very close to zero or negative because of the high respiration rates. Saturation light intensity (I_S) was only 7 µmol quanta $\cdot m^{-2} \cdot s^{-1}$ in QABEJITA and < 30 in the other streams, in contrast to intensities between 100 and 400 µmol quanta $\cdot m^{-2} \cdot s^{-1}$ are often found in the literature (Table 8.2). The estimate of α was 5-fold greater in QABEJITA, suggesting greater shade adaptation and/or nutrient supply, or both, there. When GPP was normalized for total daily PAR (GPP/PAR, data expressed as g $O_2 \cdot$mol quanta photons$^{-1} \cdot d^{-1}$) streams ranked as follows: QABEJITA (0.103) > QTRC4 (0.037) > QATI8 (0.011), but differences were not statistically significant (ANOVA, $p > 0.05$).

Table 8.2. Data from photosynthesis irradiation curve analyses. PS_{max} = maximum rate of photosynthesis in the absence of photoinhibition (µg $O_2 \cdot m^{-2} \cdot s^{-1}$), α = regression slope (g O_2/mol quanta photons, β' = analog for ecosystem respiration (µg $O_2 \cdot m^{-2} \cdot s^{-1}$), I_S = saturation intensity (µmol quanta photons $\cdot m^{-2} \cdot s^{-1}$).

Stream	Date	PS_{max}	α	β'	I_S
QATI 8	17-Aug-06	.	.	.	.
	18-Aug-06	4.22	0.00014	-48.33	27
QABEJITA	21-Aug-06	6.50	0.00082	-167.11	7
	22-Aug-06	.	.	.	.
QTRC4	26-Aug-06	3.74	0.00020	-20.67	17
	27-Aug-06	-3.29	0.00012	-21.00	25

Ecosystem respiration (ER_{24}) averaged 4.21, 15.03 and 1.88 g $O_2 \cdot m^{-2} \cdot d^{-1}$ in QATI8, QABEJITA and QTRC4, respectively (Fig. 8.4). Differences between the three streams were statistically significant (ANOVA, $p < 0.001$ and Scheffe MRT: $p \leq 0.001$). Median dissolved O_2 saturation values were 53% and 50% in QATI8 and QABEJITA respectively, but 85% in QTRC4, and the difference between each lowland stream and QTRC4 was statistically significant (ANOVA, $p < 0.05$, Scheffe MRT, $p < 0.05$). Warm water temperature and high respiration rates both contribute to these low saturation values but only the correlation with respiration was statistically significant ($r = -0.815$, $p < 0.05$).

Because respiration predominated metabolism in these streams, water-column metabolism was evaluated using it as a percentage of total system respiration during the same time period. Water column respiration was only a minor percentage of total ecosystem respiration, amounting to 0.1% in QABEJITA, 8.5% in QATI8 and 3.9% in QTRC4. Thus, system metabolic activity could be attributed primarily to the benthic community.

Net daily metabolism (NDM = GPP - ER_{24}) in QATI8, QABEJITA and QTRC4 equaled -4.20, -14.92, and -1.80 g $O_2 \cdot m^{-2} \cdot d^{-1}$, respectively (Fig. 8.4). These values reflect the excess of respiration and indicate a net consumption of energy in these streams at the times measurements were made. NDM values were ~ 2 to 7 times more negative in the lowland streams than QTRC4, and the differences between streams were statistically significant (ANOVA, $p < 0.001$) with QABEJITA differing from both QATI8 and QTRC4 (Scheffe MRT, $p < 0.001$). The relative amounts of production and respiration expressed as P/R ratios (GPP/ER_{24}) were 0.0006, 0.008, and 0.042 for QATI8, QABEJITA, and QTRC4, respectively (Fig. 8.4). The data for the lowland streams are some of the lowest P/R ratios observed. These data did not differ statistically, but were very close to being significant (ANOVA, $p = 0.055$, Scheffe MRT (QATI8 vs. QTRC4), $p = 0.06$).

Discussion

To our knowledge, these are the only data concerning ecosystem functions in streams of this region. In fact, there are few data concerning ecosystem metabolism for streams in the tropics where most interest has focused on large rivers. While these data are preliminary, some differences and similarities among the three study streams, as well as between the three study streams and streams studied elsewhere, emerge from this study.

The contrast between the two lowland streams was striking. The greater GPP in QABEJITA than in QATI8 is conceivably due to the difference in watershed land use. While the riparian area was forested along both study reaches, watershed land use was predominantly large animal agriculture at QABEJITA and cattle had access to the stream in at least one location. In contrast, the watershed was forested at QATI8. Although NO_3-N and o-PO_4-P concentrations were approximately 50% lower at QABEJITA, total alkalinity and NH_4-N were ~ 3-fold and 2-fold greater, respectively, in QABEJITA. Ammonium is a preferred nitrogen source for algae which may have contributed to the higher productivity at QABEJITA. In addition to watershed sources of NH_4, the high respiration in QABEJITA may generate anaerobic microenvironments in an otherwise aerobic environment, allowing nitrate respiration to NH_4 to occur.

Respiration rates were 2 – 7 times higher in the lowland streams than in QTRC4. It is possible that the floc cover type was an important site of respiration. This floc was found in both lowland

streams, but not in QTRC4. Separate measures of respiration associated with that material are warranted in future work. Daily median % O_2 saturation values in the lowland streams (~50%) were lower than any encountered in temperate zone streams where 85% saturation (the median saturation value for QTRC4) would be considered low. Hyporheic respiration made a significant contribution to total system respiration in some streams (Grimm and Fisher 1984, Mulholland et al. 1997). However, the transient storage zones in both lowland streams were relatively small, as was exchange with the hyporheic, making this an unlikely explanation for the high respiration in QABEJITA. The lower concentrations of o-PO_4 and NO_3 in QABEJITA than in QATI8 also are consistent with the high respiration rate if they reflect greater metabolic activity of heterotrophic microbes and simultaneous demand for N and P.

Most ecosystem metabolism occurred on or in the streambed with little activity attributable to the water column. In this respect these streams behaved like most other streams previously studied, from small to large in size. Mosses were encountered only in QTRC4, presumably because the soft substrata predominating in the other streams were not conducive to development there.

These data can be compared to values for one other tropical headwater stream, Qbda. Bisley, a heavily shaded tributary of the Rio Nameyes, Puerto Rico (Ortiz-Zayas, et al 2005). GPP data for Qbda. Bisley averaged 0.19 g $O_2 \cdot m^{-2} \cdot d^{-1}$, with little annual variation. GPP data for all of the streams studied here are lower than this. The ER_{24} rates in the two lowland streams were 2- and 6-fold greater than in Qbda. Bisley (2.44 g $O_2 \cdot m^{-2} \cdot d^{-1}$), although respiration in QTRC4 was close (77%) of the value for Qbda. Bisley.

Comparisons can also be made between these data and measures made using the same techniques in forested reaches of three temperate zone streams in southeastern Pennsylvania (PA) with width, depth and light input similar to those for the Peruvian streams. Restricting data for comparison to measures made during warm weather in PA gives mean values for GPP, ER_{24} and NDM of 0.11, 3.48 and –3.37 g $O_2 \cdot m^{-2} \cdot d^{-1}$, respectively, and an average P/R ratio of 0.062 (Bott et al. 2006). GPP in QTRC4 was reasonably similar (71%) to the mean for the PA streams and QABEJITA equaled that value, but the data for QATI8 was much lower (3% of that mean). Respiration rates in the lowland streams were 1.2 – 4.3-fold higher than in the PA streams but the respiration rate in QTRC4 was only 54 % of the mean for the PA streams. While the P/R ratio for QTRC4 was slightly higher than the value for the PA streams with similar light and discharge, the ratios for QABEJITA and QATI8 were extraordinarily low. The P/R ratio for QATI8 is the lowest measured relative to results from similar work. These metabolic data reflect a dependence on the s watershed for energy inputs. Leaf litter accounted for from 36.8 to 40.7% of the cover type encountered in each of these streams. In addition, the DOC concentration was also extremely high at QATI8 and moderately high at QABEJITA. Biodegradable DOC concentrations also were 2 to 4 fold greater in the lowland streams than in QTRC4 (Appendix 3). Periphyton chlorophyll concentrations in these streams were lower than those in the PA streams, where the average was 7 mg/m^2. This low biomass does not appear to be the result of grazing because microscopic examination indicated few algal cells associated with macroinvertebrate gut contents (D. Funk, personal communication).

While low light levels resulting from dense tree canopy cover certainly contribute to the low algal densities and low GPP measured here, comparisons can be made betwen temperate and tropical streams with similar light levels. At present, lower algal biomass and GPP in these tropical streams are attributed to lower nutrient concentrations. While NH_4-N concentrations

were similar in both the PA and Peruvian streams, concentrations of NO_3-N, PO_4-P, DOC, and BDOC were 8-83, 2-4, 10-20, and 0.5 – 4 fold lower, respectively in the Peruvian streams and conductivity was 10-20 fold lower.

In summary, these data, while preliminary in nature, indicate that (1) algal densities and GPP were low in these streams at least at this time of year, (2) all of these streams were strongly heterotrophic and receive considerable energy subsidies from their surrounding watersheds, (3) agriculture has a quantifiable impact on lowland stream metabolism resulting in increased GPP and a higher P/R ratio, even though NDM is still highly negative.

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