Characterization of pond effluents and biological and physicochemical assessment of receiving waters in Ghana

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ABSTRACT

This study was carried out to characterize ponds and aquaculture systems, and also to determine both the potential and actual impacts of pond aquaculture effluents on receiving stream quality in the Ashanti and Brong Ahafo regions of Ghana. Water, fish and macroinvertebrate samples were collected from upstream, downstream and nearby reference streams of, and questionnaires administered to, 32 farms. Total settleable solids were higher in ponds than reference streams ($p = 0.0166$); suspended solids was higher in ponds than reference streams ($p = 0.0159$) and upstream ($p = 0.0361$); and total phosphorus was higher in ponds than reference ($p = 0.0274$) and upstream ($p = 0.0269$). Total nitrogen was most clearly higher in ponds than all other locations: $p = 0.0016$, 0.0086 and 0.0154 for the differences between ponds and reference, upstream, and downstream respectively. BOD_5 level was also higher in ponds than all locations ($p = 0.0048$, 0.0009, and 0.0012 respectively). Also, non-guarding fish species were more abundant in reference streams than downstream ($p = 0.0214$) and upstream ($p = 0.0251$), and sand-detritus spawning fish were less predominant in reference streams than upstream ($p = 0.0222$) and marginally less in downstream locations ($p = 0.0539$). A possible subsidy-stress response within study streams was also observed. Hence, ponds are potential sources of these water quality variables to receiving streams. Effluent-receiving streams, generally, were not much different from reference streams in terms of most the metrics of community structure and function used in the comparisons. Hence, even though receiving streams in Central Ghana may not be severely impacted by aquaculture effluents at the moment,

the management of pond effluents will determine the scale of future impact. Vegetable, cereal, and livestock farming could serve as additional sources of fecal streptococci and coliform bacteria and nutrient-enrichment within the study area, besides aquaculture, and so these industries must also be included in efforts to minimize pollution of these streams.

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General introduction

The decline in global capture fisheries stocks has given the impetus to the aquaculture industry, which has seen a prolific growth over the last half-century. Most ocean stocks are now recognized as overfished, and aquaculture (fish farming) is being promoted all over the world to make up the deficit in demand for fish and fish products. The aquaculture industry in the sub-Saharan Africa region, like other parts of the world, continues to grow, albeit slowly. FAO (2007) has reported in its State of the World's Fisheries and Aquaculture report that even though there are signs that the rate of growth for global aquaculture may have peaked, high growth rates may continue for some regions and species, such as the sub-Saharan African region and tilapia, respectively.

Unfortunately, the growth of aquaculture has been commensurate with the deterioration of aquaculture pond effluent-receiving streams. Fish farming is characterized by the use and management of inputs like feed and fertilizer, with a potential for the spread of pathogens. There is also a high rate of generation of waste material, which includes organic matter, nutrients, and suspended solids in ponds, and this directly impacts on the quality of receiving streams through oxygen depletion, eutrophication, and turbidity (Beasley and Allen 1974; Tucker et al. 1979; Shireman and Cichra 1994; Naylor et al. 2000; Yin and Li 2003).

Nitrogen and phosphorus are the key nutrients generated in aquaculture systems (Boyd and Massaut 1999). Inefficiency of nitrogen and phosphorus use from feeds into fish biomass (Tucker et al. 2000) indicates that much of the remaining nutrient load is lost to the pond ecosystem as fish waste products (Stephen and Harris 2004). High concentrations of these nutrients can harm fish and aquatic ecosystems (Losordo et al. 1992; Tucker 1996). It has also been demonstrated that nutrients are associated with elevated total suspended solids (TSS) concentrations (Cripps 1992; Schwartz and Boyd 1994; Boyd et al. 1998; Teichert-Coddington et al. 1999). Even though cultured fish within the system may not be significantly

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affected by the poor water quality events, if this water was discharged into a receiving stream, it could pose a significant threat to natural biota (Boyd and Massaut 1999; Naylor et al. 2000; Piedrahita 2003; Stephens and Farris 2004). Either nitrogen or phosphorus, both of which are abundant in aquaculture pond effluents, has been shown to be limiting in different freshwater systems (Vallentyne 1974; Setaro and Melack 1984).

The enrichment of nitrogen and phosphorous in effluent-receiving streams as a result of pond effluents has the potential to set-off subsidy-stress responses in these systems, based on which of these nutrients is limiting in that ecosystem (Odum 1979). A subsidy-stress response is characterized by an initial increase in biotic metrics due to the augmentation of a particular variable, which is limiting in a particular system, and then an eventual drop in these metrics as levels of this variable exceeds a threshold (Odum 1979). This response is deceptive at the subsidy stage of augmentation and managers might consider the structure and function of such a system to be sound, which might result in the augmentation of this limiting variable to escape detection until the stress stage when the system is severely affected (Odum 1979).

Pathogens can also be transported in pond effluents into receiving streams (Goldburg and Triplett 1997). This is particularly common in aquaculture systems that depend on natural organic fertilizers (Boyd and Massaut 1999). One such system is the semi-intensive system of pond aquaculture that is seen in most sub-Saharan African countries (FAO 2009). A microbial study on common feeds and organic fertilizers used in the Ghanaian fish ponds yielded alarming results. Three of the feeds (biscuit waste, groundnut husk, and dried termite) and three of the organic fertilizers (cow manure, pig manure, and poultry manure) contained significant counts of fecal coliforms. Addtionally, four out of 11 feeds (biscuit waste, cassava, groundnut husk, and termites) and all organic fertilizers (poultry manure, cow manure, pig manure, and cow blood) contained fecal streptococci (Ampofo and Clerk 2003),

which can infect humans. These and many other pathogens may be transported in effluents into receiving waters.

Biological pollution of receiving streams from aquaculture, such as the introduction of non-native species to natural ecosystems, can harm ecosystems by altering species composition or reducing biodiversity (Courtenay and Williams 1992; Mottram 1996; Goldburg and Triplett 1997). These species may feed on native species, compete with native species for food and for space, modify or destroy habitat of native species, and introduce new diseases and parasites (Krueger and May 1991). By 1990, as many as 291 species of inland fishes had been transferred outside their native ranges into 148 countries through aquaculture (Welcomme 1992).

In recent years, there has been a lot of public outcry at the perceived impacts of fish farming on natural aquatic ecosystems (e.g. Goldburg and Triplett 1997) which has led to the imposition of a number of unpleasant restrictions on the aquaculture industry in a number of countries. A number of effects of aquaculture operations on receiving streams have been documented, as indicated in preceding paragraphs, but without a good understanding of the differences that exist among the different aquaculture systems and without a good knowledge of the background conditions that exist in effluent-receiving streams, there is a tendency to lump all aquaculture systems together, and to exaggerate the impact of the industry on the quality of natural aquatic ecosystems.

A clear distinction exists between 'potential' and 'actual' pond effluents. Potential effluent quality refers to that determined from pond water samples collected from inside the pond and not from an actual pipe discharge (Stevens and Farris 2004). This is particularly useful in studying aquaculture pond effluents since these ponds are seldom drained.

Aquatic biota themselves provide the most reliable signals of the effects of pollutants or habitat alteration, providing the basis for direct biological assessment and monitoring

(Karr and Chu 1999). Biomonitoring is the systematic use of living organisms to evaluate changes in environmental or water quality (Cairns and van der Schalie 1980). It is obvious that water chemistry alone will not be enough to determine the effects of aquaculture pond effluents on the quality of receiving waters. Water chemistry is altered only for a short period after the release of effluents into a lotic water-body, but the "very existence of aquatic living systems integrates everything that has happened where they live, as well as what has happened upstream and upland" (Karr and Chu 1999). This is a feasible and low-cost alternative or complement to chemical measurements and toxicological bioassays, and as such, should be developed for resource-poor countries (Thorne and Williams 1997). Previous successes in the application of biological monitoring have been documented from West Africa under the Onchocerciasis Control Program, where fish and benthic macroinvertebrates were used to monitor the effects of pesticides on aquatic communities of rivers (Leveque et al. 2003).

In this thesis, I examine the effects of pond aquaculture on natural aquatic ecosystems in Ghana, which is located in sub-Saharan Africa. Chapter one examines the general potential physicochemical and microbial impacts of aquaculture operations on effluent-receiving streams in the study area by comparing concentrations of physicochemical and microbial variables among aquaculture ponds, stream reaches upstream and downstream of fish farms and reference stream locations. I also examine the different aquaculture pond typologies that exist in the study area, and the physicochemical impacts of these unique pond types on the quality of receiving streams.

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Chapter 1: Characterization of Potential Pond Effluents and Physicochemical Assessment of Effluent-Receiving Waters in Central Ghana

Abstract. – This study was conducted to determine the potential physicochemical and microbial impacts of pond aquaculture on pond effluent-receiving streams in the Ashanti and Brong Ahafo regions of Ghana. Only a small portion of the nutrients in feed is retained in cultured fish biomass. The remainder is lost to the pond system as uneaten feed, fecal solids, and dissolved nutrients. The interaction of pond aquaculture with the aquatic environment has the potential to be detrimental to the natural environment and, in turn, hurt the industry which depends on the same water sources for survival. Hence, a combination of standard questionnaire survey, field sampling, and laboratory analyses of pond water samples, samples from upstream and downstream of fish farms, and reference stream water were used to generate needed data. Water quality variables considered included nutrients, solids and bacteria. Results indicate that aquaculture ponds had significantly higher levels of most physicochemical variables considered in comparison to upstream reference locations. Levels of total settleable solids were higher in ponds than reference streams ($p = 0.0166$); suspended solids was higher in ponds than reference streams ($p = 0.0159$) and upstream ($p = 0.0361$); and total phosphorus was higher in ponds than reference ($p = 0.0274$) and upstream ($p = 0.0269$). Total nitrogen was most clearly higher in ponds than all other locations: $p = 0.0016$, 0.0086, and 0.0154 for the differences between ponds and reference, upstream, and downstream respectively. BOD_5 level was also higher in ponds than all locations: $p = 0.0048$, 0.0009, and 0.0012, for the differences between ponds and reference, upstream, and downstream, respectively. Temperature was significantly higher in ponds than reference streams ($p = 0.0402$). Also, all study ponds belonged to one of six general pond types, with about 92% of all the 292 ponds studied belonging to either the *Oreochromis* monoculture, or the *Oreochromis − Clarias* polyculture pond type. Hence, any actual impacts on the receiving

streams in the future will depend on how pond effluents are managed, since this study

considered the potential, and not the actual, pond effluents. Additionally, the management of

effluents in the study area can focus on the six unique pond types to facilitate the process.

Introduction

The aquaculture industry is unique in the sense of being a potential polluter of natural water bodies with effluents (Goldburg and Triplett 1997; Naylor et al. 2000) as well as a user of polluted water (Hopkins et al. 1995). Baired et al. (1996) argue that owing to its tight linkage with natural aquatic ecosystems, aquaculture has the potential to have a much more profound impact on [aquatic] environmental quality than terrestrial farming systems of similar size. Nevertheless, aquaculture continues to be a reliable source of fish for the several uses over the years and it is practiced today to some extent in every country of the world (Twarowska et al. 1997). The worldwide decline of ocean fisheries stocks has provided the impetus for rapid growth in fish and shellfish farming, especially now when the general consensus is that ocean fisheries stocks are being overfished (NRC 1999). World aquaculture (food fish and aquatic plants) has experienced a rapid and sustained growth over the past half-century. From a production of below 1 million tonnes in the early 1950s, the industry produced 59.4 million tonnes, with a value of US$70.3 billion, in 2004. The annual growth rate for global aquaculture (8.8 percent per year since 1970; FAO 2005) is much higher than that for capture fisheries (1.2%) and for terrestrial farmed meat production systems (2.8%, FAO 2005; 2007). Farmed fish currently account for over a third (43%) of all fish directly consumed by humans (FAO 2007). With the current high rate of global population growth the reliance on farmed fish production as an important source of protein is likely to increase (Naylor et al. 2000; FAO 2007).

Aquaculture involves "the enclosure of fish in a secure system under conditions in which they can thrive" (Naylor et al. 2000). This scenario allows for portions of the life cycles of cultured species to be altered with the aim of maximizing biomass. There are three general, typical intervention methods: extensive, semi-intensive and intensive aquaculture, characterized by exclusion of predators and control of competitors; enhancement of food

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supply; and the provision of all nutritional requirements, respectively (Naylor et al 2000). As aquaculture production intensifies, there is a greater use and management of inputs such as feed, and this also results in an increase in the potential for the spread of pathogens. The rate of waste generation within the system also increases. The concentration of organic matter, nutrients, and suspended solids in ponds shoots up, and this directly increases oxygen demand, eutrophication, and turbidity in receiving waters (Beasley and Allen 1974; Shireman and Cichra 1994; Naylor et al. 2000; Lin and Yi 2003). This is especially the case in developing countries where there is a high reliance on organic fertilizer and natural feeding in the mostly semi-intensive systems (Diana et al 1997; Ofori 2000). Four main components of aquaculture waste water are of interest; nutrients (including nitrogen (N) and phosphorus (P)), Biochemical Oxygen Demand (BOD), suspended solids, and pathogens (Cripps and Kelly 1996).

Up to 80% of feed ingested by fish is released to the pond environment as fecal solids and dissolved nutrients and organic matter, with just about 20% retained as fish biomass (Boyd and Tucker 1998; Tucker and Hargreaves 2003). According to Pillay (1992), these figures vary for the different fish species and aquaculture systems, as well as with environmental conditions and feed quality. Left in the system, these solids could generate additional oxygen demand, carbon dioxide and ammonia nitrogen when undergoing bacterial decomposition (Twarowska et al. 1997) and, if released into the natural environment, can be detrimental to aquatic habitats (Naylor et al. 2000; Piedrahita 2003; Stephens and Farris 2004). The method of harvesting from an earthen pond can have a huge bearing on the concentration of settleable solids (Maillard et al. 2005). This and the heavy algal blooms, common in the tropics, may result in the discharge of huge volumes of suspended solids that will contribute to high turbidity and biochemical oxygen demand (BOD) in natural systems.

Nitrogen and phosphorus are the key nutrients generated in aquaculture systems (Boyd and Massaut 1999). Inefficiency of nitrogen and phosphorus use from feeds into catfish biomass indicates that much of the remaining nutrient load is lost to the pond ecosystem as fish waste products (Stephen and Harris 2004). High concentrations of these nutrients can harm both fish and receiving aquatic ecosystems (Losordo et al. 1989; Tucker et al.1996). Phytoplanktons make use of these nutrients, resulting in the elevation of chlorophyll-a levels (Boyd 1979). Nutrients are also associated with elevated total suspended solids (TSS) concentrations (Schwartz and Boyd 1994; Boyd and Tucker 1998; Teichert-Coddington et al. 1999). Even though cultured fish within the system may not be significantly affected by the poor water quality events, if this water was discharged into a receiving stream, it could pose a significant threat to natural biota (Boyd and Massaut 1999; Naylor et al. 2000; Piedrahita 2003; Stephens and Farris 2004). Therefore, nitrogen and phosphorus were major concerns in the rule-making process for aquaculture effluents by the United States Environmental Protection Agency (USEPA) (Boyd and Queiroz 2001).

The spread of pathogens on fish farms occurs through the employment of particular management practices on aquaculture farms, like the use of natural organic fertilizers in fish ponds (Boyd and Massaut 1999). Therefore, fish and fish wastes may contain human pathogens (Midtvedt and Lingaas 1992; Smith et al. 1994). The gradual release of nutrients from these natural organic materials has made them the preferred fertilizer in pond aquaculture (Diana et al 1997). In many instances, these organic fertilizers are wastes from livestock farms and other industries, making them far less costly than inorganic fertilizers, but at the same time, more susceptible to infection by bacteria and other microorganisms. Ampofo and Clerk (2003) found that three common fish feeds in Ghana (biscuit waste, groundnut husk, and dried termite) and three organic fertilizers (cow manure, pig manure, and poultry manure) contained significant counts of fecal coliform. Additionally, four out of

11 feeds (biscuit waste, cassava, groundnut husk, and termites) and all organic fertilizers (poultry manure, cow manure, pig manure, and cow blood) contained fecal streptococci (Ampofo and Clerk 2003). These and other pathogens may be transported in effluents into receiving waters (Goldburg and Triplett 1997).

Environmental impacts of aquaculture on aquatic ecosystems are related to the species cultured, location of installations, intensity of operations, the morphology, limnology and hydrology and trophic status and assimilative capacity of the receiving water (Costa-Pierce 1996; Cripps and Kelly 1996; Boyd and Queiroz 2001). Thus, for example ponds located in relatively pristine watersheds are likely to alter receiving waters to a larger extent than those located in heavily agricultural watersheds. Consequently, to understand the potential threats of pond effluents it is necessary to characterize the background quality of the receiving water as well. Based on data synthesis, Costa-Pierce (1996) concluded that during normal operations of channel catfish ponds, total phosphorus (TP) releases are comparable to precipitation whereas during harvesting, mean TP discharges are comparable to concentration in runoff from intensive agriculture. The wide range of factors determining pollution potential of aquaculture necessitates a different way of categorizing aquaculture systems, even for those that culture the same or similar species; categorization will need to include management practices, such as stocking, draining and harvesting regimes. In Africa for instance, it is already recognized that the strategies for addressing problems arising from small-scale and large-scale commercial aquaculture operations will probably be different (Jamu and Brummett 2004). Environmental management practices for pond aquaculture will be most effective if developed for specific systems that have differing pollution potentials.

Without a good understanding of the differences among aquaculture systems, there is the tendency on the part of environmental advocacy groups and consequently decision makers to lump all systems together and exaggerate the impact of the industry. Such was the

case after the publication of 'murky waters' by the Environmental Defense Fund and the subsequent decision by the USEPA to regulate virtually all aquaculture operations in the United States (Boyd and Tucker 2000). It is only a matter of time before the growing aquaculture industry in Africa will be confronted with competition for water and regulation of effluent discharges. Characterization of effluents would allow for a proactive management of the environmental effects of aquaculture. Proactive action will forestall the restrictive regulations that could result from regulatory agencies acting on insufficient or exaggerated assessments of the industry (Cripps and Kelly 1996).

Even though the impacts of fish pond effluents on receiving streams are beginning to be documented in recent years, most of these studies have been conducted in developed countries where the aquaculture industry is already at an intensive stage. The goal of this study was to evaluate the potential effects of pond aquaculture effluents on receiving streams in a developing country located in the sub-Saharan Africa region, where aquaculture is still extensive to semi-intensive. Ghana has a relatively young aquaculture industry, and is mostly located within the moist semi-deciduous forest zone, which is common to the sub-Saharan Africa region.

The specific objectives of this study are to:

1. characterize potential pond effluent quality

2. characterize receiving and reference stream quality in terms of nutrients, suspended solids, and pathogens

3. categorize ponds into unique typologies

4. characterize potential pond effluents by pond typologies

Methods

Study area

The study was conducted in the Ashanti and Brong-Ahafo Regions (figure 1.1). These two regions host most of the pond aquaculture operations in Ghana. Centrally located side-by-side in the middle belt of Ghana with Kumasi and Sunyani as their capitals, respectively, these two are among the most populated of the 10 regions of the country. The regions lie between longitudes 0.15W and 2.25W, and latitudes 5.50N and 7.46N, with more than half of this area located within the moist, semi-deciduous forest zone between 150 and 300m above sea level. The regions have an average annual rainfall of 1270mm. Ghana has two rainy seasons; the major season starts in March, with a peak in May, and the minor starts from July with a peak in August. The average daily temperature across the regions is approximately 27°C. The climate of the region is similar to that of most of the forest zones of West Africa. The study regions are drained by the Rivers Offin, Pra, Tano, Mankran and Owabi, and Lake Bosomtwe. There are several other smaller rivers and streams which serve both domestic and industrial purposes. Common species cultured include several species of tilapia such as *Oreochromis niloticus, Tilapia zillii*, *Sarotherodon galilaeus* and *Hemichromis fasciatus* (Cichlidae)*; Heterotis niloticus* (Osteoglossidae) and the catfishes, including *Clarias gariepinus* and *Heterobranchus bidorsalis* (Clariidae) (FAO 2009).

Major study design components included a) administration of a survey to characterize farms, farmers, ponds, and management practices in the Ashanti and Brong Ahafo regions, b) sampling of ponds, receiving, and reference streams in the Ashanti region for physicochemical and microbial assessment, c) a post-stratification of ponds by cluster analysis of survey data, and d) characterization of potential effluent quality by pond types.

Site selection

In the process of selection of farms and ponds for the study, it became apparent that a formal survey was needed since most vital data on farms were not systematically documented or accessible. A four-page survey was developed and administered in person to 32 farms and farmers, who were voluntary participants. Questions in the survey covered demographics of farmers and farm-level practices, including feeding, fertilizing, and harvesting regimes, and a detailed documentation of information on all ponds on each farm. Detailed pond-level information included size, source of input water, drainage design and frequency of effluent releases, species currently stocked, and stocking densities.

As anticipated, draining was not a frequent event and therefore I focused on characterizing potential, rather than actual, effluent quality. Of the 32 farms surveyed, 12 in the Ashanti Region were chosen for potential effluent quality and biological assessment studies. The restriction of laboratory studies to the Ashanti region was based on logistical considerations, in particular, accessibility of farms by road and travel time required back to the laboratory while maintaining the integrity of samples. Three ponds were randomly selected from each of the 12 farms for sampling (i.e., a total of 36 ponds). I applied the control impact design (Karr and Chu 1999), using the site-specific reference (Thorne and Williams 1997) for comparisons between sites. Upstream and downstream sampling stations within 100m of each farm were established on the receiving streams. A reach on a stream closest to each farm and similar in size to the receiving stream, but with no apparent influence of aquaculture was identified as a site-specific reference site.

Sampling

We collected water samples from pond, upstream, downstream, and reference sites from May to July of 2009. At upstream sites a 2.75-L sample was collected at approximately 0.3m below the water surface for physicochemical analysis and a 0.5-L sample was also

taken for microbiological analysis. Within pond samples were vertically stratified, one about 0.3m below the surface (limnetic) and the other about 0.3m from the bottom (benthic). At downstream stations, three replicate samples were taken at 0m, 5m and 100m from the farm. The same volume of water was collected from every station, and almost all samples were collected between 0800 and 1400 (appendix A), transported to the laboratory and analyzed within 24 hours. Water temperature, pH, pressure, conductivity, total dissolved solids, salinity and dissolved oxygen were determined on-site with a portable multi-parameter water quality meter (HANNA HI9828). Laboratory analysis of water samples followed the standard procedures in Clesceri et al. (1998) for the following variables: total nitrogen (TN) (Macro-Kjeldahl method), total phosphorus (TP) (acid persulfate digestion method), total suspended solids (glass fibre filtration), total settleable solids (gravimetric method), and 5-day biochemical oxygen demand (BOD_5) (20^oC incubation). Total fecal streptococci and coliform counts also followed standard methods (Clesceri et al. 1998) and the most probable number of bacteria was determined based on Lindquist (2008).

Data analysis

Statistical analyses were preceded by a detailed graphical analysis in Minitab®15. The optimal number of pond types was identified by the K-means clustering procedure. This procedure relies on the differences and similarities that exist within a group of observations, to create subgroups based on set criteria, with the aim of minimizing 'within-subgroup' variability (MacQueen 1967). The management practices that went into the clustering procedure were: pond size, type of species cultured, number of species cultured, stocking density, number of harvests in a year, and draining or no draining with harvest (appendix B). These management practices served as criteria, on which the grouping of the total 292 ponds was based.

A mixed-effects ANOVA with farm as random blocks and fixed location effects was the main model, using the Tukey procedure for post-hoc analyses of location effects. Other analyses simplified to t-tests or paired t-tests. Statistical significance was decided as $p \leq 0.05$ but marginal situations up to $p \leq 0.15$ were noted because of the relatively small sample sizes, and the consequent low power in some tests to detect significant differences unequivocally.

Results

Characterization of potential pond effluent, receiving and reference stream quality

Neither the vertical position in the pond or distance downstream of farms appeared to show much variation in physicochemical and microbial levels except for higher limnetic BOD_5 in ponds (Figure 1.2 & 1.3). This made it possible to pool samples and work with averages of the samples taken from these locations in subsequent comparisons with upstream and reference streams. Temperature, settleable and suspended solids, total phosphorus and total nitrogen, and BOD_5 showed differences among locations whereas fecal coliform and streptococci and the remaining ancillary variables did not significantly differ (Figure 1.4). Post-hoc analysis with Tukey simultaneous tests showed that settleable solids were higher in ponds than reference streams ($p = 0.0166$) and marginally higher than upstream ($p = 0.0707$) and downstream ($p = 0.1102$) sites. Suspended solids followed a close pattern, being higher in ponds than reference streams ($p = 0.0159$) and upstream ($p = 0.0361$) and marginally higher than downstream ($p = 0.0711$) sites. Total phosphorus was higher in ponds than reference ($p = 0.0274$) and upstream ($p = 0.0269$) and marginally higher than downstream ($p = 0.1364$) sites. Total nitrogen was most clearly higher in ponds than all other locations: $p = 0.0016$, 0.0086, and 0.0154 for the differences between ponds and reference, upstream, and downstream respectively. BOD_5 was also higher in ponds than all locations: $p = 0.0048$, 0.0009, and 0.0012, for the differences between ponds and reference, upstream, and downstream, respectively. Temperature was significantly higher in ponds than reference

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streams (p = 0.0402) and marginally higher in ponds than downstream (p = 0.0518). No other significant differences were observed between any pairs of locations for these or any other variables. Actual means of these water quality variables can be seen in Figure 1.4.

Categorization of pond typologies and characterization of potential pond effluents by pond type

The iterative process to determine optimal number of clusters to describe types of ponds showed, as expected, a declining trend of the sum of squared distance from group centroids ('the residuals') with increasing number of clusters (Figure 1.5). Since too many or too few clusters are not desirable I sought to optimize the number of clusters by choosing the number at which error started declining at a decreasing rate. Six clusters were decided from the total of 292 ponds as optimum because there were steep declines in error up to six clusters after which the error actually increased before flattening out.

Table 1 describes the typical characteristics of each cluster or pond type and the number of ponds falling into that cluster. Type 1 is typically a large pond containing *Clarias* in a polyculture with *Oreochromis* and up to 3 other species at a medium to high stocking density, which sees two annual harvest events, mostly by draining. Ponds in cluster 2 are generally smaller without *Oreochromis*, but with *Clarias* and one other species stocked at medium to high densities, which are completely drained for harvest, at least twice annually. Cluster 3 consists of small ponds with *Oreochromis* at medium to high stocking densities, which experiences up to 3 annual harvest events, mostly without draining. Cluster 4 is characterized by a very small pond size with a high stocking density for a monoculture of *Clarias* that is 'harvested' more than three times in a year, without draining. Ponds in cluster 5 are generally small in size, most are under construction, and so have not yet been stocked. Cluster 6 is made up of small ponds with a polyculture of *Oreochromis*, *Clarias* and possibly

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one other species, stocked at medium to high densities that sees 2 to 3 harvest events in a year, with draining about half the time. The additional species that characterize some clusters besides *Clarias* and *Oreochromis* were commonly *Heterotis niloticus*, *Parachanna obscura* (Channidae) and species of the genus *Chrysichthys* (Claroteidae).

The two most common types of ponds were 3 (*Oreochromis* monoculture) and 6 (*Oreochromis-Clarias* polyculture). Not surprisingly, 33 out of 36 of the ponds sampled belonged to one or the other of these two types. Therefore, I focused on these two types of ponds to characterize potential effluent quality by pond type (Table 2; Figure 6). No significant differences were found in the microbial levels of the two types of ponds, although streptococci were moderately high across pond types compared to coliform. Salinity was significantly higher in pond type 3 ($p = 0.046$) and temperature ($p = 0.057$), BOD_5 ($p = 0.083$), and total nitrogen ($p = 0.135$) were only marginally higher in type 6.

Discussion

The potential effluent quality of ponds was found to differ from that of the other locations, as expected. Temperature, total nitrogen, total phosphorus, total settleable solids, total suspended solids and BOD_5 were all in higher levels in ponds than in receiving streams. Bodary et al (2004) reported similar trends from baitfish ponds in Central Arkansas. This was expected because these ponds are actively managed, with fertilization and daily feeding carried out. The activity of concentrated fish in ponds would also be expected to keep solids suspended even in the absence of harvesting activities. Higher temperatures in ponds can be explained by standing water exposed to the sun. Statistically, there was no evidence that the elevated levels of nutrients, solids, and oxygen demand on farms caused high levels of these variables downstream. Nevertheless, I noticed a pattern of pond water and downstream water being the least statistically distinguishable compared to pond versus upstream or reference. Silapajarn and Boyd (2005) observed that streams into which effluents flowed directly from

catfish ponds had higher concentrations of suspended solids, turbidity, nutrients, and biochemical oxygen demand, compared to reference streams in the same Alabama watershed. Hence, due to the fact that the potential, and not the actual effluent quality was characterized, and also due to the fact that levels of variables at downstream locations were closest to that of ponds, how effluents are managed over time may well make the difference in avoiding future detectable effects of ponds on receiving stream water quality.

Fecal streptococci and coliform bacteria are known to be in high levels in semi-intensive fish ponds (Ampofo and Clerk 2003), and I expected significantly higher levels of bacteria in pond water samples compared to water samples taken from upstream, downstream and reference locations. For the levels of bacteria in these three locations to have matched that of ponds, then bacteria levels had to be alarmingly high in these three locations. I observed several piggeries, sheep and poultry farms within the study area. These industries served as the main sources of organic fertilizer for fish pond operations. Hence, farm wastes, mostly fecal matter, could have been washed in run-off into streams, leading to the high levels of bacteria in these streams.

Some of the patterns in management practices on the study farms, which influenced the clustering of ponds into six types, appear to have intuitive explanations. All pond types with *Oreochromis* and *Clarias* culture (types 1, 2, 3, 4 and 6), with the exception of type 4, also saw some pond draining with harvest. *Oreochromis* is known to hide in the pond bottom out of the reach of harvesting seines (Lin and Yi 2002), and so ponds either have to be seined several times or drained during harvesting. Fish farms with an abundance of water resources, such as my study farms, are expected to choose partial or complete draining during harvest of *Oreochromis*. The quality of water in *Clarias* ponds has been shown to decrease rapidly, as a result of the characteristic high feeding levels in these ponds (de Graaf, G. and H. Janssen, 1996). Two recommended solutions are reducing feeding levels and flushing the system with

fresh water (de Graaf, G. and Janssen 1996). Hence, it is only normal for fish farmers to change the water in *Clarias* ponds before each production cycle.

The characteristics of type 4 ponds, such as extremely small sizes, high stocking density, and a large number of harvest events without draining, resemble the situation in *Clarias* nursery ponds and tanks. In the study area, fry production was more common for *Clarias* than for *Oreochromis* culture. Most *Oreochromis* farmers start with a broodstock and rely on the prolific breeding of this species to sustain the stock. Since *Clarias* does not reproduce spontaneously in captivity (de Graaf, G. and Janssen 1996; Dyk and Pieterse 2008), *Clarias* ponds have to be stocked with fry for each production cycle.

Salinity was the only effluent component that appeared to differ between the two most common pond types (Table 1.2; figure 1.5). I did not come up with any evidence of a particular management practice influencing the salinity of pond water, but *Oreochromis* and indeed most tilapine fish have been shown to be tolerant of high salinity levels (Stickney 1986; Tian et al. 2001; Al-Harbi and Uddin 2005; Kamal and Mair 2005). Hence, a probable explanation for the higher salinity levels in *Oreochromis*-monoculture ponds is that other culture species could not thrive in the high-salinity environments, giving the farmers no option but to culture *Oreochromis* alone. At least for these two types of ponds (*Oreochromis*-only and *Clarias-Oreochromis* polyculture) management does not appear to vary enough to result in differences in water quality. I have no data to make the same conclusion across all the pond types. It will be interesting in future studies to collect data for all six pond types. In particular, pond type 1 that are larger and have more than two species appear to be the most intensively managed and, though not as common, would need to be studied closely in future investigations.

A direct link between management practices and downstream water quality was not obvious in this study for three reasons. Firstly, farms were quite similar in various ways and

did not present sufficient contrast for comparing effects of practices. Secondly, the downstream did not appear to have been altered significantly as concluded from other analyses (see chapter 2), also adding to the range issue in receiving water. Finally, sample size was too small for rigorous farm level analysis and even non-parametric analyses had shortcomings of low power. I think the concept was laid out clearly by my approach in this study and future studies should expand sample sizes, howbeit at much higher cost, to afford the needed analytical rigor. All three problems outlined above will be ameliorated by using a larger sample size for the water quality and farm survey studies.

<u>Conclusions</u>

1. Ponds in the Ashanti and Brong Ahafo regions of Ghana generally hold a different, poorer water quality compared to receiving and reference streams.

2. There were six main categories of ponds in Ashanti and Brong Ahafo regions, even though most ponds belonged to either of two pond types – *Oreochromis* monoculture and *Oreochromis-Clarias* polyculture.

3. Any potential effects in the future will depend on how effluents are managed, including effluent treatment, and frequency and volume of releases.

4. Other industries like livestock farming must be considered in efforts to reduce the deterioration of stream water quality

Table 1.1 - Typical characteristics of six clusters (types of ponds or culture systems) defined by pond size, species cultured, stocking density, and harvesting practices (N = 292).

Pond type	Average Pond Size (m^2)	Oreochromis present	Clarias present	Number of Species	Stocking density	Annual harvests	Drain for Harvest?
Type 1 (n = 13)	8,249	Yes (92%)	Yes	2 - 5	Medium to High	2 times	77%
Type 2 (n = 8)	1,574	No	Yes	1 - 2	Medium to High	2 or more	100%
Type 3 (n = 151)	415	Yes	No	1	Medium to High	2 - 3	41%
Type 4 (n = 17)	261	No	Yes	1	High	More than 3	No
Type 5 (n = 26)	1,067	No	No	0	Not stocked	N/A	N/A
Type 6 (n = 77)	824	Yes	Yes	2 - 3	Medium to High	2 - 3	47%

Table 1.2 – Physicochemical and microbial levels in the two most common types of ponds (*Oreochromis* monoculture - types 3 and *Oreochromis* and *Clarias* polyculture)

Variable	Pond Type	N	Mean	SE of Mean
Streptococci				
(count/100ml)	Type 3	14	2800	420
	Type 6	19	3032	479
Coliforms				
(Count/100ml)	Type 3	14	241	171
	Type 6	19	682	481
Settleable Solids				
(ml/L)	Type 3	14	0.31	0.09
	Type 6	19	0.47	0.19
Suspended Solids				
(mg/L)	Type 3	14	86.2	15.3
	Type 6	19	79.6	15.3
Total Phosphates				
(mg/L)	Type 3	14	0.60	0.12
	Type 6	19	0.64	0.07
Total Nitrogen				
(mg/L)	Type 3	14	1.960	0.388
	Type 6	19	2.992	0.879
BOD_5				
(mg/L)	Type 3	14	5.8	1.0
	Type 6	19	8.5	1.1
pH				
	Type 3	14	7.2	0.2
	Type 6	19	7.1	0.3
Temperature				
(^{o}C)	Type 3	14	27.0	0.3
	Type 6	19	28.0	0.3
Conductivity				
(mS/cm)	Type 3	14	102.2	22.3
	Type 6	19	132.3	17.7
TDS				
(mg/L)	Type 3	14	51.2	11.5
	Type 6	19	66.0	8.8
Salinity				
(%)	Type 3	14	0.15	0.05
	Type 6	19	0.06	0.01
Dissolved Oxygen				
(mg/L)	Type 3	14	1.9	0.41
	Type 6	19	1.5	0.36
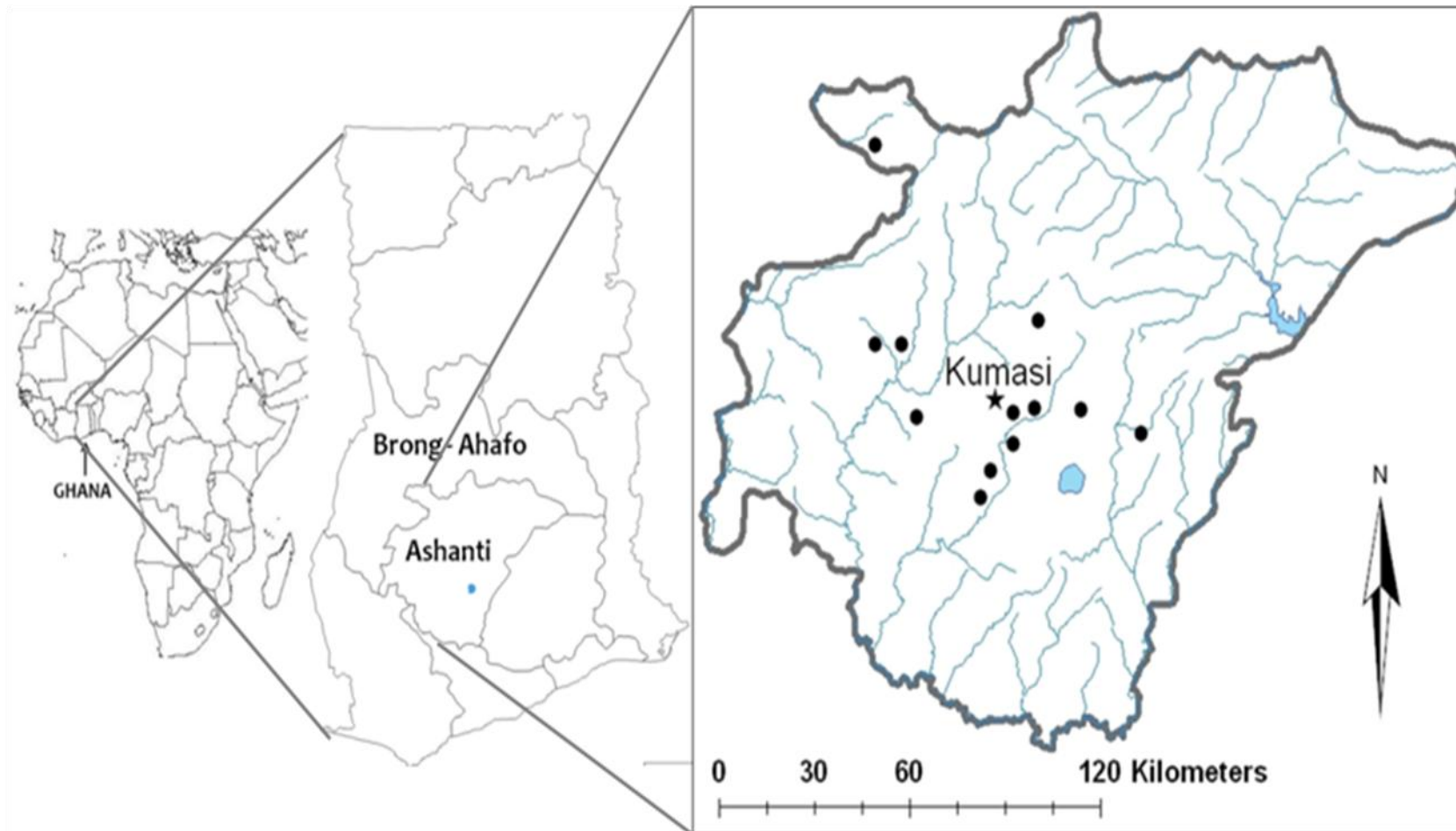
Figure 1.1 – Relative location of sampled locations. Questionnaires were administered to 32 fish farms from the Brong-Ahafo and Ashanti regions of Ghana. Out of this number, water, fish and macroinvertebrate samples were taken from fish ponds, effluent-receiving, and reference streams from, or close to 12 farms (indicated as bullets) in the Ashanti Region.
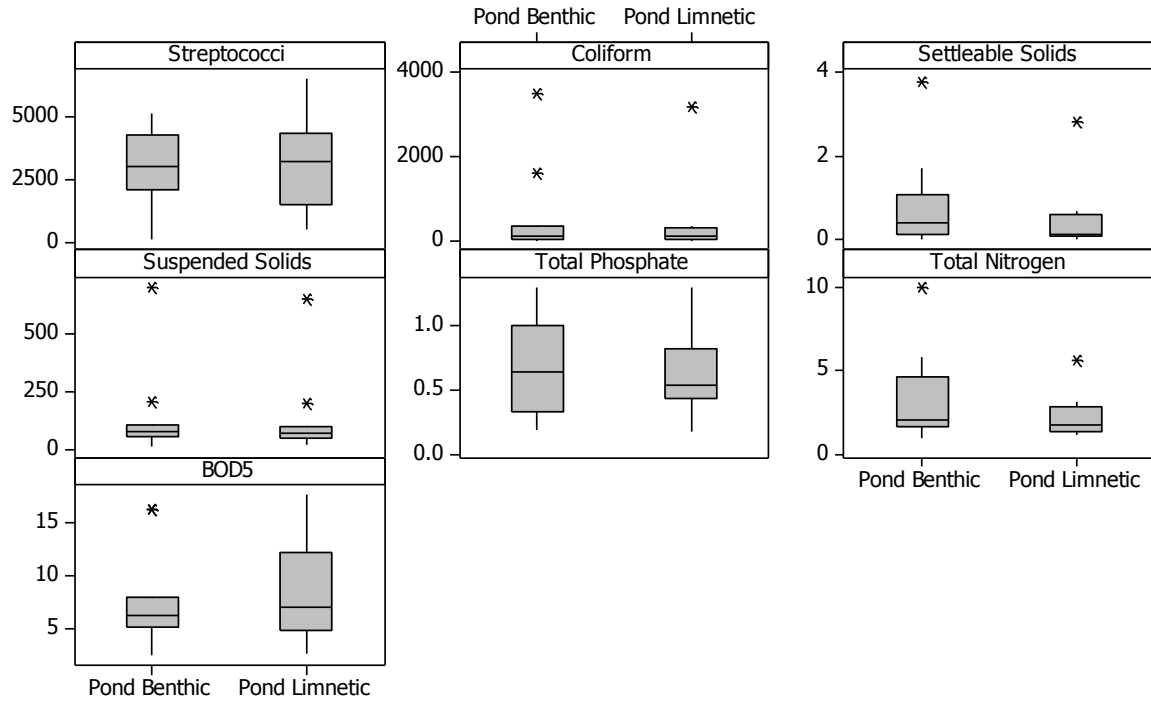
Figure 1.2- Comparison of microbial and physicochemical levels as a function of vertical location in the pond
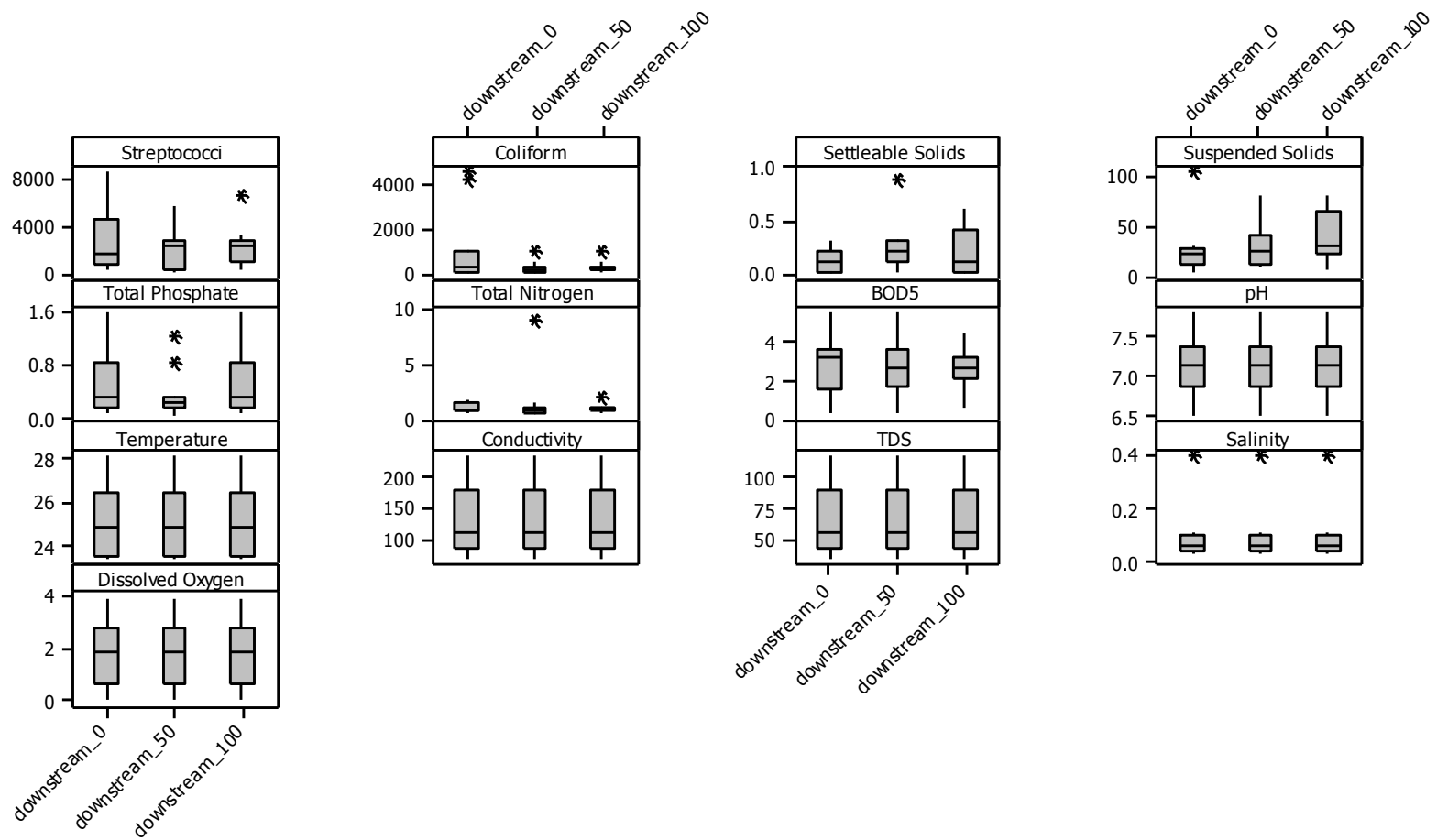
Figure 1.3 - Comparison of microbial and physicochemical levels as a function of distance downstream of farms
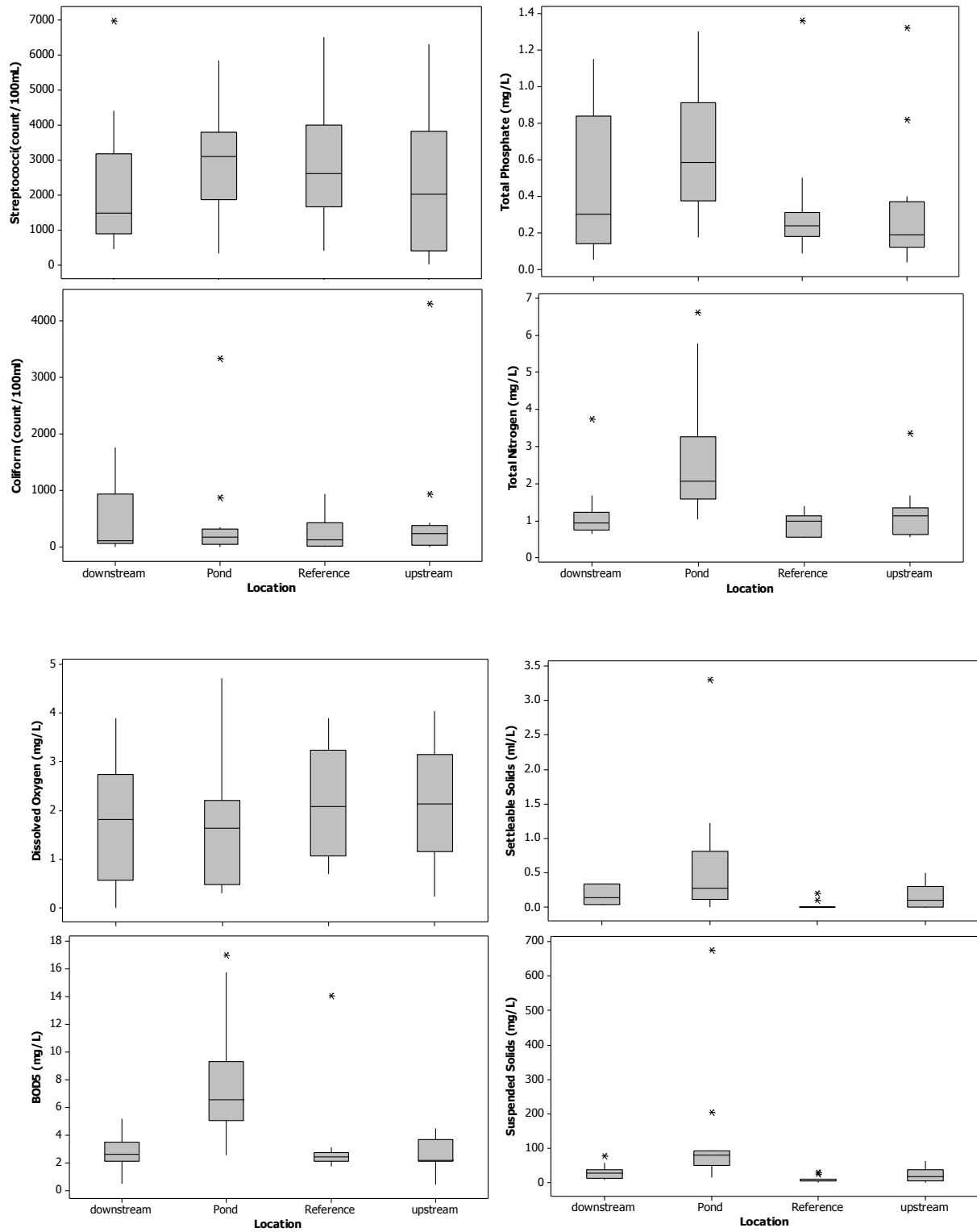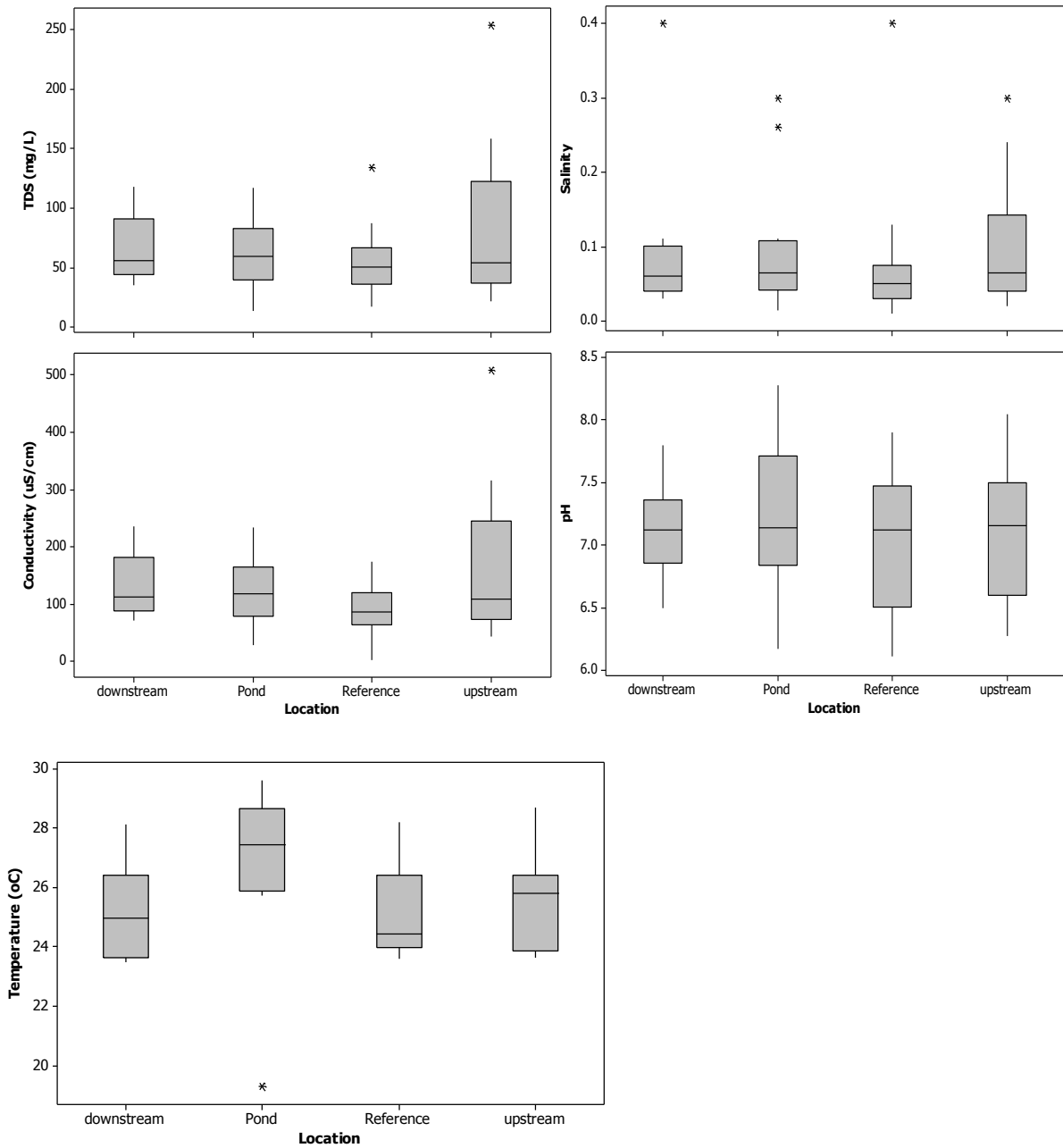
Figure 1.4 – Levels of microbial and physicochemical variables in ponds, upstream, downstream, and reference locations.
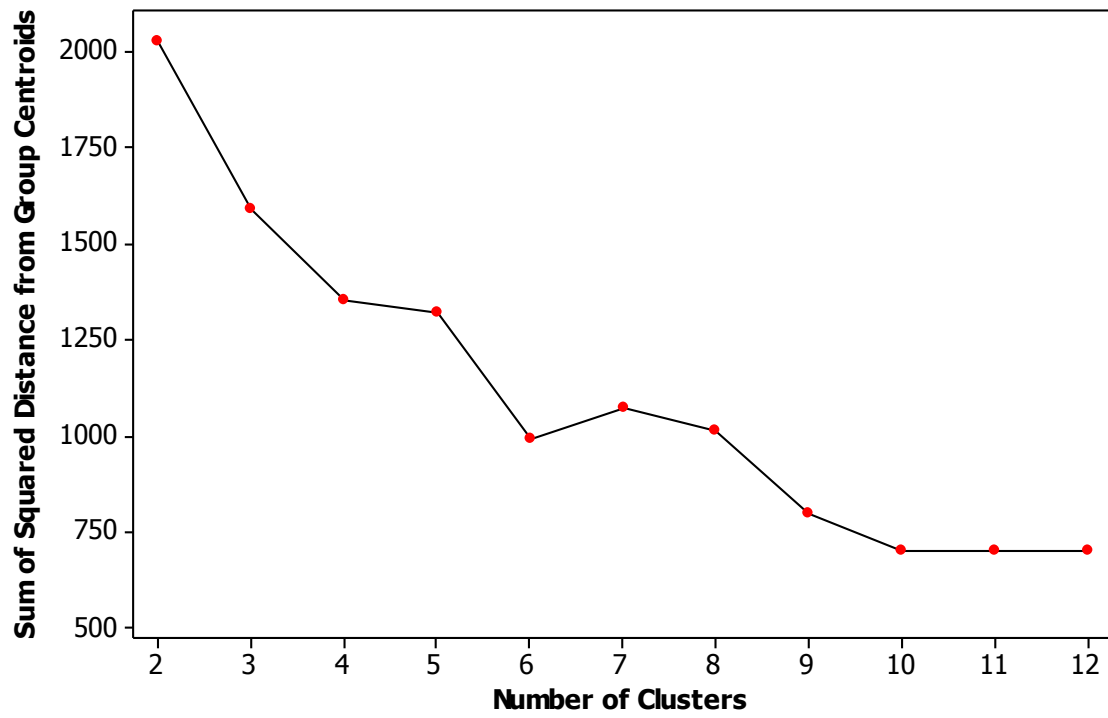
Figure 1.5 - Rate of reduction in residual error with increasing number of clusters to describe pond types.
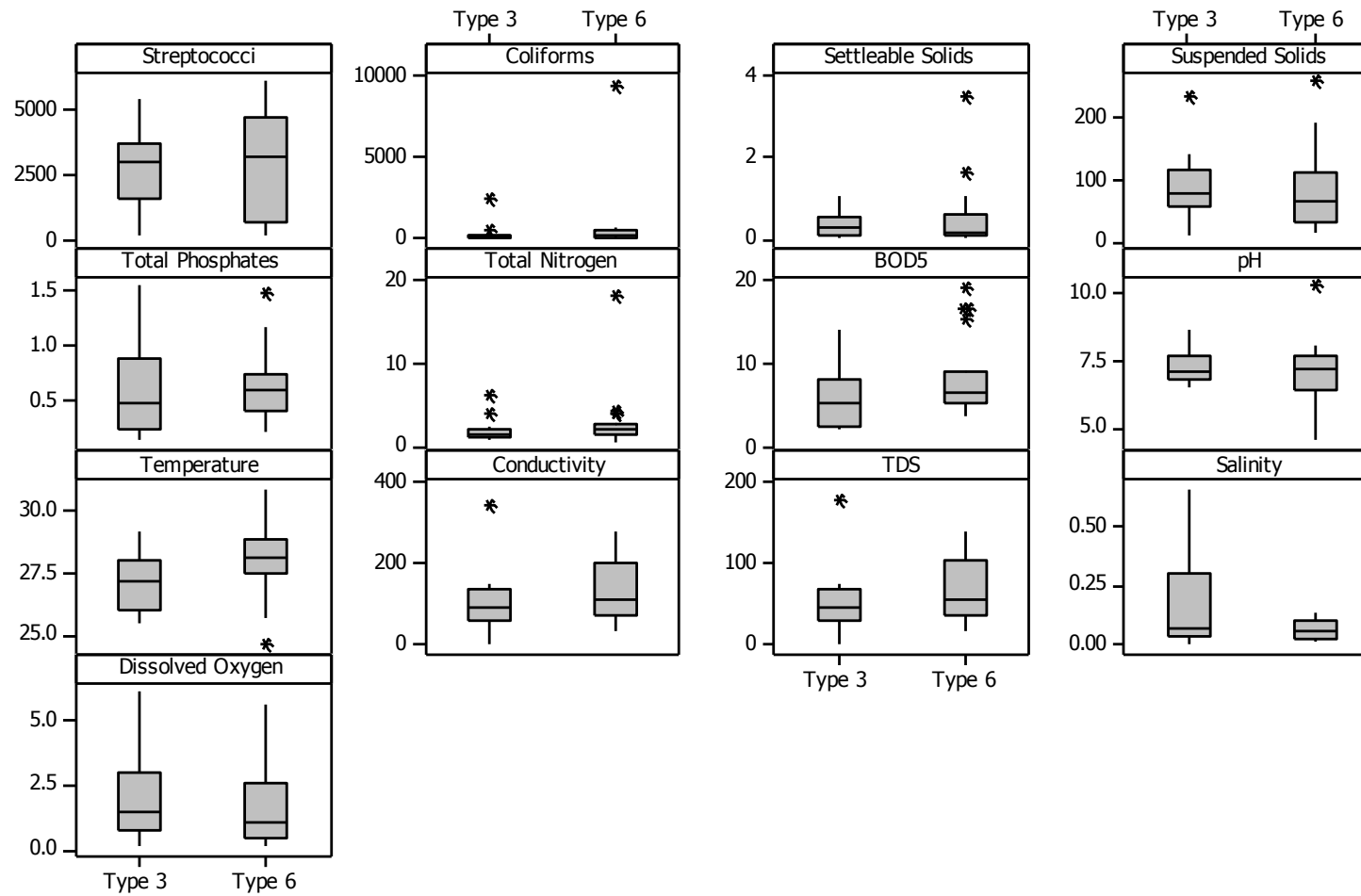
Figure 1.6 – Comparison of microbial and physicochemical levels in the two most common types of ponds.

Chapter 2: Biological effects of aquaculture ponds on receiving streams using structural and functional composition of fish and macroinvertebrate assemblages

Abstract. – This study was conducted to determine the biological impacts of aquaculture operations on effluent-receiving streams in the Ashanti Region of Ghana. Biomonitoring of stream water quality is quickly becoming recognized due to the many advantages to the use of stream biota, especially fish and benthic macroinvertebrates to determine stream condition. I collected water, fish and benthic macroinvertebrate samples from 12 aquaculture effluent-receiving streams upstream and downstream of fish farms and 12 reference streams between May and August of 2009, and then calculated structural and functional metrics for both fish and macroinvertebrates. Non-guarding fish species were more abundant in reference streams than downstream ($p = 0.0214$) and upstream ($p = 0.0251$), and sand-detritus spawning fish were less predominant in reference streams than upstream ($p = 0.0222$) and marginally less in downstream locations ($p = 0.0539$). A possible subsidy-stress response of macroinvertebrate family richness and abundance was also observed, with nutrient augmentation from other industries apart from aquaculture likely. Generally, there were only marginal differences among locations downstream and upstream of fish farms and in reference streams in terms of several metrics considered. Hence, the scale of impact in the future will depend not only on the management of nutrient augmentation from pond effluents, and nutrient discharges from other industries like fruit and vegetable farming within the study area must also be considered.

Introduction

The global decline in capture fisheries stocks has provided the impetus for the accelerated development of aquaculture, which is practiced, in one form or another, in every country (Twarowska et al 1997). World aquaculture (food fish and plants) has grown from an annual production of below 1 million tonnes in the early 1950s, to 59.4 million tonnes, with a value of US$70.3 billion, in 2004 (FAO 2005). Even though there are visible signs of the peaking of the global aquaculture growth rate, high growth rates may still continue for some regions and species, such as the sub-Saharan Africa region and tilapia, respectively (FAO 2007).

Despite the fact that the aquaculture industry has proven to be a reliable source of fish over the years, its growth has been commensurate with the deterioration of pond effluent-receiving waters. In recent years the negative impacts of fish farming on natural ecosystems have come under severe public criticism (e.g., Goldburg and Triplett 1997; Naylor et al. 2000). Only a small percentage of ingested feed is retained in fish biomass, with the rest lost to the pond environment as fecal solids, uneaten feed and dissolved nutrients (Boyd and Tucker 1998; Tucker and Hargreaves 2003). Hence, the concentration of organic matter, nutrients, and suspended solids in ponds go up, and when pond effluents are drained oxygen demand, eutrophication, and turbidity in receiving waters are directly impacted due to the mostly organic pollution from fish ponds (Beasley and Allen 1974; Shireman and Cichra 1994; Naylor et al. 2000; Lin and Yi 2003).

Nitrogen and phosphorus, both abundant in fish pond effluents, could be limiting in freshwater systems (Vallentyne 1974; Setaro and Melack 1984). Nutrient enrichment of streams and rivers has been shown to set off a subsidy-stress response in these systems. This response is characterized by an initial increase in ecological metrics due to the increase in a

non-toxic perturbation such as nutrient enrichment, followed by a decline in these metrics as the perturbation increases beyond a particular threshold (Odum et al. 1979).

Biological pollution, which is the introduction of unwanted non-native species to natural ecosystems from aquaculture facilities, can harm ecosystems by altering species composition or by reducing biodiversity (Courtenay and Williams 1992; Mottram 1996; Goldburg and Triplett 1997). These species may feed on native species, compete with native species for food and for space, modify or destroy habitat of native species, and introduce new diseases and parasites (Krueger and May 1991). Welcomme (1992) found that at least 291 species of inland fishes have been transferred outside their native ranges into 148 countries, through aquaculture. The search for a balance between growth and intensification of aquaculture and protection of natural fisheries and the environment continues.

In recent years, water quality criteria have been established by consolidating the view that an aquatic ecosystem in which structure and functions [ecological integrity] are not disrupted possesses a quality which is immediately suitable, or suitable after simple treatment, for a variety of uses (Biney 1997). Such is a receiving water body whose assimilative capacity for pollutants has not been exceeded. Unfortunately, assessing the effect of aquaculture effluents on a receiving stream is often difficult because the cause-and-effect relationship between discharge and ecological impacts is not always obvious (Tucker et al. 2002).

It has been established that aquatic biota themselves provide the most reliable signals of the effects of pollutants or habitat alteration, providing the basis for direct biological assessment and monitoring (Karr and Chu 1999). According to Cairns and van der Schalie (1980), biomonitoring is the "systematic use of living organisms to evaluate changes in environmental or water quality." This is a feasible and low-cost alternative or complement to chemical measurements and toxicological bioassays, and as such, should be developed for

resource-poor countries. Previous successes in application of biological monitoring have been documented from West Africa under the Onchocerciasis Control Program, where fish and benthic macroinvertebrates were used to monitor the effects of larvicides on aquatic communities of rivers (Leveque et al. 2003). Closely related to biomonitoring is 'bioassessment', which is the evaluation of the biological condition of a waterbody that uses biological surveys and other direct measurements of resident biota in surface waters (MPCA 2010).

A number of aquatic organisms have been proposed and used in assessing water quality, but macroinvertebrates (Hellawell 1986) and fish (Karr 1981; Shamsudin 1988) are the two most recommended and utilized biological indicators. The advantages to using either of these organisms have made them popular (Rosenberg and Resh 1993). Macroinvertebrates and fish are ubiquitous in river systems and in the different habitats of each of these systems, and so they are exposed to the various environmental perturbations at these locations (Illies 1961; Lenat et al. 1980; Scardi et al 2006). These organisms are also relatively easy to collect and identify (Depaw et al 2006; Scardi et al 2006), and several methods of data analysis have been developed for fish and macroinvertebrate biomonitoring (e.g., Hilsehoff 1977; Karr 1981; Resh and Jackson 1993).

There are some limitations to the use of fish and macroinvertebrates in biomonitoring, however. Macroinvertebrates are known to be distributed non-randomly, and so a large number of samples is needed to achieve the required precision (Hellawell 1986; Abel 1989; Depaw et al 2006). Also, the distribution of macroinvertebrates is influenced by other factors besides water quality, such as current velocity, nature of substrate and the seasonality of life cycles (Suess 1982; Tachet et al. 2002). These factors could result in the establishment of different macroinvertebrate communities at different sites with identical water quality (Giller and Malmqvist 1998). Fishing gears are known to be selective, depending on fish size or

environment (Scardi et al 2006). Some species of fish are also highly tolerant to some pollutants that could kill other aquatic organisms, and fish can move laterally to avoid some disturbances (Scardi et al 2006). These limitations notwithstanding, the advantages of using fish and macroinvertebrates, far outweigh the disadvantages (Scardi et al. 2006), and there are recommended ways to deal with these challenges (e.g. Rosenberg and Resh 1993).

It is obvious that water chemistry alone is inadequate in determining the effects of aquaculture pond effluents on the quality of receiving waters. Water chemistry is altered only for a short period after the release of effluents into a lotic water-body, but "the very existence of aquatic living systems integrates everything that has happened where they live, as well as what has happened upstream and upland" (Karr and Chu 1999).

Biomonitoring is particularly useful in developing countries as it frequently involves low cost and has low technical requirements (Thorne and Williams 1997). Hence, this study was aimed at determining the effects of aquaculture effluents on the structure and function of receiving stream macroinvertebrate and fish communities, as a proxy to the impacts of these effluents on receiving stream quality. The specific objectives were to:

1. Examine community condition metrics between receiving and reference streams.

2. Investigate relationships between water quality and biotic condition of streams.

Ghana is located on the western coast of Africa, and is delineated by latitudes 4°12' N and 11°7' N and longitude 1°12' E and 3°14' W (FAO 2009). Considering the country's abundant water resources, the potential for freshwater and brackish-water aquaculture and culture based fisheries, cannot be over-emphasized. In addition to several rivers, lakes and the Atlantic Ocean on the South, Ghana has the Volta Lake which, at 8600 sq.km (WHO 2002), is on record as the largest man-made reservoir on earth. Nevertheless, demand for fish has always exceeded supply in Ghana, with an annual demand of approximately 600,000 tonnes and total landings (marine and freshwater) of around 357,600 tonnes (WRI 2001), making

Ghana a net importer of fish. Aquaculture is regarded as a means to counter this imbalance (Ofori 2000), and due to the profitability of the aquaculture industry and the availability of streams for pond operations, the number of fish farms in Ghana is rapidly increasing, with already-existing farms intensifying operations. A study of the biological impacts of aquaculture effluents on receiving streams is most appropriate in these early stages of the aquaculture industry in a sub-Saharan African country, such as Ghana.

Methods

Study area

This study was conducted in the centrally located Ashanti Region of Ghana (figure 1.1). With about three-quarters of the region in the moist, semi-deciduous forest zone between 150 and 300 metres above sea level, it records an average annual rainfall of 1270mm. It has two rainy seasons; the major rainy season starts in March, peaking in May, and the minor from July with a peak in August, tapering off in November. The period from December to February is dry, hot, and dusty. The average daily temperature is approximately 27 degrees Celsius. The region is drained by the rivers Offin, Prah, Afram and Mankran, and the lake, Bosomtwe, among others. There are several smaller rivers and streams which serve both domestic and industrial purposes. As such, Ghana's Ashanti Region has, arguably, the most aquaculture activity in the country. This study is representative of the country and the West African sub-region, most of which lies in this same forest zone and has similar characteristics as listed above. Species cultured include several tilapine species such as *Oreochromis niloticus, Tilapia zillii*, *Sarotherodon galilaeus* and *Hemichromis fasciatus* (Cichlidae); *Heterotis niloticus* (Osteoglossidae) and the catfishes, *Clarias gariepinus* and *Heterobranchus bidorsalis* (Clariidae), in mostly semi-intensive systems (FAO 2009).

Field and laboratory methods

The control impact design (Karr and Chu 1999) was employed. In this type of design the abundance, or other characteristic, of a particular taxon is measured at unaffected control sites and at sites affected by an impact. Thorne and Williams (1997) concluded that in developing countries where information on the nature of pristine communities is often rare, comparisons with appropriate reference sites are the only yardstick by which water quality classifications can be made in the absence of a sufficient historical database. Water, fish and macroinvertebrate samples were collected 50 - 100m upstream and downstream of each of the 12 selected farms between May and August of 2009. An additional stream segment close to each designated study segment, with similar characteristics, that is not influenced by aquaculture was selected as a paired control (i.e., site-specific reference). This was also sampled for water, fish and macroinvertebrates.

Water samples were collected between 0800 and 1400 (appendix A), and analyzed within 24 hours of collection. Laboratory analysis of water samples followed the standard procedures in Clesceri et al. (1998) for total nitrogen (TN) (Macro-Kjeldahl method), total phosphorus (TP) (acid persulfate digestion method) and 5-day biochemical oxygen demand (BOD$_5$) (20°C incubation).

A reconnaissance study in the study area revealed that for fish sampling, a multi-gear, non-electroshocking approach was most appropriate. This was primarily due to two factors. Firstly, the muddy bottoms and high turbidity of study streams made electroshocking inappropriate. Also, the fact that several reaches were not wadable made seining alone an inadequate method. As such, seining was combined with traps and hook-and-line to get fish samples. A conscious effort was made to even out both effort and time for all sites as much as possible. Each 50m study stream reach was seined four times against the current, with a crew member stomping the stream upstream of the seine to drive fish into the seine. The use

of fish traps and hook-and-lines was fortuitous; whenever we chanced on any locals fishing at any site, we took an inventory of their catch.

For macroinvertebrate sampling, jab samples were collected 50 – 100m upstream and downstream of farms, and also from reference streams, using a standard rectangular dip net (500um mesh size). The recommended number of sampled benthic macroinvertebrate individuals for the biomonitoring of a site is 100 – 300 (J. R. Voshell *pers comm.*). So for this study I determined the number of jab samples which will result in this number of individuals as 25, and collected this from each site. The effort (number of samples) focused on each type of microhabitat at each site depended mainly on the occurrence and size of that microhabitat in relation that of other microhabitats at that site. The 25 jab samples for each site was then composited into one sample to represent that site.

Fish identifications were based on established keys such as Holden and Reed (1972), Dankwa et al. (1999), and Fishbase descriptions. Functional and reproductive traits of fish species in assemblages were determined from several sources such as Breder and Rosen (1966), Davies and Walker (1986), Lowe-McConnel (1987) and Romand (2006). Fish traits determined were: bearers, mouthbrooders, nester-guarders, guarders, nesters, broodhiders, nonguarders, sand-detritus spawners, vegetation spawners, substrate-indifferent spawners, open-water spawner, detritivore-herbivore, carnivore, and omnivore. Community metrics such as species richness and abundance were also calculated for fish samples. Proportional abundances of fish were used in all calculations to standardize the catch per unit effort due to the variability of fishing gear and stream widths.

Macroinvertebrates were identified complete to taxonomic family, even though some taxa, such as Hirudinea, Decapoda and Oligochaeta, were left at a higher taxonomic level due to the paucity of information on those. Identifications were based on recognized keys, including Dijoux et al. (1982), Brown (1994) and Merritt et al. (2008). I calculated Family

richness and abundance, Ephemeroptera, Tricoptera, Plecoptera (EPT) and Chironomid taxa metrics. EPT are recognized as orders that are sensitive to pollution (Lenat 1988), while Chironomidae are tolerant to pollution.

I also calculated the Hilsenhoff Biotic (HBI) indices (Hilsenhoff 1977) and the Shannon – Wiener diversity index (H') (Shannon 1948). The HBI combines the pollution tolerance scores and the relative abundance of taxa in to determine the level of organic pollution at a site (Zimmerman 1993). The HBI is calculated as:

$$HBI = \frac{\Sigma n i a i}{N}$$

where ni = number of individuals of a taxa i, ai = pollution tolerance score of taxa i, N = total individuals in sample. Pollution tolerance scores for benthic macroinvertebrates were compiled (and inverted in some cases) from studies such as Hilsenhoff (1988); Chessman (1995); Barbour et al. (1999); and Bode et al. (2002) (see table 2). The Shannon-Wiener Index (H') incorporates the taxa richness and abundance of a site to determine the biodiversity of that site (Shannon 1948). H' is calculated as:

$$H' = -\sum_{i=1}^{S} (pi \; In \; pi) - [\frac{S-1}{2Ni}]$$

where Ni = number of individuals in taxa i, S = total number of taxa, N = total number of all individuals, pi = relative abundance of each taxa.

I modified a number of macroinvertebrate metrics with the aim of improving their sensitivity to perturbation. The modified metrics were: Non-airbreather HBI, non-airbreather abundance, non-airbreather richness, non-airbreather percent abundance, airbreather richness, airbreather percent abundance, sensitive taxa richness, sensitive taxa percent abundance, tolerant taxa richness, tolerant taxa percent abundance. I based modifications on ability of

taxa to 'breathe' atmospheric air (Krivosheina 2005; Merrit et al 2008), and also on the tolerance score of the taxa, where I designated $0-4$ as sensitive, and $7-10$ as tolerant to organic pollution. I expected air-breathers to be more tolerant since they will not be affected by decreases in dissolved oxygen as a result of organic pollution. All metrics and indices used in this study can be found in table 2.1.

Statistical analysis

A mixed-effects ANOVA with farm as random blocks and fixed location effects was the main model, using the Tukey procedure for post-hoc analyses of location effects. Statistical significance was decided as $p \leq 0.05$ but marginal situations up to $p \leq 0.15$ were noted because of inherent variability in biological data, the relatively small sample sizes, and the consequent low power in some tests to detect significant differences unequivocally. I also examined the correlation of biological variables with the concentrations of the three physicochemical variables – nitrogen, phosphorus and BOD_5. Proportional abundances of fish were used in all calculations to off-set the disparity in fish sampling methods.

Results

Nonguarder fish species were significantly more abundant in reference streams than downstream ($p = 0.0214$) and upstream ($p = 0.0251$) (figure 2.3), and sand-detritus spawners were significantly less predominant in reference streams than upstream (p = 0.0222) and marginally less in downstream locations (p = 0.0539) (figure 2.3). The Shannon – Weiner diversity index for macroinvertebrates ranged from 0.45 to 0.92 on a scale of 0–1 and averaged 0.74 (figure 2.4). HBI values, which also did not differ significantly among the three locations, ranged from 4.7 to 7.4, on a scale of 0 to 10 (figure 2.5). When atmospheric air-breathing macroinvertebrate taxa were eliminated, HBI values ranged 4.2 to 8.75.

An examination of the correlation of TN, TP and BOD_5 with macroinvertebrate metrics revealed trends in most of these metrics associated with levels of these three

physicochemical variables (appendix C). BOD_5 was negatively correlated to almost all metrics. Both total phosphorus and total nitrogen showed strong correlations with various metrics, either way, even though nitrogen had stronger correlations with metrics compared to phosphorus. While increasing nitrogen levels was associated with increasing macroinvertebrate richness and richness of tolerant species, levels of the N: P ratio was inversely related to the richness and proportional abundance of EPT, Non-air breathing and sensitive taxa (figure 2.6; appendix C).

A total of 28 different fish species (table 1) and 54 benthic macroinvertebrate taxa (table 2) were identified within the study area. No stoneflies (Plecoptera) were found within the study area. Fish species richness in sampled assemblages ranged from 1 to 10, with an average of 4 species (figure 1). Macroinvertebrate taxa (mostly family) richness in a given sample ranged from 9 to 26 with an average of 17 (figure 1). These and other structural and functional metrics for both fish and macroinvertebrates showed no significant differences among upstream, downstream, and reference streams, with the exception of two fish reproductive metrics – percent nonguarder species and percent of species that are sand-detritus spawners.

Discussion

The degree of parental care in fish is known to be directly related to the level of perturbation of a site (Burcher et al 2008). Hence, bearers and mouthbrooding fish are expected to be more abundant in more impacted sites (Burcher et al 2008), since young are not exposed as much to perturbation as fish with other traits. Fish with other traits are expected to either emigrate to more conducive sites or die out. On the other hand, sites with more pristine conditions are known to be characterized by fish with a low degree of parental care. For example, the non-guarding ethological fish trait is characteristic of ecosystems that achieve stability through repeated, unpredictable cycles, such as rivers subject to episodic

floods (Bruton and Merron 1990). While I anticipated a higher degree of perturbation at locations downstream of fish farms in my study area due to the high probability of pollution by aquaculture effluents, I expected reference stream locations to be more pristine. As such, I expected a higher proportional abundance of bearers and mouth brooding fish at downstream locations and a higher abundance of non-guarding fish species in reference streams. The availability of a particular spawning substrate is also expected to determine the abundance of particular fish species at a site. Since I observed a prolific growth of macrophytes and algae downstream of farms as a result of the likely pond effluent-enriched nutrients levels in those locations, I expected this to ethologically encourage a higher abundance of vegetation-spawning fish species at these locations.

Fish ecological traits are associated with the availability of particular food items at a site. For example, I expected a higher abundance of the more generalist detritivore-herbivore fish species at downstream sites (Burcher et al 2008), also due to the prolific growth of macrophytes and algae. All ecological and most ethological traits calculated showed no significant differences among locations. I observed more predictable conditions upstream and downstream of study farms as a result of embankments constructed upstream of these farms to facilitate aquaculture operations, and the regulated flow of water downstream of farms. Hence the natural, more unpredictable flood events only occurred in the reference streams, where the abundance of non-guarding fish species was significantly higher than that of the other two locations. Also, due to the fact that my study streams were relatively small, high elevation (approx. mean = 240m ASL, appendix A) streams, I expected fluvial deposition to be scarce (Schumm et al. 1987), but the constructed embankments upstream, and the level of settleable solids downstream (see chapter 1), coupled with the abundance of macrophytes in the nutrient-rich stream reaches below farms, seemed to facilitate deposition of sediment at these locations. These factors could have led to the higher abundance of sand-detritus

spawning fish species at upstream and downstream sites (Balon 1975), unlike the more erosional reference streams, where sand-detritus spawners were significantly less abundant.

The generally similar upstream, downstream and reference locations, in terms of the proportional abundance of fish with the ecological and ethological traits considered suggests that either aquaculture, which was my point of comparison among locations, has had only minimal impact on pond effluent-receiving streams, or there are other possible sources of organic pollution in the watershed that evened out the impacts of aquaculture on stream water quality.

HBI values were intermediate, indicating that the quality of my study streams ranged from good, with some organic pollution, to fairly poor, with significant organic pollution (Hilsenhoff 1977). Also, increasing BOD_5 levels was associated with a decrease in almost all macroinvertebrate metrics. These results point to the fact that that study streams were possibly impacted by organic pollution (not necessarily from aquaculture alone). As such, I expected low macroinvertebrate diversity, but the Shannon-Wiener diversity index (H') for benthic macroinvertebrates indicated high average macroinvertebrate diversity from study streams. H' is directly related to the richness of taxa at a site (Lenat and Crawford 1994) but it does not show whether increases in taxa richness at a site is from sensitive or non-sensitive taxa. It was observed that EPT, non-air breathing and sensitive taxa were replaced by the more tolerant ones, and so the increases in metrics associated with increases in the nitrogen and the N: P ratio was most likely from tolerant, but not sensitive species.

The high diversity of benthic macroinvertebrates, with the moderate organic pollution (indicated by HBI), is indicative of a possible subsidy-stress response. This response is a reaction of a particular ecosystem to the augmentation of a limiting variable in a particular system (Odum 1979). Nitrogen was strongly correlated with most of the macroinvertebrate metrics used in this study. Chapter 1 indicated that total nitrogen was the one variable most

clearly higher in fish ponds compared to reference, upstream, and downstream locations ($p =$ 0.0016, 0.0086, and 0.0154 respectively) in the study area. Hence, nitrogen could be limiting in the study streams.

Generally, biodiversity decreases with increasing stress due to the disappearance of sensitive species (Resh and Jackson 1993). This implies that theoretically, additional increases in the stress (possibly from nitrogen enrichment) on the study streams will result in a reduction in biodiversity along the inverse relationship that exist between system stress and biodiversity (figure 2.7), in line with the subsidy stress response concept (Odum 1979). The subsidy-stress response has been identified in a number of studies. Niyogi (2007) found subsidy-stress relationships between some biotic responses, including benthic macroinvertebrate richness, and nutrient index in agriculture-impacted streams in New Zealand. King (2007) also identified this response in 8 out of 10 taxonomic groups in the Florida Everglades, to phosphorus enrichment.

Nitrogen has been shown to be limiting in some tropical freshwater systems (Setaro and Melack 1984), and it is abundant in aquaculture pond effluents (Diana et al 1997) and also in run-off from tree and vegetable farms (Withers and Lord 2002; Ullah and Faulkner 2006). My results indicate that nitrogen, which could be limiting in the study streams, could play a regulatory role (subsidy or stress) on biodiversity and other ecosystem metrics within streams in the study area (Riley et al. 2003, Graca et al. 2004). Since my study area was largely rural, other possible sources of nutrients were few. Intensive terrestrial agriculture (cultivation of maize, vegetables, fruits and cash crops, such as cocoa) (Akrasi and Ansa-Asare 2008) is common in the study area, and it could be a major contributor of nitrogen into both receiving and reference streams, from herbicide and inorganic fertilizer waste run-off (Niyogi 2007). I also observed a large number of piggeries, sheep and poultry farms within

the study area. Farm wastes, mostly fecal matter, could be washed into these streams, leading to nutrient enrichment of the streams.

There was no evidence of biological pollution from aquaculture facilities within the study area. All sampled fish were native to the drainages from which they were collected (Dankwa et al. 1999). I identified 28 fish species within my study area. Dankwa et al. (1999) took an inventory of fish in Ghana and came up with 157 as the total number of species within the country. My lower fish species richness was expected since Ghana's Volta River alone has 121 species with a number of endemics (Dankwa et al. 1999), and this river system was outside of my study area. I learned of some fish escapes from aquaculture facilities due to pond flooding or broken embankments, but since only native species were cultured, it was difficult to confirm this from my species list.

I also found between 9 and 26 macroinvertebrate families in the different study locations with a total family count of 54. Thorne and Williams (2007) found that macroinvertebrate family richness of the Odaw River in Ghana to be between 3 and 15 for the different sites, with 19 families in all, but while I took 25 jab samples from each of my 36 sites, they took just 6 surber samples from each of their 5 sites. Additionally, my study area was largely rural, within the moist semi-deciduous forest zone to the north of theirs, which was largely within the urban, likely more-impacted portions of the coastal savanna (Lenat and Crawford 1994). Kuusela (1979) found that the number of species in a Finnish river increased with the number of samples collected. MacArthur and Wilson (1967) also found that number of species was positively correlated with the size of the sampling area. Therefore considering my larger study area and number of sites and samples, it was expected that I have more families (Allan 1995).

Conclusions

The general similarity observed between effluent-receiving and reference streams, in terms of biotic condition, points to the fact that either the impact of aquaculture on receiving streams was insignificant, or that there were other industries, such as terrestrial agriculture, within the watershed that were also contributing stress to these streams, besides aquaculture. Also, it was shown that theoretically, further nutrient augmentation could result in sharp drops in stream community metrics such as biodiversity (Odum et al. 1979). Hence, attempts to manage nutrient levels in the study streams must target all source industries in addition to aquaculture.

Table 2.1 – Metrics used in the study of biotic condition of effluent-receiving streams in the Ashanti Region of Ghana.

Metric	
Fish	**Meaning**
Bearer	Fry are born; not hatched.
Mouthbrooder	Either the male or female, or both parents keep eggs and fry in the mouth for varying lengths of time.
Nester-guarder	These build and guard nests, eggs and fry.
Guarder	Nests are not always built, but eggs and fry are guarded.
Nester	Nests are built, but egg and fry guarding and tending not guaranteed.
Broodhider	An attempt is made to hide clutch, but there is no guarding.
Nonguarder	There is no egg-tending or guarding after spawning.
Sand-detritus spawner	Requires sand or detrital substrate for spawning; psammophil.
Vegetation spawner	Requires living macrophytes (e.g. leaves, etc) as spawning substrate; phytophil.
Substrate-indifferent spawner	Has no specific substrate requirement for spawning.
Open-water spawner	Spawns freely in the water column.
Detritivore-herbivore	Feeds on plant matter or decaying material.
Carnivore	Feeds on fish, macroinvertebrates or other animals.
Omnivore	Has no specific feeding material requirement.
Species richness	Number of unique fish species found in a specified area.
Macroinvertebrate	
Family richness	Number of unique macroinvertebrate families found in a specified area.
Family abundance	Number of individuals belonging to a particular family.
Dominance	Degree to which particular taxa out-number others at a site.
EPT richness	Number of families belonging to the orders; Ephemeroptera, Plecoptera and Tricoptera, found in a specified area.
EPT : Chironomid	Ratio of proportional abundance of Ephemeroptera, Plecoptera and Tricoptera families to the abundance of Chiromomidae in a specified area.

EPT abundance	Percentage of total number of individuals belonging to the orders; Ephemeroptera, Plecoptera and Tricoptera.
Chironomid abundance	Percentage of total number of individuals belonging to the family; Chironomidae.
Shannon-Wiener Index	Combines family richness and relative abundance of particular families to determine the diversity of a site.
Hilsenhoff Biotic Index	Combines the pollution tolerance scores and relative abundances of the different families at a site to determine the level of organic pollution at a site.
Non-airbreather HBI	HBI calculation based solely on families that rely on dissolved oxygen.
Non-airbreather abundance	Number of individuals of families that rely on dissolved oxygen.
Non-airbreather richness	Number of unique families that rely on dissolved oxygen.
Non-airbreather percent abundance	Proportional abundance of the number of individuals that rely on dissolved oxygen, in a specified area.
Airbreather richness	Number of unique families that use atmospheric oxygen.
Airbreather percent abundance	Proportional abundance of the number of individuals that breathe atmospheric oxygen.
Sensitive taxa richness	Number of unique families that have pollution tolerance scores 0-4
Sensitive taxa percent abundance	Proportional abundance of the number of individuals that have pollution tolerance scores 0-4
Tolerant taxa richness	Number of unique families that have pollution tolerance scores 7-10
Tolerant taxa percent abundance	Proportional abundance of the number of individuals that have pollution tolerance scores 7-10

Table 2.2 – Species data on sampled 28 fish species, with references for ecological and ethological information

Species name	Common name	Family	Food Habits	Ref.*	Ethological guild	Ref.*	Spawning substrate	Ref.*	Occurrence (out of 36 sites)
Alestes baremoze	Silverside	Characidae	omnivore	8, 3, 4	nonguarder	8	open water	8	1
Aphyosemion petersii	African lyretail	Cyprinodontidae	omnivore	3	broodhider	8	sand/detritus	1	1
Aplocheilichthys normani	Norman's lampeye	Cyprinodontidae	carnivore	7, 8	nonguarder	1	vegetation	1	7
Barbus ablabes	Ablabe's barb	Cyprinidae	herbivore	8	nonguarder	8, 1	vegetation	1	6
Barbus bynni occidentalis	Niger barb	Cyprinidae	omnivore	3	nonguarder	1	vegetation	1	1
Barbus macrops	Blackstripe barb	Cyprinidae	carnivore	8, 4	nonguarder	1	vegetation	1	24
Barbus trispilos	Three-blotched barb	Cyprinidae	omnivore	3	nonguarder	1	vegetation	1	12
Brycinus brevis	African tetra	Characidae	omnivore	5	nonguarder	1	vegetation	1, 3	1
Brycinus longipinnis	Longfin tetra	Characidae	detritivore	8	nonguarder	8, 1	vegetation	1, 3	1
Chromidotilapia guentheri	Guenther's mouthbrooder	Cichlidae	herbivore	8	mouthbrooder	8, 3	indifferent	3	6
Clarias gariepinus	North African catfish	Clariidae	omnivore	8, 3	nonguarder	8, 5	vegetation	1	2
Clarias sp.	-	Clariidae	omnivore	8, 3	nonguarder	8, 5	vegetation	1	4
Epiplatys chaperi	Toothed carp	Cyprinodontidae	omnivore	3	nonguarder	8, 1	sand/detritus	1	2
Epiplatys dageti	Redchin panchax	Cyprinodontidae	omnivore	3	nonguarder	8, 1	vegetation	1	6
Epiplatys sexfasciatus	Sixbar panchax	Cyprinodontidae	carnivore	8	nonguarder	8, 1	vegetation	1	3
Hemichromis	African	Cichlidae	carnivore	8,3	nester/guarder	8, 1,	sand/detritus	1	3

Species	Common name	Family	Diet	Ref	Reproduction	Ref	Substrate	Ref	No.
bimaculatus	jewelfish				3				
Hemichromis fasciatus	Banded jewelfish	Cichlidae	carnivore	8, 3, 4	nester/guarder	9, 1	sand/detritus	8, 1	5
Heterobranchus sp	-	Clariidae	carnivore	8	nonguarder	6	sand/detritus	6	4
Hydrocynus forskalii	Tigerfish	Characidae	carnivore	8, 3, 4	nonguarder	1, 3	vegetation	1, 3	1
Micralestes occidentalis	Sharptooth tetra	Characidae	omnivore	3, 4	nonguarder	4	vegetation	4	4
Oreochromis niloticus	Nile tilapia	Cichlidae	omnivore	8, 4	bearer	8	indifferent	8	9
Parachanna obscura	African snakehead	Channidae	carnivore	8	guarders	1	vegetation	1	9
Poecilid species	Livebearers	Poeciliidae	insectivore	2	bearer	8, 1	indifferent	8, 1	2
Sarotherodon galilaeus galilaeus	Mango Tilapia	Cichlidae	detritivore	8, 3, 4	mouthbrooder	8, 3	indifferent	8	2
Tilapia dageti	-	Cichlidae	omnivore	4	nester	1	sand/detritus	1	12
Tilapia zilli	Redbelly tilapia	Cichlidae	omnivore	8, 4	nester	1, 3	sand/detritus	1	2

*Ref. = References: 1 - Breder and Rosen (1966); 2 - Meffe (1985); 3 - Davies and Walker (1986); 4 - Lowe-McConnel (1987); 5 - Bruton (1995); 6 - Poncin et al (2002); 7 - Romand (2006); 8 – Fishbase.org

Table 2.3 – List of 54 macroinvertebrate families showing pollution tolerance scores

Sampled taxa	Family	Pollution tolerance score	Reference	Occurrence (out of 36 sites)
Ephemeroptera	Baetidae	4	1, 4, 8	27
	Caenidae	7	1, 8	16
	Leptophlebiidae	2	1, 4, 8	7
	Polymitarcyidae	2	1, 5, 8	2
	Heptageniidae	4	1, 4, 8	7
	Oligoneuriidae	2	1, 5, 8	2
Trichoptera	Limnephelidae	4	1, 8	2
	Leptoceridae	4	1, 5, 8	4
	Hydropsychidae	4	1, 5, 8	7
Odonata	Aeshnidae	3	1, 5, 8	4
	Calopterygidae	5	1, 8	8
	Chlorocyphidae	5	12	2
	Coenagrionidae	9	1, 9	30
	Libellulidae	9	1, 8	32
	Gomphidae	1	1, 8	7
Coleoptera	Elmidae	4	1, 5, 8	9
	Dytiscidae	5	5, 9	26
	Gyrinidae	4	4, 5, 6	13
	Haliplidae	5	5, 6	3
	Hydrophilidae	5	5, 9	25
	Scirtidae	5	4, 5, 9	20
Hemiptera	Belostomatidae	5	12	30
	Corixidae	5	5, 7	12
	Gerridae	5	6	27
	Hydrometridae	5	6, 4	8
	Naucoridae	5	6	12
	Nepidae	5	6	23
	Pleidae	5	11	11
	Notonectidae	5	11	21
	Veliidae	5	11	26
Diptera	Ceratopogonidae	6	1, 5, 8	9
	Chironomidae	8	1, 5, 6, 8	31
	Culicidae	8	5, 6, 10	2
	Ephydridae	6	1, 5	3
	Sciomyzidae	8	6	2
	Simuliidae	6	1, 5, 6, 8	5
	Stratiomyidae	7	5, 6, 9	4

	Syrphidae	10	1, 5, 6, 8	3
	Tabanidae	6	1, 8	3
	Tipulidae	3	1, 5, 8	2
Gastropoda	Physidae	5	4	25
	Planorbidae	7	4, 5, 7	16
	Pleuroceridae	6	5, 7	17
	Potamididae	6	5	7
	Thiaridae	7	4	10
Decapoda	Atyidae	6	3	17
	Potamonautidae	6	5	11
Others				
Lepidoptera	Crambidae	5	9	7
Hirudinea		10	5	16
Isopoda		8	1, 5, 7	2
Bivalvia	Mussel	8	5, 7	2
Oligochaeta		8	5, 6	17
Hydracarina		6	9	4
Collembola		5	5	2

References: 1-Hilsenhoff (1988); 2-Spark (1993); 3-Sharma et al. (2006); 4-SWCSMH (2008); 5-Gabriels (2009); 6-Barbour (1999); 7-Hauer and Lamberti (1996); 8-Bode et al. (1996); 9-Bode et al. (2002); 10- Sivaramakrishnan (2000); 11-Chessman (1995)
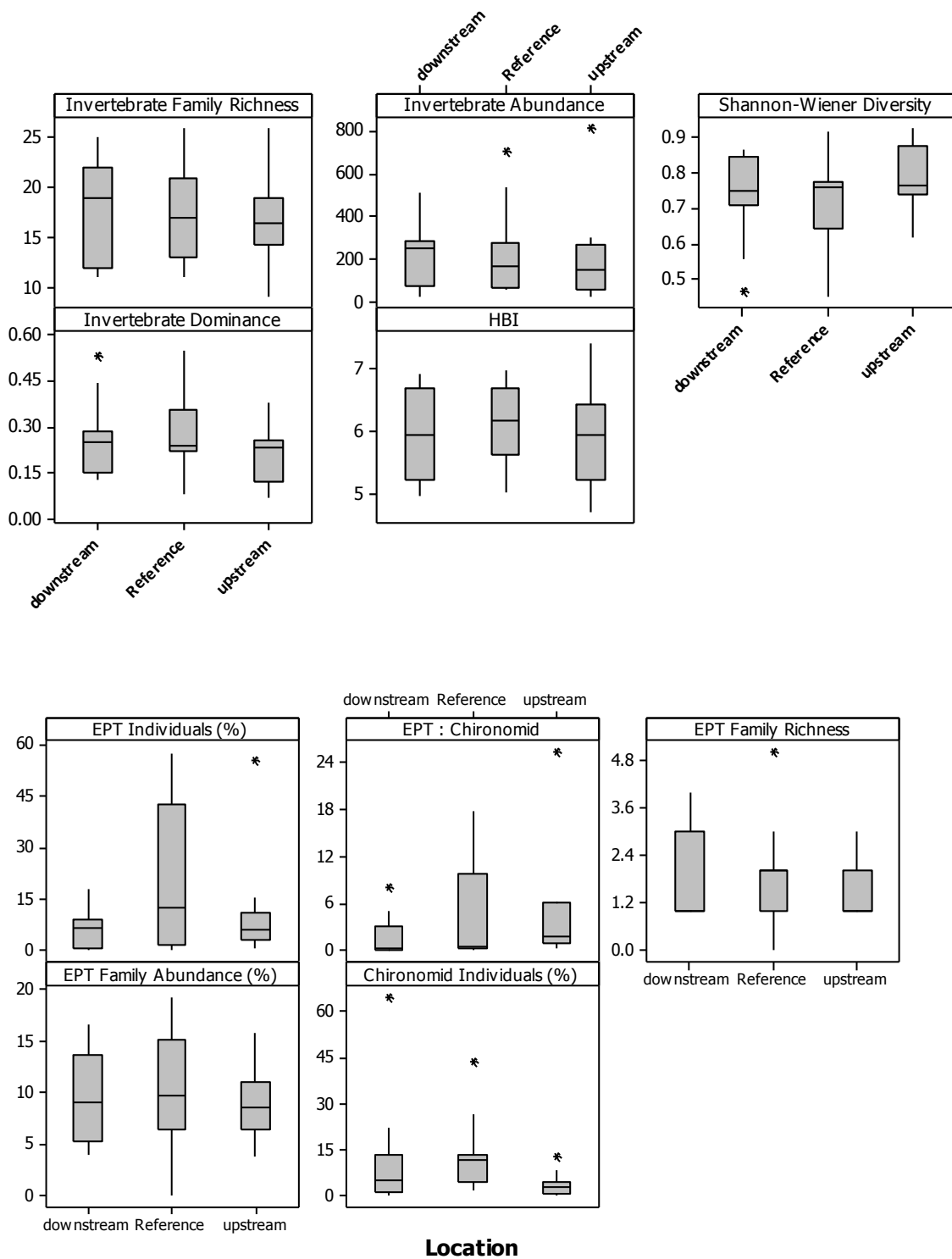
Figure 2.1 – Comparison of benthic macroinvertebrate metrics and indices among downstream, reference and upstream locations.
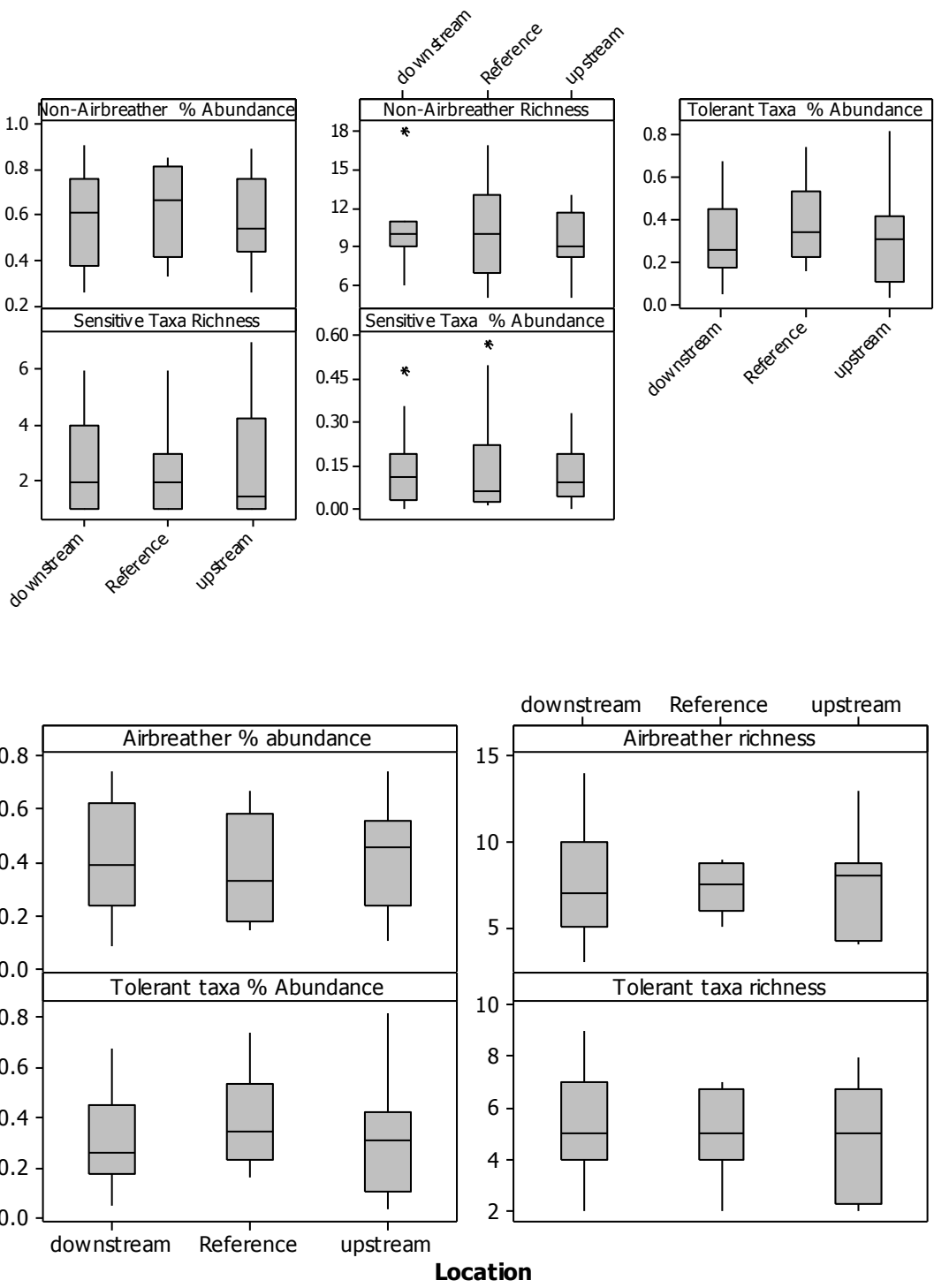
Figure 2.2 – Comparison of modified macroinvertebrate metrics and indices among downstream, reference and upstream locations.
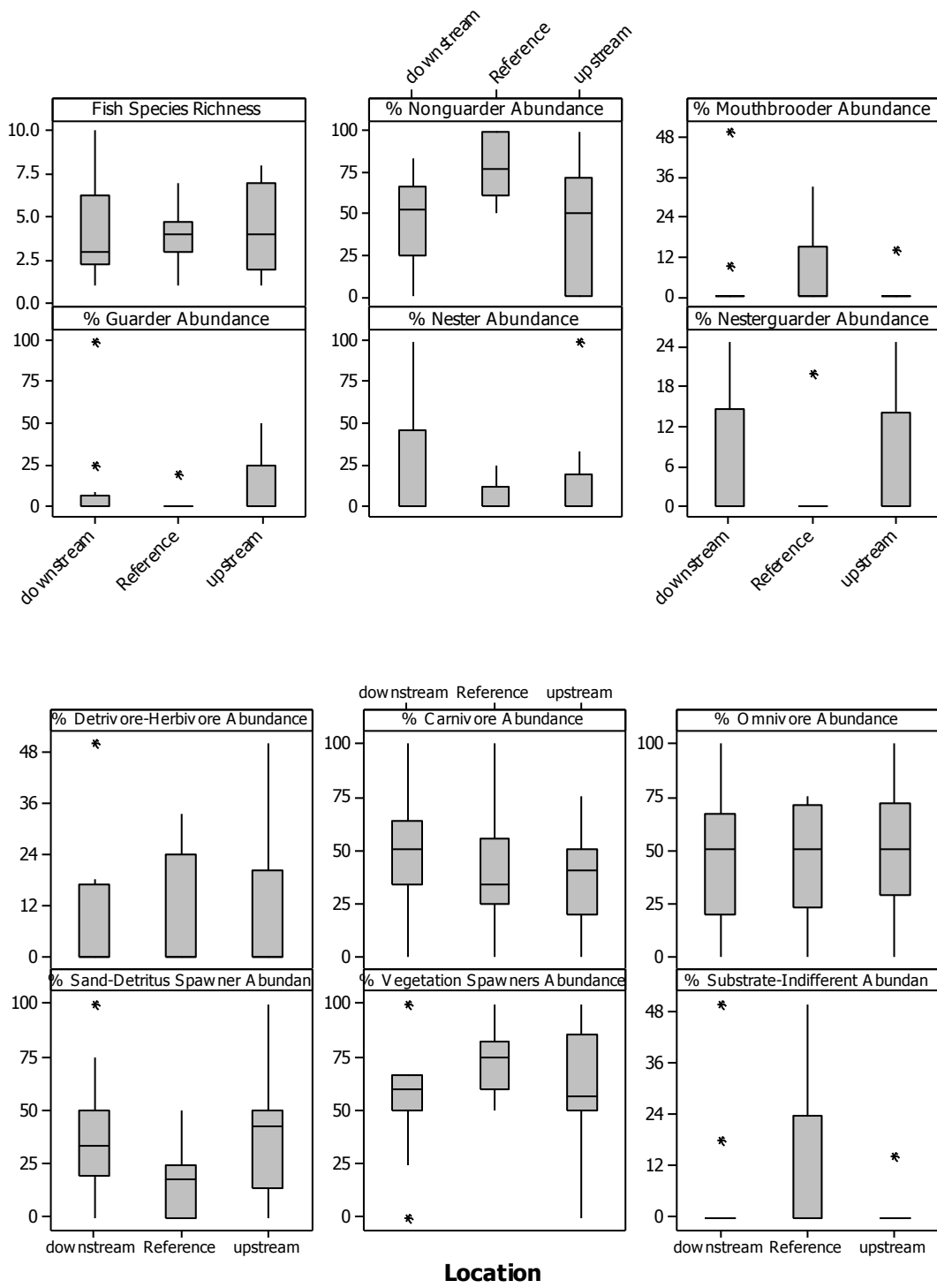
Figure 2.3 – Comparison of fish species proportional abundance metrics among downstream, reference and upstream locations.
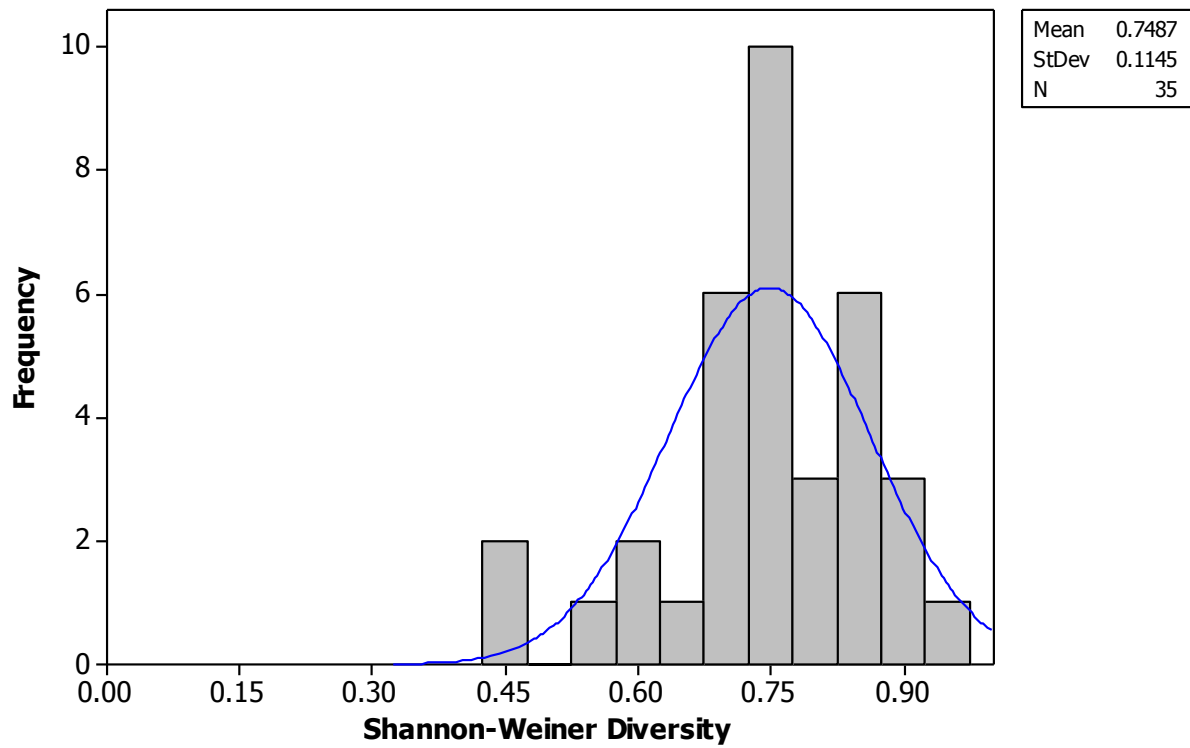
Figure 2.4 – Spread of diversity values for 36 sampled sites in Central Ghana.
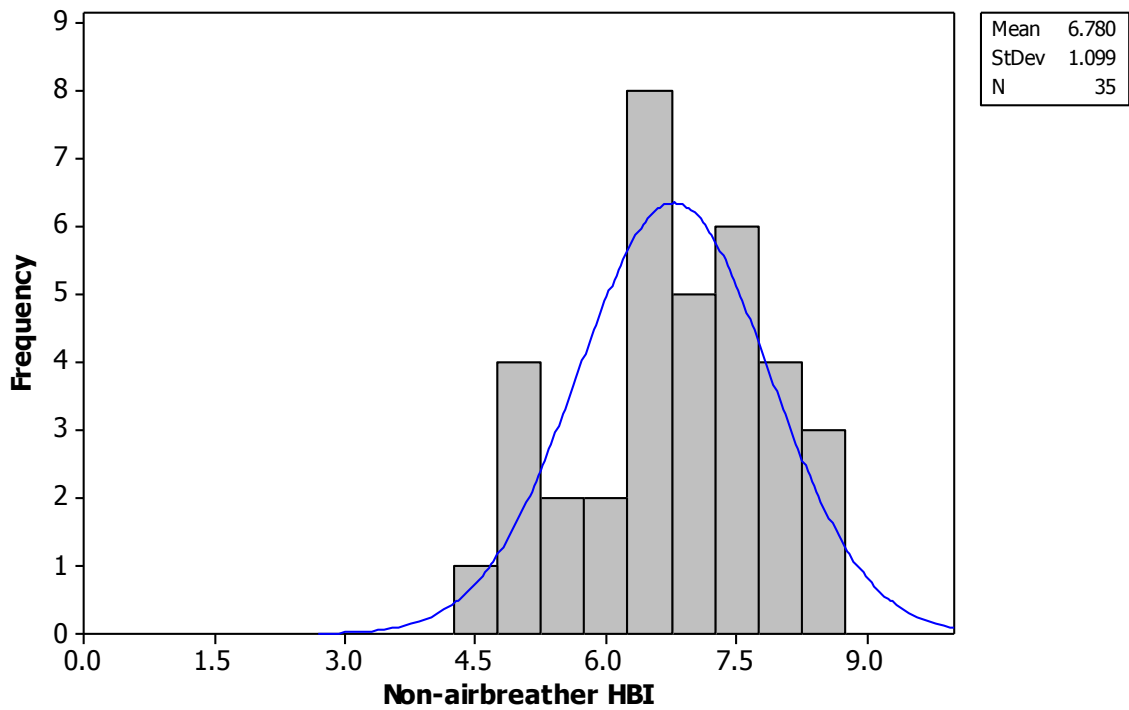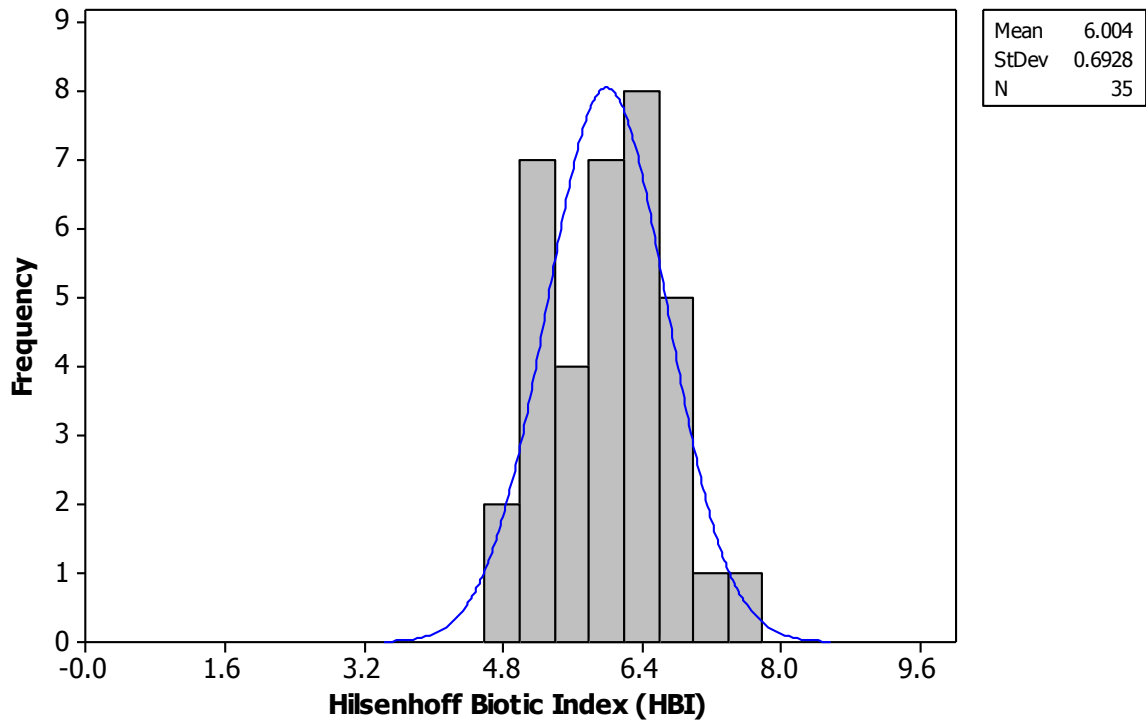
Figure 2.5 – Hilsenhoff (family) Biotic Index values for 36 sampled sites in Central Ghana. Top: all taxa; bottom: air-breathing taxa eliminated.
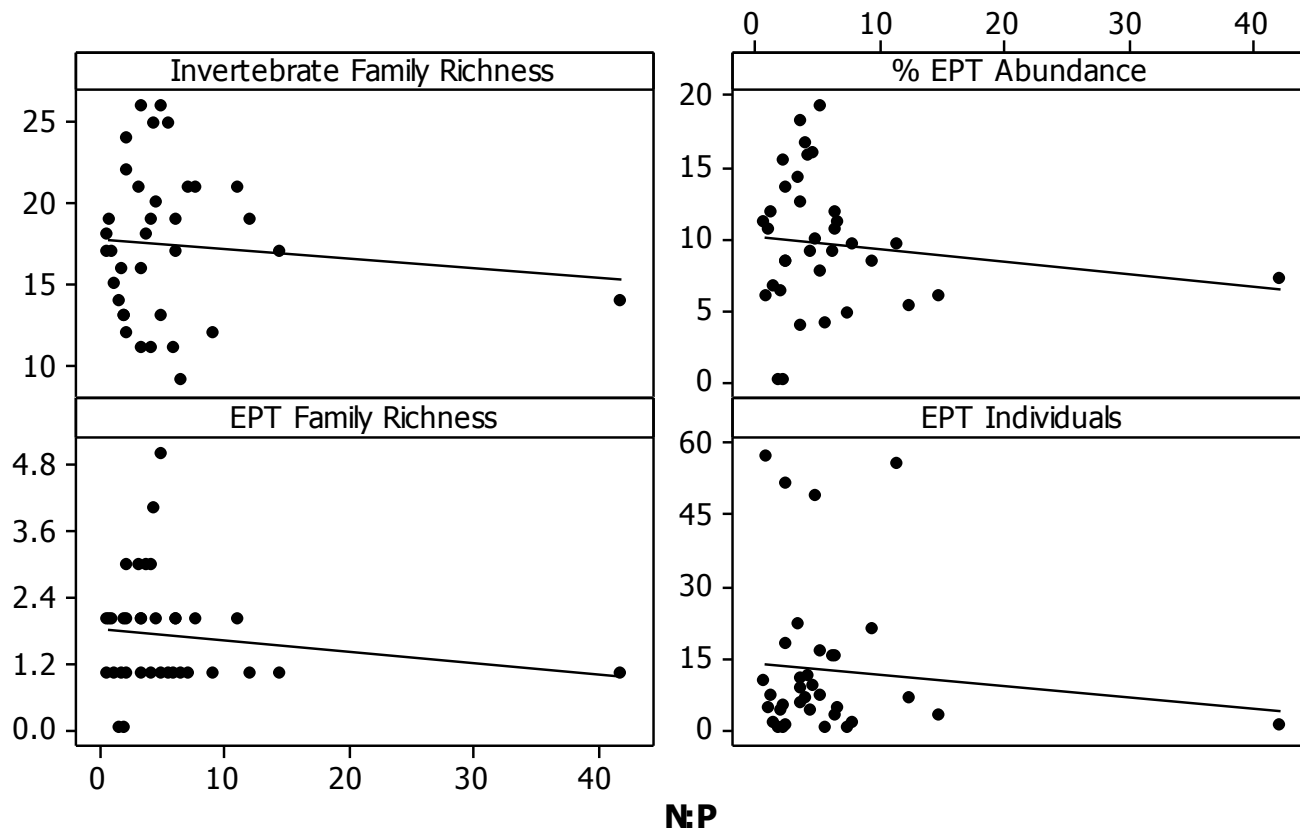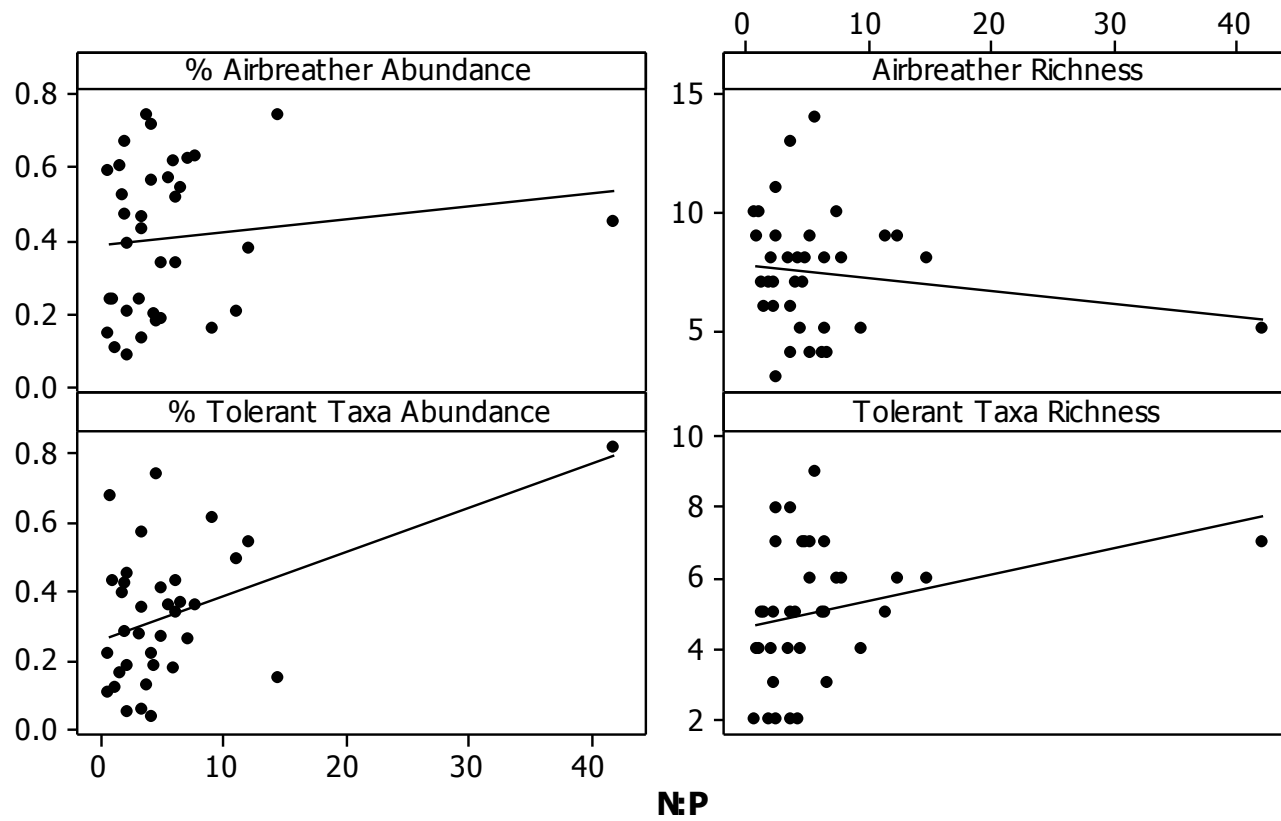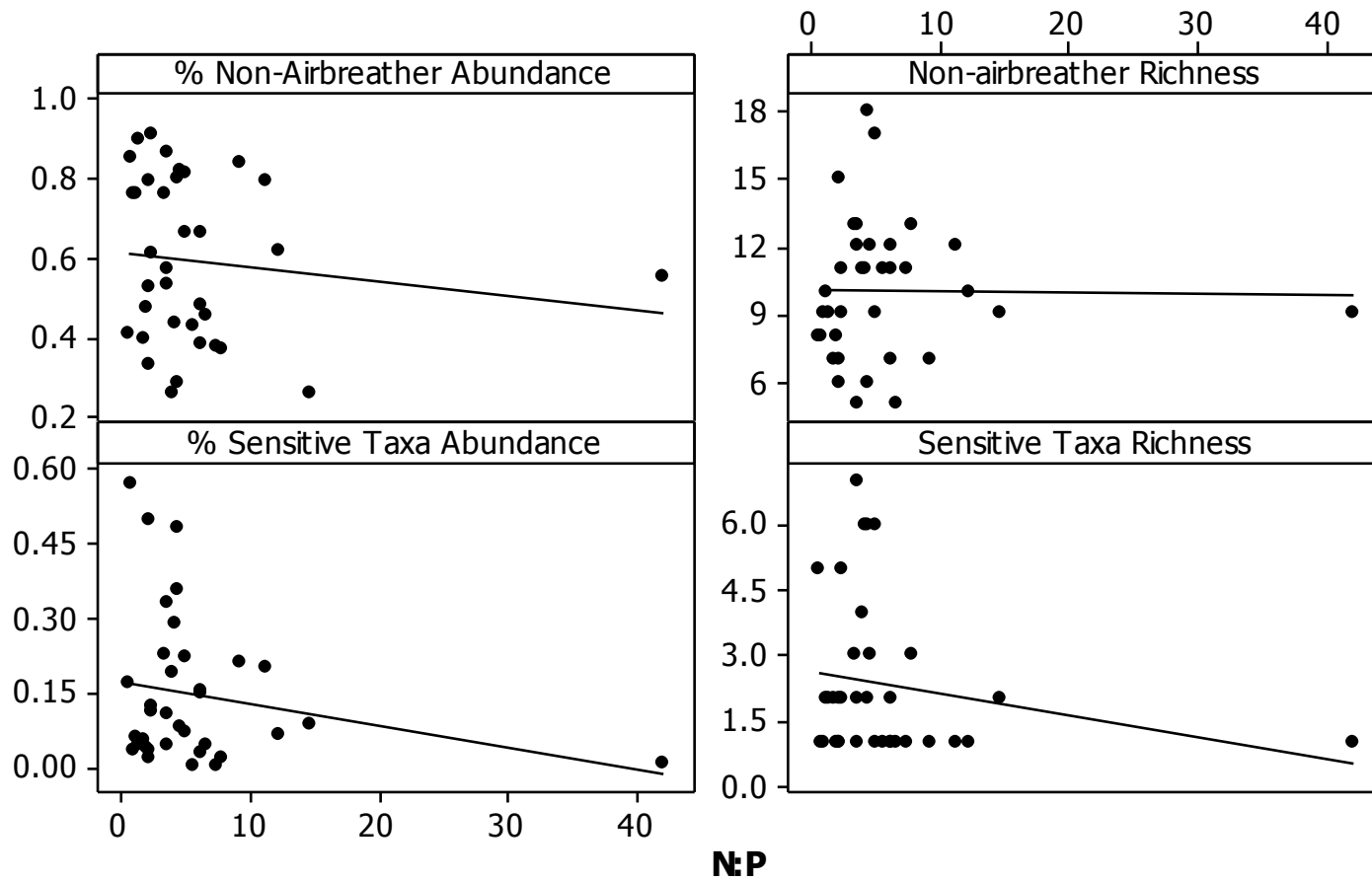
Figure 2.6 – Relationship between benthic macroinvertebrate metrics of biotic condition and levels of the N: P ratio.
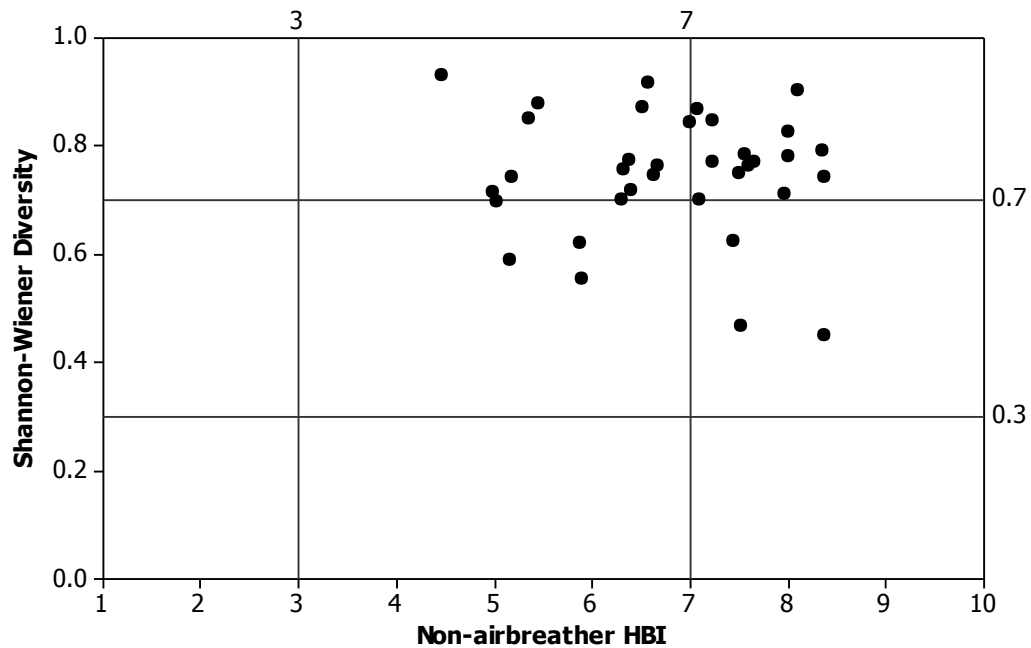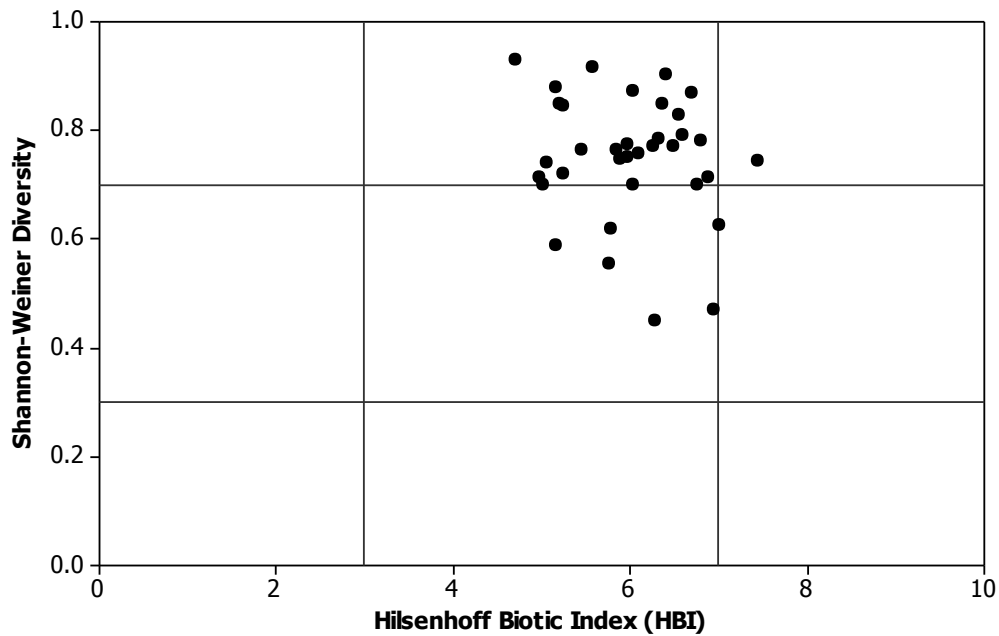
Figure 2.7 – Relationship between benthic invertebrate diversity and perturbation (HBI) in Central Ghana streams. An intermediate level of perturbation corresponds to a high diversity (subsidy-stress response). Increases in perturbation will drive benthic macroinvertebrate diversity down along the inverse relationship that exists between diversity and perturbation. Top: all macroinvertebrates; bottom: Atmospheric air-breathers eliminated.

Summary and general conclusions

Total nitrogen, total phosphorus, BOD$_5$ levels, and that of other physicochemical and microbial variables at sites upstream of aquaculture farms and at reference sites closely matched those of downstream sites, with concentrations being highest at downstream sites. Concentrations of variables at downstream sites were also closest to the high levels in fish ponds. This implied that fish farms actually contributed to the concentrations of these variables in stream reaches downstream of the farms, but bioassessment indicated that the impact of aquaculture on streams was either marginal or closely matched by other polluting industries within the watershed. None of the 12 farms drained their ponds within the study period, and I confirmed that draining was not a common event. However, it was common to find a large volume of overflow from fish ponds during heavy rainfall. Due to the possible subsidy-stress response that was identified within streams, further nutrient augmentation could result in sharp drops in stream community metrics. Also, since there are other possible polluting industries within the study area, such as fruit, vegetable and livestock farms, any attempts to manage nutrient levels in the study streams should target all potential source industries in addition to aquaculture.

Future studies, apart from increasing samples sizes and extent of study area, could focus on the determination of indicator fish and macroinvertebrate species, along with pollution tolerance scores within the study area and the West African sub-region. The fact that all bioassessment metrics used in this study were based on studies done mostly in temperate or arid regions of the world could have implications for their sensitivity to the unique conditions of my study area. *Epiplatys dageti* and Coenagrionidae are two examples of potential indicator taxa from Central Ghana and the West African sub-region. The probability of finding *E. dageti* was

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seen to decrease with total nitrogen levels (figure 2.9). Also, the proportional abundance of the odonate family Coenagionidae was also observed to be inversely related to total phosphorus levels (figure 2.10).
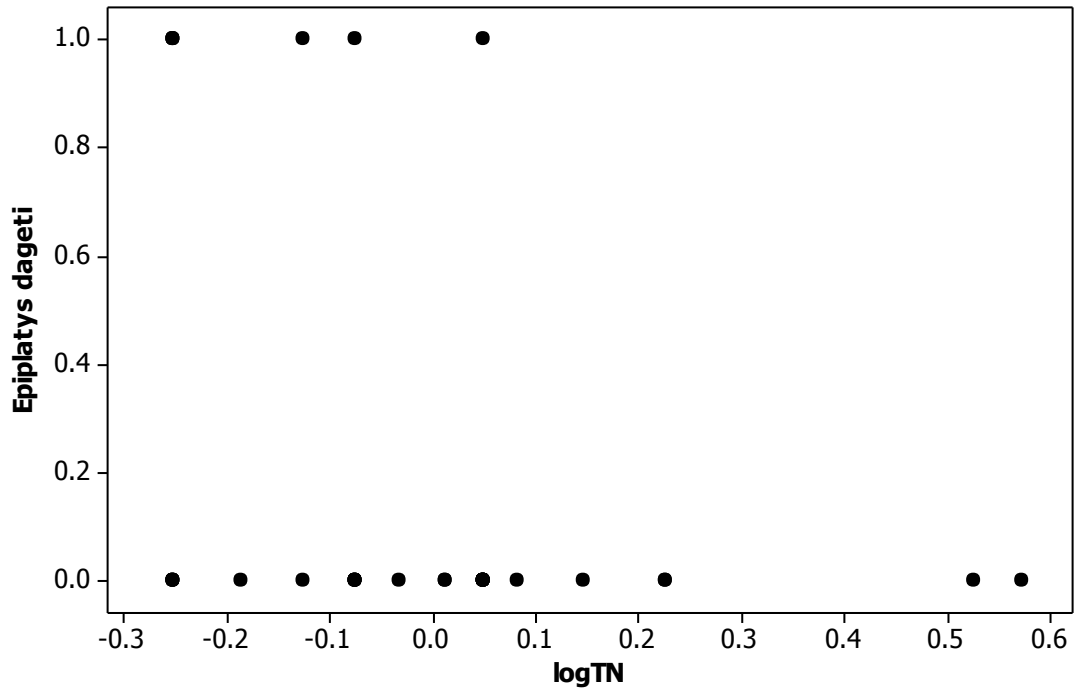
Figure 2.8 – Presence-absence of the cyprinodont, *Epiplatys dageti* in response to levels of total nitrogen.
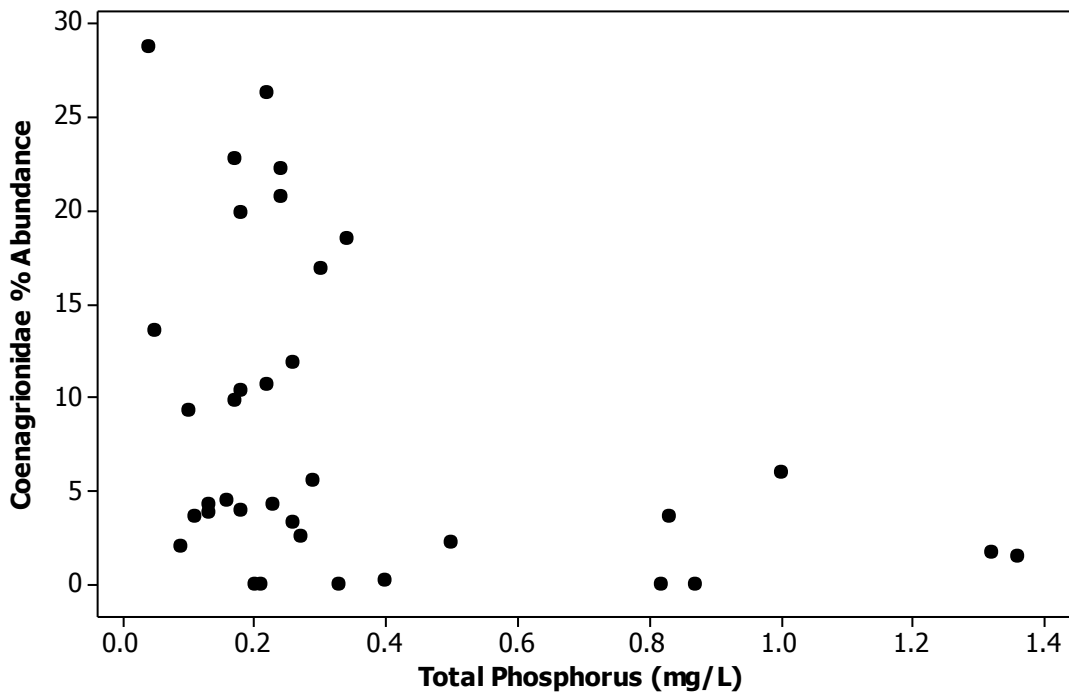
Figure 2.9 – Relationship between the proportional abundance of the odonate, Coenagrionidae and concentration of total phosphorus.

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Appendices

APPENDIX A. – List of 12 sites sampled for physicochemical and biological analysis, showing time of sampling for each sampled location.

Site Code	Time	Location	GPS Northing	GPS Westing	Elevation (m)
A	0932	downstream	06.40.964	001.34.328	255.4
A	1215	pond 1	06.39.933	001.34.386	260.0
A	1250	pond 2	06.39.951	001.34.365	251.4
A	1315	pond 3	06.39.964	001.34.424	248.3
A	1346	upstream	06.40.002	001.34.543	178.3
A	0950	reference	06.41.613	001.32.074	255.1
B	0858	pond 1	06.40.661	001.30.084	251.9
B	0907	pond 2	06.40.626	001.30.044	255.5
B	0914	pond 3	06.40.600	001.30.072	252.2
B	0948	downstream	06.40.578	001.30.079	279.3
B	1217	upstream	06.40.709	001.30.027	255.1
B	0844	reference	06.41.509	001.31.395	267.7
C	0854	pond 1	06.40.403	001.22.093	219.8
C	0858	pond 2	06.40.432	001.22.163	215.2
C	0901	pond 3	06.40.448	001.22.115	219.7
C	1015	downstream	06.40.376	001.22.202	216.0
C	1319	upstream	06.40.436	001.22.010	556.2
C	1530	reference	06.40.370	001.23.621	220.5
D	0902	pond 1	06.35.411	001.34.272	217.2
D	0911	pond 2	06.35.419	001.34.297	243.5
D	0914	pond 3	06.35.418	001.34.308	243.9
D	0927	downstream	06.35.421	001.34.368	217.6
D	1110	upstream	06.34.206	001.34.206	243.2
D	1315	reference	06.35.495	001.34.206	221.3
E	1005	pond 1	06.49.753	001.57.849	208.7
E	1021	pond 2	06.49.740	001.57.828	209.2
E	1010	pond 3	06.49.734	001.57.774	203.9
E	1033	downstream	06.49.734	001.51.750	193.9
E	1159	upstream	06.49.811	001.52.067	170.2
E	1400	reference	06.50.134	001.57.706	208.7
F	1008	pond 1	06.49.840	001.53.407	215.8
F	1014	pond 2	06.49.839	001.53.395	213.0
F	1021	pond 3	06.49.832	001.53.378	213.5
F	1045	downstream	06.49.850	001.53.646	210.0
F	1243	upstream	06.49.813	001.53.334	216.5
F	1412	reference	06.51.281	001.55.027	221.4
G	820	pond 1	06.53.118	001.30.143	278.2

G	828	pond 2	06.53.150	001.30.146	286.1
G	835	pond 3	06.53.133	001.30.163	277.8
G	911	downstream	06.53.259	001.30.314	280.8
G	1025	upstream	06.53.090	001.20.123	284.1
G	1121	reference	06.54.339	001.29.844	279.5
H	905	pond 1	06.31.547	001.38.158	217.1
H	918	pond 2	06.31.561	001.38.095	212.5
H	928	pond 3	06.31.551	001.38.088	208.4
H	1020	downstream	06.31.576	001.38.039	202.4
H	1209	upstream	06.31.445	001.38.401	207.3
H	1315	reference	06.31.818	001.37.872	207.3
I	831	pond 1	06.27.822	001.37.880	186.6
I	857	pond 2	06.27.807	001.37.815	178.5
I	904	pond 3	06.27.861	001.37.811	179.1
I	932	upstream	06.27.941	001.37.953	182.6
I	1212	reference	06.27.817	001.38.883	186.1
J	930	pond 1	07.18.366	001.57.913	309.0
J	938	pond 2	07.18.381	001.57.918	306.0
J	948	pond 3	07.18.407	001.57.923	304.0
J	1039	downstream	07.18.347	001.57.851	292.0
J	1116	upstream	07.18.434	001.57.909	285.0
J	1330	reference	07.20.176	001.58.992	275.0
K	1037	pond 1	06.36.896	001.12.634	258.9
K	1057	pond 2	06.36.935	001.12.657	214.8
K	1121	pond 3	06.36.952	001.12.653	230.7
K	937	upstream	06.36.986	001.12.631	223.2
K	930	downstream	06.36.960	001.12.680	227.0
K	1012	reference	06.42.489	001.07.930	287.4
L	822	pond 1	06.39.348	001.50.841	210.8
L	830	pond 2	06.39.416	001.50.783	214.8
L	833	pond 3	06.39.434	001.50.783	216.6
L	1112	upstream	06.39.354	001.50.871	215.9
L	900	downstream	06.39.463	001.50.792	212.6
L	1227	reference	06.38.902	001.54.311	211.6

APPENDIX B. - Correlation of management practices with physicochemical and microbial levels downstream of farms. The correlation coefficient is nonparametric (Spearman's rank type).

	Strep	Coli	Settleable Solids	TSS	TP	TN	BOD5	pH	Temp	Conductivity	TDS	Salinity	DO
Age of farm	-0.33	-0.36	-0.34	-0.21	0.06	-0.13	**-0.55**	-0.06	-0.21	-0.32	-0.32	-0.01	0.33
Number of ponds	0.02	-0.37	0.35	0.39	0.05	0.24	-0.25	**-0.56**	-0.11	**-0.82**	**-0.82**	-0.36	-0.35
Integrated farming	-0.26	0.41	-0.02	0.14	-0.06	0.13	0.15	0.36	-0.12	**0.60**	**0.60**	0.27	0.48
Frequency of feeding	-0.31	-0.30	-0.23	-0.15	-0.27	-0.02	-0.49	-0.47	0.05	-0.42	-0.42	**-0.53**	0.36
Average pond size	-0.18	-0.37	-0.39	**-0.53**	0.41	**0.59**	0.40	**0.51**	0.40	0.35	0.35	0.14	-0.09
Drain to Harvest	0.08	0.25	0.08	0.04	**-0.66**	-0.37	-0.04	-0.30	0.12	-0.06	-0.06	-0.32	0.12
Frequency of effluent releases	0.34	0.18	0.49	0.36	-0.15	0.29	0.07	-0.34	0.36	-0.18	-0.18	-0.47	-0.28
Proportion of ponds with top release	0.11	-0.02	-0.19	-0.12	-0.06	-0.12	-0.16	-0.04	-0.33	-0.15	-0.15	-0.19	**0.53**
Bottom Release	0.06	-0.05	0.07	0.07	-0.02	0.07	0.03	0.21	0.28	-0.05	-0.05	-0.08	-0.10
Water Reuse	0.18	-0.02	0.14	0.30	0.32	0.30	0.42	0.22	-0.01	0.04	0.04	-0.16	0.04
Species Present	-0.02	-0.12	0.41	0.38	0.45	**0.63**	-0.36	0.07	0.12	-0.21	-0.21	0.08	-0.24

APPENDIX C. – Pearson's correlation/p-values of macroinvertebrate metrics and indices with N: P ratio, nitrogen and phosphorus. The correlation coefficient is nonparametric.

	N:P	Nitrogen (mg/L)	Phosphorus (mg/L
Nitrogen (mg/L)	0.300		
	0.080		
Phosphorus (mg/L	**-0.399**	0.173	
	0.018	0.319	
BOD5 (mg/L	-0.055	0.106	0.044
	0.753	0.545	0.801
Invert Rich	-0.089	**0.352**	0.155
	0.613	0.038	0.375
Inver Abund	0.120	0.207	-0.010
	0.492	0.233	0.953
%EPT Indiv	-0.101	-0.041	0.177
	0.563	0.816	0.310
%Chiron Indiv	-0.058	-0.118	0.203
	0.741	0.499	0.243
EPT:Chironomid	-0.031	-0.042	-0.087
	0.862	0.814	0.628
EPT Rich	-0.141	0.205	0.081
	0.419	0.237	0.644
% EPT Abund	-0.132	0.038	0.004
	0.449	0.829	0.984
Diversity	-0.026	-0.153	-0.226
	0.883	0.379	0.193
Dominance	0.026	0.153	0.226
	0.883	0.379	0.193
HBI	**0.386**	-0.150	-0.219
	0.022	0.391	0.207
Non-airbr HBI	0.320	-0.129	-0.353
	0.061	0.458	0.037
Non-airbr Abund	0.045	0.173	0.116
	0.799	0.320	0.507
Non-airbr Rich	-0.008	**0.401**	0.009
	0.963	0.017	0.961
Airbr Rich	-0.153	0.161	0.274
	0.380	0.355	0.111
% Airbr Abund	0.126	0.040	-0.277
	0.472	0.817	0.107
% Non-Airbr Abun	-0.126	-0.040	0.277
	0.472	0.817	0.107
% Sens Taxa Abun	-0.213	0.191	0.314

	0.220	0.271	0.066
% Tol Taxa Abund	**0.457**	-0.038	-0.163
	0.006	0.827	0.350
Sens Taxa Rich	-0.199	0.157	0.114
	0.251	0.369	0.513
Tol Taxa Rich	0.283	**0.425**	-0.163
	0.100	0.011	0.349

	BOD5 (mg/L	Invert Rich	Inver Abund
Invert Rich	0.073		
	0.675		
Inver Abund	-0.009	0.640	
	0.959	0.000	
%EPT Indiv	-0.190	0.288	0.132
	0.273	0.093	0.450
%Chiron Indiv	-0.015	-0.099	0.085
	0.930	0.572	0.627
EPT:Chironomid	-0.168	0.324	0.056
	0.349	0.065	0.756
EPT Rich	-0.208	0.548	0.054
	0.230	0.001	0.760
% EPT Abund	-0.360	0.119	-0.202
	0.034	0.497	0.245
Diversity	0.169	-0.226	-0.510
	0.332	0.192	0.002
Dominance	-0.169	0.226	0.510
	0.332	0.192	0.002
HBI	-0.187	-0.106	0.305
	0.283	0.544	0.074
Non-airbr HBI	-0.139	-0.198	0.239
	0.427	0.255	0.166
Non-airbr Abund	-0.055	0.626	0.906
	0.755	0.000	0.000
Non-airbr Rich	0.034	0.864	0.467
	0.845	0.000	0.005
Airbr Rich	0.093	0.791	0.611
	0.593	0.000	0.000
% Airbr Abund	0.107	-0.183	-0.141
	0.540	0.294	0.419
% Non-Airbr Abun	-0.107	0.183	0.141
	0.540	0.294	0.419
% Sens Taxa Abun	-0.060	0.233	-0.130
	0.731	0.178	0.456

% Tol Taxa Abund	-0.224	0.029	0.355
	0.195	0.866	0.036
Sens Taxa Rich	0.106	0.299	-0.050
	0.546	0.082	0.775
Tol Taxa Rich	-0.139	0.596	0.626
	0.426	0.000	0.000

	%EPT Indiv	%Chiron Indiv	EPT:Chironomid
%Chiron Indiv	-0.046		
	0.794		
EPT:Chironomid	0.767	-0.305	
	0.000	0.085	
EPT Rich	0.196	-0.125	0.340
	0.259	0.473	0.053
% EPT Abund	0.086	-0.059	0.201
	0.622	0.735	0.262
Diversity	-0.215	-0.370	-0.087
	0.215	0.029	0.631
Dominance	0.215	0.370	0.087
	0.215	0.029	0.631
HBI	-0.128	0.455	-0.132
	0.465	0.006	0.463
Non-airbr HBI	-0.362	0.325	-0.338
	0.033	0.057	0.055
Non-airbr Abund	0.359	0.112	0.249
	0.034	0.520	0.162
Non-airbr Rich	0.263	-0.248	0.379
	0.126	0.151	0.030
Airbr Rich	0.210	0.119	0.148
	0.225	0.495	0.412
% Airbr Abund	-0.473	-0.162	-0.337
	0.004	0.353	0.055
% Non-Airbr Abun	0.473	0.162	0.337
	0.004	0.353	0.055
% Sens Taxa Abun	0.579	-0.182	0.317
	0.000	0.296	0.072
% Tol Taxa Abund	0.154	0.507	0.111
	0.377	0.002	0.537
Sens Taxa Rich	-0.067	-0.384	0.171
	0.703	0.023	0.341
Tol Taxa Rich	0.154	-0.065	0.133
	0.376	0.709	0.460

	EPT Rich	% EPT Abund	Diversity
% EPT Abund	0.862		
	0.000		
Diversity	-0.061	0.029	
	0.727	0.871	
Dominance	0.061	-0.029	-1.000
	0.727	0.871	*
HBI	-0.188	-0.142	-0.185
	0.280	0.416	0.288
Non-airbr HBI	-0.306	-0.207	-0.078
	0.074	0.232	0.655
Non-airbr Abund	0.158	-0.107	-0.565
	0.364	0.540	0.000
Non-airbr Rich	0.697	0.319	-0.265
	0.000	0.062	0.123
Airbr Rich	0.162	-0.170	-0.093
	0.353	0.330	0.596
% Airbr Abund	-0.227	-0.115	0.415
	0.189	0.512	0.013
% Non-Airbr Abun	0.227	0.115	-0.415
	0.189	0.512	0.013
% Sens Taxa Abun	0.362	0.258	-0.058
	0.033	0.135	0.742
% Tol Taxa Abund	-0.040	-0.038	-0.217
	0.818	0.827	0.210
Sens Taxa Rich	0.617	0.498	-0.014
	0.000	0.002	0.937
Tol Taxa Rich	0.231	0.016	-0.150
	0.181	0.926	0.389

	Dominance	HBI	Non-airbr HBI
HBI	0.185		
	0.288		
Non-airbr HBI	0.078	0.841	
	0.655	0.000	
Non-airbr Abund	0.565	0.304	0.070
	0.000	0.075	0.688
Non-airbr Rich	0.265	-0.204	-0.338
	0.123	0.241	0.047
Airbr Rich	0.093	0.052	0.047
	0.596	0.766	0.788
% Airbr Abund	-0.415	-0.145	0.313

	0.013	0.405	0.067
% Non-Airbr Abun	0.415	0.145	-0.313
	0.013	0.405	0.067
% Sens Taxa Abun	0.058	-0.664	-0.745
	0.742	0.000	0.000
% Tol Taxa Abund	0.217	0.902	0.694
	0.210	0.000	0.000
Sens Taxa Rich	0.014	-0.531	-0.595
	0.937	0.001	0.000
Tol Taxa Rich	0.150	0.299	0.249
	0.389	0.081	0.148

	Non-airbr Abund	Non-airbr Rich	Airbr Rich
Non-airbr Rich	0.523		
	0.001		
Airbr Rich	0.516	0.375	
	0.002	0.027	
% Airbr Abund	-0.471	-0.347	0.086
	0.004	0.041	0.624
% Non-Airbr Abun	0.471	0.347	-0.086
	0.004	0.041	0.624
% Sens Taxa Abun	0.052	0.362	-0.012
	0.769	0.033	0.948
% Tol Taxa Abund	0.405	-0.081	0.153
	0.016	0.643	0.380
Sens Taxa Rich	0.035	0.529	-0.093
	0.842	0.001	0.593
Tol Taxa Rich	0.564	0.494	0.497
	0.000	0.003	0.002

	% Airbr Abund	% Non-Airbr Abun	% Sens Taxa Abun
% Non-Airbr Abun	-1.000		
	*		
% Sens Taxa Abun	-0.354	0.354	
	0.037	0.037	
% Tol Taxa Abund	-0.186	0.186	-0.396
	0.284	0.284	0.018
Sens Taxa Rich	-0.204	0.204	0.418
	0.239	0.239	0.012
Tol Taxa Rich	-0.013	0.013	-0.095
	0.942	0.942	0.586

	% Tol Taxa Abund	Sens Taxa Rich

Sens Taxa Rich	-0.496	
	0.002	
Tol Taxa Rich	0.400	-0.265
	0.017	0.124