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Environmental Sustainability at High Altitude in Mexico: A Case Study of Rainbow Trout Culture and Water Quality at High Altitude

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Abstract: A long-term international collaborative effort joined scientists and students from the University of North Texas (UNT) and the Universidad Autónoma del Estado de México (UAEM), Toluca, to investigate the health of farmed rainbow trout (Onchorynchus mykiss) at high altitude (9642 ft) in central Mexico. This study was conducted in the Corral de Piedra watershed in Mexico State, which is the major source of clean water to nearby towns, to Mexico City, and to the expanding rainbow trout aquaculture industry. In aquaculture, stress is of central concern because stressors, which accompany intensive fish husbandry, result in compromised fish health, decrease growth and productivity, and promote disease. Stress can be quantified by measuring concentrations of four common physiological indices (glucose, lactate, cortisol and antioxidants), levels of which rise in the blood in response to stressors. Blood samples were collected in the field from farmed fish at each of five farms in the watershed: Dos Potrillos (D), Corral de Piedra (C), El Arroya (E), Piedra Ancha (P) and Tizapa (T). Three farms; C, E and P had high fish stress levels, while farm D and T had significantly lower levels. In conjunction with the fish stress analysis, nine parameters of water quality, as well as details of each farm were recorded. While it was expected that water quality would be the principal factor influencing fish stress, the results showed little significant differences in water quality among the farms compared to the Los Hoyos River. It was concluded that the one-time sampling instance of this study might not have been sufficient to resolve differences in water quality among the farms. In fact farm quality, that is; location in the watershed, farming management and pond construction, may have a significant effect on fish stress in the short-term, and will consequently influence the future of environmental sustainability of aquaculture at high-altitude in Mexico.

Keywords: Water Usage, Stress Indicators, Ecological Physiology, Trout Aquaculture, Mexico, Conservation

INTRODUCTION

The present study is one critical part of a long-term international collaborative effort among scientists and students from the University of North Texas (UNT) and the Universidad Autónoma del Estado de México (UAEM), Toluca; to determine the environmental sustainability of rainbow trout (*Onchorynchus mykiss*) aquaculture in a rural, ecologically diverse, high-altitude (9642 ft) region of Mexico.

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Mexico has been designated as a mega-diversity country, one of only seventeen in the world, and one of three in North America along with the United States and Columbia with coastlines on the Atlantic and Pacific Oceans (Llorente-Bousquets and Ocegueda, 2008). The present study was conducted in the Corral de Piedra microbasin and watershed in the central state of Mexico. The Corral de Piedra watershed represents a unique ecosystem because it lies in the Mexican Transvolcanic Belt (MTVB), an area that is geologically ancient at over 3.5 to 7 million years old, which has one of the highest animal and plant diversities in the world (FAO, 2009).

In the Corral de Piedra watershed, endemic fishes, amphibians and snakes inhabit the spring fed Los Hoyos River system, but compete for water with fish farms, cattle and with the demands for drinking water from nearby towns, such as Valle de Bravo, where Lake Via de Bravo supplies 20% of drinking water to Mexico City (Mendez-Sanchez, pers. comm.). In addition to its unique natural history the Corral de Piedra watershed is the location of the largest rainbow trout aquaculture industry in the country (2800 tons in 2008, Subdelegacion de Pesca, 2008).

Aquaculture is the fastest growing industry in the world, and in Mexico aquaculture contributes more than 12% to total fish production. This will likely grow to 40% within the next 15 years if provided the right support and expertise (FAO, 2009). Most trout production in Mexico is conducted on small, locally owned farms, often surrounded by conservation areas. For example, the protected area or water sanctuary named "Parque Estatal Santuario del Agua Presa Corral de Piedra", which surrounds the Corral de Piedra watershed. For trout aquaculture in the Corral de Piedra watershed as throughout the globe, stress is of major concern, as the stressors, which accompany intensive fish husbandry result in compromised fish growth and health, and likely promote disease in the farms and the surrounding natural areas.

Stress here is defined as the response of an organism, to any demand placed on it such that it causes an extension of a physiological state beyond the normal resting state (Iwama et al., 2006). An animal's response to stress may be either adaptive (fish adjust to the stressors) to regain homeostasis (balance), or maladaptive (fish compromise their performance) and eventually die. Stress induced changes can be grouped into three levels of responses; primary, secondary and tertiary. The present study focused only on primary and secondary responses. The primary stress response includes a rapid release of stress hormones, including adrenaline, which is flushed quickly from the blood; and cortisol, which remains elevated for several hours (Gamperl et al., 1994). The secondary response results in changes in metabolic pathways and in blood chemistry, and is mediated by the stress hormones released in the primary response. Elevated levels of glucose, released from the liver to provide energy substrates to tissues for increased energy demand; and lactate, which can be an indicator of lactic acidosis (an anaerobic metabolic product) are common examples of secondary stress responses. While glucose, lactate and cortisol are commonly measured in response to stress, the present study also measured antioxidant concentrations. Antioxidant levels provide information about the ability of the system to protect and recover from stress (Barton 2002, Iwama 2006, Hunt von Herbing and Turnbough 2011).

In most intensive aquaculture settings the most common stressors are grading, transportation and vaccination. These stressors may be *in addition* to the environmental stressors such as low dissolved oxygen concentrations and temperature fluctuations (especially acute at high-altitude), and build up of wastes. Trout aquaculture is common at high-altitude in Mexico because of the lower water temperatures, compared to those at sea level. Rainbow trout is a coldwater species and requires water temps less than 68°F year-round and highly oxygenated water for optimal growth. Therefore the spring-fed, high altitude areas of the Corral de Piedra watershed are very conducive to growing trout. But despite the lower temperatures, dissolved oxygen concentrations at 9642 ft are still almost 30% below oxygen concentrations at sea level at the same temperature. Therefore, at high altitude, both human derived and environmental stressors could have a cumulative effect on sustainability of the aquaculture industry.

The primary purpose of the present study was to investigate relationships between fish stress levels, water quality and farm quality at five rural farms in the Corral de Piedra watershed;

Dos Potrillos (D), Corral de Piedra (C), El Arroya (E), Piedra Ancha (P) and Tizapa (T). The methods in the present study are unique, because of the combination of in-field measurements of glucose and lactate concentrations followed by laboratory validation and measurement of cortisol and antioxidants; quantifying for the first time the fish health in commercial farms at high altitude in Mexico.

In addition to physiological studies detailed water quality data was recorded and will help us better understand the relationships between fish stress, water supply and sustainability. Together, these data will provide information on the health of rainbow trout farmed at high altitude in rural Mexico, and may aid in evaluating the sensitivity and potential resilience of the watershed to the aquaculture industry, in a region where environmental change may be particularly acute.

Materials and Methods

This study was conducted on rainbow trout (*Onchorynchus mykiss*) farmed in the Corral de Piedra microbasin located between, 19° 10' 29" and 19° 14' 33" N; 99° 53' 27" and 100° 00' 13" W, at an average elevation of 9642 ft, in Amanalco de Becerra, Estado de México, México. Over 50 trout farms are scattered throughout the microbasin. Trout culture is carried out largely in earthen and concrete lined ponds, which are fed by springs derived from the neighboring Los Hoyos River. Five rainbow trout farms (Dos Potrillos (D), Corral de Piedra (C), El Arroya (E), Piedra Ancha (P) and Tizapa (T)) were sampled for in this study (Fig. 1). As fish had been graded in each farm, it was necessary to sample at least 3 ponds within each farm to determine stress levels in all three fish stages; alevin, juvenile and adult. Note that farm P contained only alevins and juveniles.

These farms were selected because they are part of a trout farm co-operative (INTEGRAMEX), which permitted our team of investigators access to the farms and fish in order to make our physiological stress assessments. After interviewing the farmers and accessing the farm quality, we also classified the farms. Farms D, C, E were classified as "well-managed" as fish were fed, ponds cleaned and maintained on a daily basis. Farms P and T were classified as "poorly-managed" because maintenance of the farms was more irregular and Farm P only held alevin and juvenile stages, while Farm T had been recently re-stocked due to flooding and loss of the previous year's harvest. All sampled fish were unfed prior to sampling, as the farmers had agreed to delay feeding until we had finished our sampling.

After a 15–20 min confinement period, fish were carefully held while a small non-lethal blood sample (1.0 ml) via caudal venipuncture [B-D vacutainer, 3 ml vial, sodium heparin, 22 gauge needle (BD, Franklin Lakes, NJ, U.S.A.) was collected using 3 ml heparinized syringes with a 22 gauge needle. Blood samples were immediately placed in water-ice slurry in a large cooler for field analysis prior to processing and storage. Fish total lengths (LT; ± 0.1mm) and mass (WT; ± 0.1g) were recorded and fish were then released into a recovery tank until they had completely recovered, at which point they were released back to the ponds. Blood sampling was rapid, taking less than a minute and as a result no anesthetic was used, because previous studies have shown that using anesthetic may affect the stress response (Wagner et al., 2004, Velisek et al., 2005).

After blood sample collection, glucose and lactate levels were measured on whole blood by adding 10 μl of blood to hand-held glucose (ACCU-CHEK glucose meter; Roche Diagnostic, Indianapolis, IN) and lactate (Lactate Pro LT-1710 portable

lactate analyser; Arkray Inc., Kyoto, Japan) meters. Both meters were calibrated with standards prior to analyses following manufacturer guidelines. The ACCU-CHEK and Lactate Pro have previously been validated as a reliable tool for field physiology including fishes (Pyne et al., 2000; Mizock, 2002; Venn Beecham et al., 2006; Cooke et al., 2008). But, neither meter had been evaluated for use at high altitude. The remaining whole blood was centrifuged (LW

Scientific Portable centrifuge; LW Scientific, Inc. Lawrenceville, Georgia) at 10,000 g for 5 min (following Cooke at al., 2008). Plasma samples were separated by micropipette, transferred to 1.5 ml standard eppendorf tubes and stored in a liquid nitrogen dry shipper (at a minimum of -80° C) until laboratory analyses were conducted.

Laboratory analysis for cortisol and antioxidants was carried out in the fully equipped diagnostic laboratory in the Marine Conservation and Aquatic Physiology Laboratory (MCAPL), at the University of North Texas (UNT) in Denton, Texas. In the laboratory, a microplate reader (Synergy 2, BioTek Instrument Inc., Winooski, VT), with appropriate reagents and commercial assay kits, was used for laboratory assays of plasma glucose (Cayman's Glucose Assay, Ann Arbor, MI), lactate (Eton BioScience Inc. Lactate Assay Kit), cortisol (Neogen Cortisol ELIZA kit, Lexington, KY) and antioxidants (Cayman's Antioxidant Assay, Ann Arbor, MI). All samples were assayed in duplicates.

Water Quality-Water samples were obtained from each of the 5 farms; Dos Potrillos (D), Corral de Piedra (C), El Arroyo (E), Tizapa (T), and Piedra Ancha (P). For each farm, samples of water were collected at the surface and at the bottom of each of three ponds from which trout had been sampled for stress analysis. The samples were analyzed on the same day, as the trout were collected in the field. The parameters measured in the water samples were: total phosphorus, nitrites (NO_2), nitrates (NO_3), ammonia (NH_4), hardness and suspended solids. Nitrites, nitrates, total alkalinity, total hardness and pH were measured with Mardel® (Virbac AH, Inc.) test strips, pH was measured using pHydrion® (Insta-Chek Jumbo 0–13 and Vivid 5.5–8) test strip and the other parameters were assayed using a DREL/2010 Advanced Water Quality Portable Laboratory spectrophotometer and its reagents.

The following physico-chemical properties were determined *in situ* at the surface and bottom each of the sampled ponds: temperature, conductivity and dissolved oxygen using a YSI Handheld dissolved oxygen, conductivity, salinity and temperature meter, (YSI, Model 85). Lacking a more advanced device, we calculated the input water flow rate in the ponds at each of the farms, using a simple, standardized method. This method consisted of a 5 gallon plastic bucket and a stopwatch. The time to completely fill the bucket with water was recorded three times at different locations along the input stream and flow rate was calculated dividing the volume by the time taken to fill the bucket.

Statistical Analysis-The effects of farm stage on standard length, body weight, blood lactate, plasma cortisol and total antioxidant capacity were assessed via two-way analysis of variances (ANOVA) on ranked data. Since the lack of adult fish in Farm P did not allow post-hoc Tukey's analysis, pairwise comparisons using additional Kruskal-Wallis one-way ANOVAs were performed if significances were found from the results in the two-way ANOVAs. Changes in blood glucose were also analyzed with Kruskal-Wallis test with respect to farms and stages dues to missing values in Farm P (all stages) and Farm T (adult). The correlations between standard length, blood glucose, lactate, plasma cortisol, total antioxidant capacity and body weight were assessed using Spearman's rank correlation. Multivariate analysis such as principal component analysis (PCA), and partial least square discriminant analysis were performed using STATGRAPHICS (©2012 Statpoint Technologies). PCA is a mathematical procedure that converts a set of observations of possibly correlated variables into a set of values of linearly uncorrelated variables called principal components. PCA is used in this study as a tool in exploratory data analysis so as to determine if potentially predictive relationships might exist among the stress variables (glucose, lactate, cortisol and antioxidants), water quality data and farm.

Results

Table 1 showed that there is a significant effect of both farm and fish stage on all the parameters that were measured both in the field and in the lab and developmental stage significantly affected all variables measured (Fig. 2a-f, Table 1). Across all farms, fish increased both in their body length ($P < 0.001$) and weight ($P < 0.001$) as development progressed, except in Farm P (Figs. 2a-e). Overall differences across all farms, in blood lactate ($P = 0.022$), plasma cortisol ($P = 0.031$) and total antioxidant capacity ($P < 0.001$) also occurred among stages. However, pairwise comparisons failed to detect differences among specific stages *within* any of the 5 farms. Similarly, overall differences among farms were observed in fish standard length ($P < 0.001$), weight ($P < 0.001$), blood lactate ($P < 0.001$), plasma cortisol ($P < 0.001$), and total antioxidant capacity ($P = 0.026$). For cortisol, juveniles in Farms P and C showed higher values than fish at the same stage in Farm D (Fig. 2e). Again, pairwise one-way ANOVAs failed to show differences among stages *within* any farm. Growth was compared across farms as comparisons of standard length (SL) at weight (WT) and as expected, weight varied significantly with length (r^2=0.88, P < 0.001). Weight also had a significant effect on two blood parameters; plasma cortisol (r^2=0.20, P < 0.001) and total antioxidant capacity (r^2=0.12, P=0.001), and regressed positively with both of these factors. In contrast, a negative, non-significant trend occurred between body weight and blood glucose (r^2=0.04, P=0.09). Growth rates did not differ among farms D, C, and E and were 0.073, 0.075 and 0.070 g cm^{-1} respectively, but were significantly higher in farm T, which had a growth rate of 0.084 g cm^{-1} (P<0.05), because it had the smallest fish.

Blood parameters- Before the Principal Component Analysis (PCA) was performed, the effects of body weight on each of the four blood parameters were removed by transforming the data using a regression and then using residuals for the PCA. All variables were standardized. Of the four stress parameters, only cortisol and antioxidants resulted in a significant regression (Fig. 3d & e). PCA was performed using the residuals for cortisol and antioxidants and the raw data for lactate, in order to obtain an index of stress and determine how this index varied among the farms. Note that glucose was not used in the PCA because of missing data in two farms (T and P).

Figure 4 shows a loading plot for cortisol, antioxidants and lactate. Levels of these three parameters were similar and higher among farms C, E, and P than in D and T. The representative points of the plasma samples are mapped in the space spanned by the first two principal components PC1 versus PC2. PCA showed that the two principal components accounted for 82.93% of the variability in the original data, with PC1=52% and PC2=30.9% and each of these parameters had a very high positive load/weight with the PC1 and increased in the same direction. Of the 4 stress parameters, three; cortisol, antioxidants and lactate, were good predictors of stress in rainbow trout among the farms, while glucose was a poor predictor.

Further analysis to determine intra-farm differences in trout health was conducted using only PC1 as a stress index as it accounted for most of the variability (52%) and the data was not significantly different from a normal distribution (X^2=3.83, df = 4, P=0.43). A one-way ANOVA was performed to resolve differences among stress indices among farms, which subsequently show clear differences in the extent to which the trout are stressed (F = 7.88, df=4, P < 0.00001 (Fig. 5). Figure 5 shows that three farms C, E and P contained fish that had significantly higher blood stress levels than the either farm D or farm T. Farms D and T contained fish that exhibited lower stress levels and were not significantly different from each other.

Water Quality- To compare water quality among five farms and the surrounding Los Hoyos River, all water quality variables (flow rate (ls^{-1}), temperature (°C), dissolved oxygen (mgl^{-1}), conductivity (μScm^{-1}), NO_2=Nitrite, NO_3=Nitrate, Hardness and Alkalinity ($meql^{-1}$)) were used in the PCA. The representative points of the water quality samples are mapped in the space spanned by the first two principal components PC1 versus PC2 (Fig. 6). PCA showed that the two principal components accounted for 47% of the variability in the original data, with PC1= 27% and PC2= 20%. Of the 9 water quality parameters 6 variables: flow rate, hardness, nitrite, conductivity, pH and dissolved oxygen, were good predictors of water quality among the farms.

Figure 6 shows a loading plot for all the water quality variables and all farms showed similar water quality, except the Los Hoyos River, which showed the best water quality for all variables.

Further analysis to determine intra-farm differences accessed water quality condition using only PC1 as a water quality index as it accounted for most of the variability (27%). A one-way ANOVA was performed to resolve differences in water quality among farms (F=19.29, df=5, P < 0.00001) (Fig. 7). Water quality was very similar among four of the farms; D, P, E and P and each of these farms had poorer water quality than the Los Hoyas River, which showed the best water quality (Fig. 7). Farm T, however showed significantly better water quality than the other four farms and this is likely because the Los Hoyos River has recently flooded Farm T.

Discussion

As defined by Holling (1973), resilience determines the persistence of relationships within a system and is a measure of the ability of these systems to absorb changes of state variables, driving variables, and parameters, and still persist. In this definition resilience is the property of the system and persistence or probability of extinction is the result. As investigators in the new field of sustainability science our goals are to determine which interactions are sustainable and benefit both nature and society; and develop ways to evaluate the sustainability of interactions, and if not sustainable, guide these interactions into sustainable trajectories.

The primary purpose of the present study was to determine in commercial rainbow trout farms if predictive relationships existed between fish stress levels, water quality and farm quality in five rural farms in the Corral de Piedra watershed; Dos Potrillos (D), Corral de Piedra (C), El Arroya (E), Piedra Ancha (P) and Tizapa (T). We tested the hypothesis that fish stress levels as measured by our in-field and laboratory tools could be related to water quality; or whether they were related to other factors such as the overall farm quality including; management, farm hygiene, and pond construction. Understanding the factors that impact fish stress and ultimately, fish health and growth, will provide useful information to the fish farmers of farming methods that promote productivity and economic sustainability. Further, because the fish farms in this study are closely linked with the surrounding conservation area fed by the Los Hoyos River, data from this study will provide information about the impact of trout aquaculture on the health of the surrounding watershed and water sanctuary (Parque Estatal Santuario del Agua Presa Corral de Piedra). This may in turn determine the future environmental sustainability of trout aquaculture in the watershed.

Stress in fish is an unavoidable consequence of normal farm practices, which often include; handling, sorting, grading, transport and poor water quality. Therefore, we need to understand if stress impacts fish growth and productivity. As rapid fish growth is key to productivity, any factors that constrain growth rate may impact on farmer livelihood and farm sustainability. Our observations of the five rural farms and results from the PCA analysis of stress indices; glucose, lactate, cortisol and antioxidants, showed that of the five farms, two farms; Dos Potrillos (D) and Tizapa (T), had the lowest fish stress levels. Further Tizapa (T) had significantly better water quality than the other 4 farms. Results from the PCA analysis also showed that water quality did not vary significantly among four of the farms (D, C E and P). Only the recently flooded farm, Tizapa had water quality parameters very similar to that of the surrounding Los Hoyos River system, which had the best water quality. Tizapa is located in the flood plain of the watershed and had been flooded six months prior to the present study. The farmer had lost the entire year's harvest, making it necessary for him to re-stock with fingerling trout, which grow at faster rates than adults. This then explains the higher growth rates observed in this farm compared to the other four farms that did not suffer flooding.

However, because farm D was also found to have similar fish stress levels as farm T it is likely that other factors may also have had an impact on fish stress levels. One of these factors could have been pond structure. Of the 5 farms sampled only farm D farmed trout exclusively in round ponds, while all the other farms had rectangular ponds. Past studies on pond shape have found that round ponds and tanks foster better growth, but this study is the first to suggest that better growth may result from lower fish stress. An advantage of round tanks is the self-cleaning aspect, which takes place due to centripetal forces by the circulating water. Uneaten feed and fecal wastes are moved to the center of the tank as water moves around in circular motion. Once collected these materials are removed using a "double-drain" type system. This system reduces need for maintenance and labor costs.

From the results of the study it appears that little relationship exists between fish stress levels and water quality among the farms. In short, stress levels were lower in farms D and T, compared to the other three farms; but water quality did not vary significantly among all five farms. It is possible that in this one time sampling of the farms, fish stress levels may be less related to water quality than to other farm quality traits. Differences were many among the five farms, but farm management practices, daily maintenance and pond structure appeared to be the most critical. Similarities also existed among the farms, which included no electricity, limited fish food supply and almost no precautionary sanitary practices; the latter, which has been shown to reduce or prevent disease in fish. While this study was the first opportunity to make quantitative measurements of fish health at high-altitude trout farms in Mexico, the results that fish health did not seem related to water quality was unexpected, and led us to search for other reasons for the significant differences in the observed fish stress levels among farms.

Based on our ability to instantaneously determine the levels of secondary metabolic stress responses (whole blood glucose and lactate) using "point of care" diagnostic tools (ACCUCHEK and Lactate Pro TM) right at the farm, we were confident of the accuracy of our values. Further, our detailed measurement of nine water quality parameters in the same ponds from which we measured fish stress provided excellent water quality profiles. But despite the rigorous science, little relationship was found between fish health and water quality. This suggested that the underlying reasons for the differences in fish health might be due to other, less quantifiable factors. This last finding was not surprising as these farms were in rural Mexico where access to supplies of high grade food and reliable workers is sporadic as many of these farmers were poor and their resources limited.

Most of the previous studies on farmed rainbow trout, with a few exceptions (Wells and Pankhurst, 1999) measured stress levels (whole blood glucose and lactate) in the laboratory with fish obtained from farms, but not directly at the farm or in the field. Further, no study except the present one, made an attempt to relate fish stress with farm water quality. While the use of field tools has been more commonly developed and used in fields such as conservation physiology (Wilkeski and Cooke, 2006, Cooke et al., 2008), there are few studies to date that have used these types of diagnostic tools in commercial aquaculture farms. In two studies, the first on farm raised rainbow trout (Wells and Pankhurst, 1999) and the second on Atlantic cod (*Gadus morhua*) (Brown et al., 2008), glucose and lactate meters were useful to measure plasma glucose and lactate levels in whole blood in the field and we can use these data to compare to those obtained in Mexico.

In Mexico in the present study, glucose values ranged from 4–8 mmol l^{-1} of plasma across all five farms, and were higher than values obtained from a "point of care" device, the Advantage TM meter (1–3 mmol l^{-1}), used in the study by Wells and Pankhurst (1999), but were comparable to Wells and Pankhurst's laboratory derived values of 4–6 mmole l^{-1}. Similarly lactate values in the present study ranged from 3–8 mmol l^{-1} and were higher than those obtained from the Accusport TM Lactate Meter which ranged from 4–6 mmol l^{-1}, but were comparable to laboratory derived values 4–10 mmol l^{-1} (Wells and Pankhurst, 1999). In contrast, cortisol levels in the present study ranged from about 4 ng ml^{-1} in alevins, to 70 ng ml^{-1} in adults compared to 43 ng ml^{-1} in post-stressed juvenile trout (Barton, 2002). These results for glucose, lactate and cortisol levels suggest that response to short-term stress is likely to be stage-specific with the younger stages being very sensitive to stress as shown by the relatively high levels of glucose and lactate in the blood after mild stress. However, the relatively low levels of cortisol in the blood in the alevins compared to adults may suggest that primary stress related effects are less in alevins than adults and that alevins can respond to mild stress more rapidly by releasing glucose for use in stress recovery than larger fishes.

As no other study has used total antioxidants as a blood stress indicator no comparisons with other studies could be made. However, given the strong relationship between total antioxidant concentrations and fish body mass, total antioxidants may be a good indicator for estimating the strength of the fish's repair mechanisms, and could be a better index than glucose and/or lactate. The positive correlation of both cortisol and antioxidant levels with body mass suggested that response to stressors may be related to body mass, size and ultimately trout and farm productivity. Differences in antioxidant status may partially explain species-specific vulnerability to stressors that induce oxidative stress and may help us identify whether the high altitude populations of rainbow trout are under more stress than their lowland cousins.

Results in the present study revealed that field tools are reliable for the assessment of physiological variables at high-altitude and values were comparable to previous studies conducted on rainbow trout (Wells and Pankhurst 1999). This provides opportunities in the future to conduct physiological studies at remote field locations and will enable the development of strategies for reducing the negative physiological consequences of handling and other practices associated with aquaculture. While there is a need to understand the health of commercially grown fishes in aquaculture farms, the present study is the first to quantitatively document rainbow trout health using "point of care" tools at the farm. Perhaps, more interestingly the trout farms sampled, which were located in central Mexico at high altitude, had never received detailed study, and thus made the present study the first to evaluate commercial fish health at high altitude.

Results gained from using "point of care" tools, have greatly aided in the understanding of the mechanisms underlying stress related disease and mortality in diverse organism from fish to birds and from wetlands to forests (Wilkeski and Cooke, 2006). More recently similar tools were used to determine the physiological response (elevation of glucose and lactate) under several different types of stressful conditions. For example northern pike (*Esox lucius* L.) were exposed to the stress of air exposure associated with in catch and release angling practices (Arlinghaus et al., 2009) and bonefish (*Albula vulpes*) were exposed to the stress of different capture techniques (Cook et al., 2009). In another study in farmed Atlantic cod (*Gadus morhua*) the Lactate Pro meter was used to evaluate lactate levels (Brown et al., 2008).

Despite the lower stress levels of farm D compared to the nearby farms of C and E, no significant differences in fish growth was found. As a result we concluded that while stress levels differed, they did not seem to directly affect fish growth. If stress levels may not have a significant impact on growth rate, they may indicate differences in the susceptibility to disease. In fact, one of the farms, Farm C had recently had to treat several fish for a fungal disease and often used antibiotic washes to reduce disease.

One of the most difficult challenges is to select good metrics that reflect the effects of environmental change. Differences in stress variables may provide reliable indices by which we can begin quantitatively to access whole system change in response to environmental perturbation. While reductionist approaches typical in physiology may be useful to understand how each component is changing within the whole, we must also the study the entire system as an integrated community.

Despite the relatively primitive nature, the farms were relatively productive, collectively harvesting a total of 20 tons of fish annually (Hunt von Herbing et al., 2010). Moreover, while some farms were owned and managed by one owner, several of the farms are owned by multiple families, e.g. Corral de Piedra farm is owed by 12 families and the revenue of this farm (about $27,000) provide the livelihood of over 100 family members and relatives (Abele Contraras, personal communication). Other farms have hired workers at very low wages ($10 per day) who feed the fish and take care of the farms (Hunt von Herbing et al., 2010). Thus the farms provide real life examples of the diversity of aquaculture practices that exist in every nation in the world and present an opportunity for valuable learning experiences from scientific, cultural and social perspectives. However, Howverfuture studies need to include a longer-term monitoring period in order to investigate effects of seasonality on the relationship between fish health, water quality and farm quality.

In summary, despite the more favorable lower temperatures at high-altitude for trout aquaculture, environmental sustainability of aquaculture in Corral de Piedra microbasin may be difficult to maintain, without the use of antibiotics and other pharmaceuticals to treat disease. Unfortunately, use of pharmaceuticals to control disease in the fish farms will destroy the fragile balance the farms have with the surrounding nature and water sanctuaries and will be ultimately environmentally unsustainable.

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Variables	SL (cm) $n = 98$		BW (g) $n = 98$		Lac (mmol/l) $n = 80$		Cort (ng/ml) $n = 83$		Antioxi (mM of Trolox) $n = 80$		Glu (mg/dl) $n = 66$	
	F	*P*	*F*	*P*	*F*	*P*	*F*	*P*	*F*	*P*	*F*	*P*
Farm	17.2	<0.001	15.9	<0.001	6.6	<0.001	22	<0.001	2.9	0.026		
Stage	205.8	<0.001	229.4	<0.001	4.1	0.022	14.2	<0.001	9	<0.001		
Farm-Stage											3.1	0.003

Table 1. Statistics of the effects of farm and stage on standard length (SL), body weight (BW), lactate (Lac), cortisol (Cort), total antioxidant capacity (Antioxi) and glucose (Glu) levels. Two-way ANOVA on ranks was used for all variables except for glucose, which was analyzed using Kruskal-Wallis test. α = 0.05.

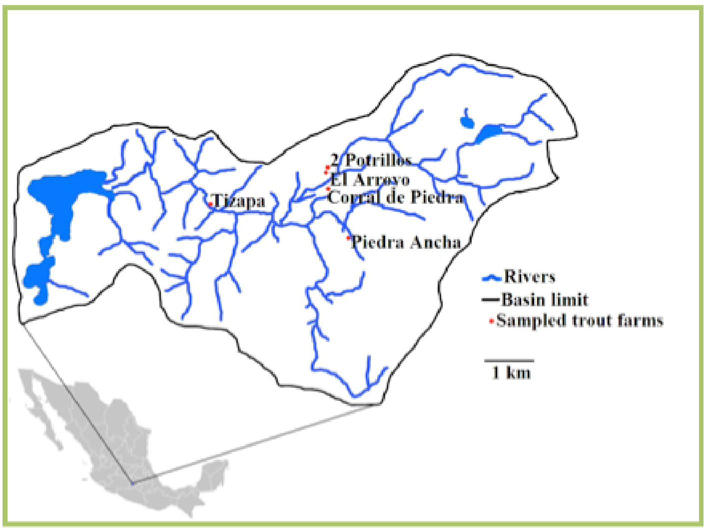

Figure 1: Map of the Location of the Five Fish Farms (Dos Potrillos, Corral de Piedra, El Arroya, Piedra Ancha and Tizapa) Sampled in this Study, in the Corral de Piedra watershed, Mexico.Map also Shows the Los Hoyos River Watershed

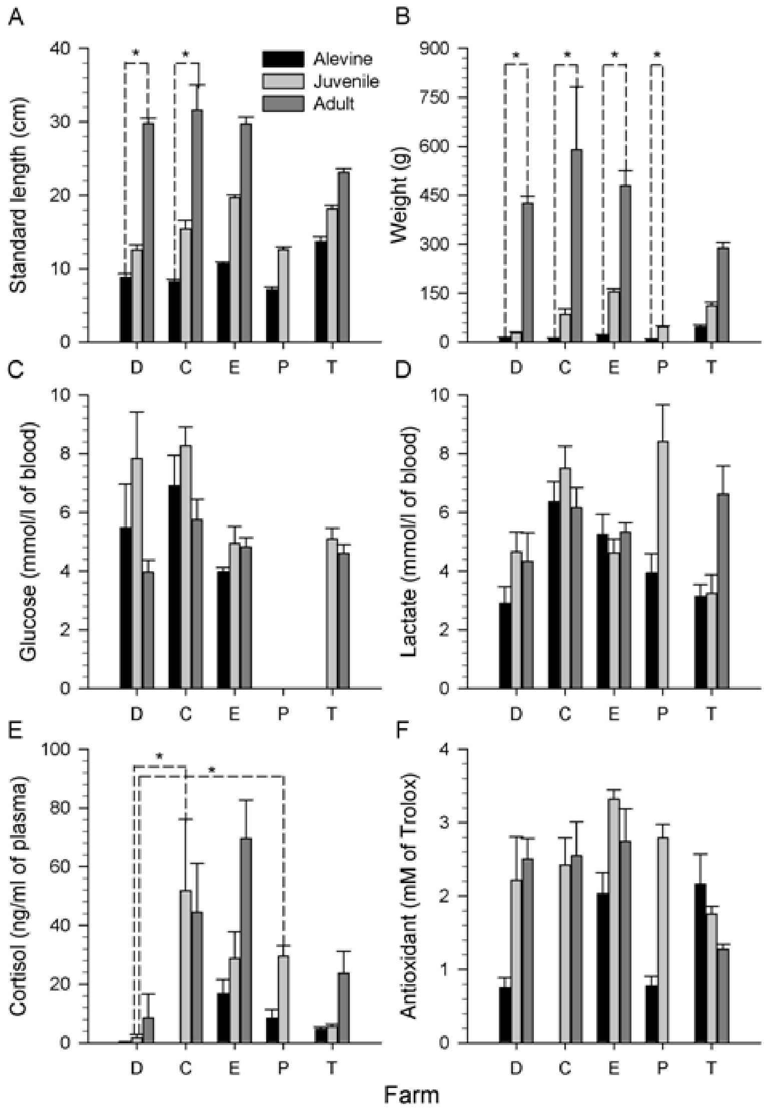

Figure 2: Graphs of; a) Standard Length (cm), b) weight (g), c) blood glucose Concentrations (mmol/l), d) Lactate Concentrations (mmol/l), e) cortisol (ng/ml) of plasma, and f) antioxidant enzymes mM of Trolox, for 3 fish stages (alevin, juvenile, and adult) in 5 fish farms D = Dos Potrillos, C = Corral de Piedra, E = El Arroya, P= Piedra Ancha and T=Tizapa. Dotted Lines Represent Statistically Significant different Values (P < 0.05)

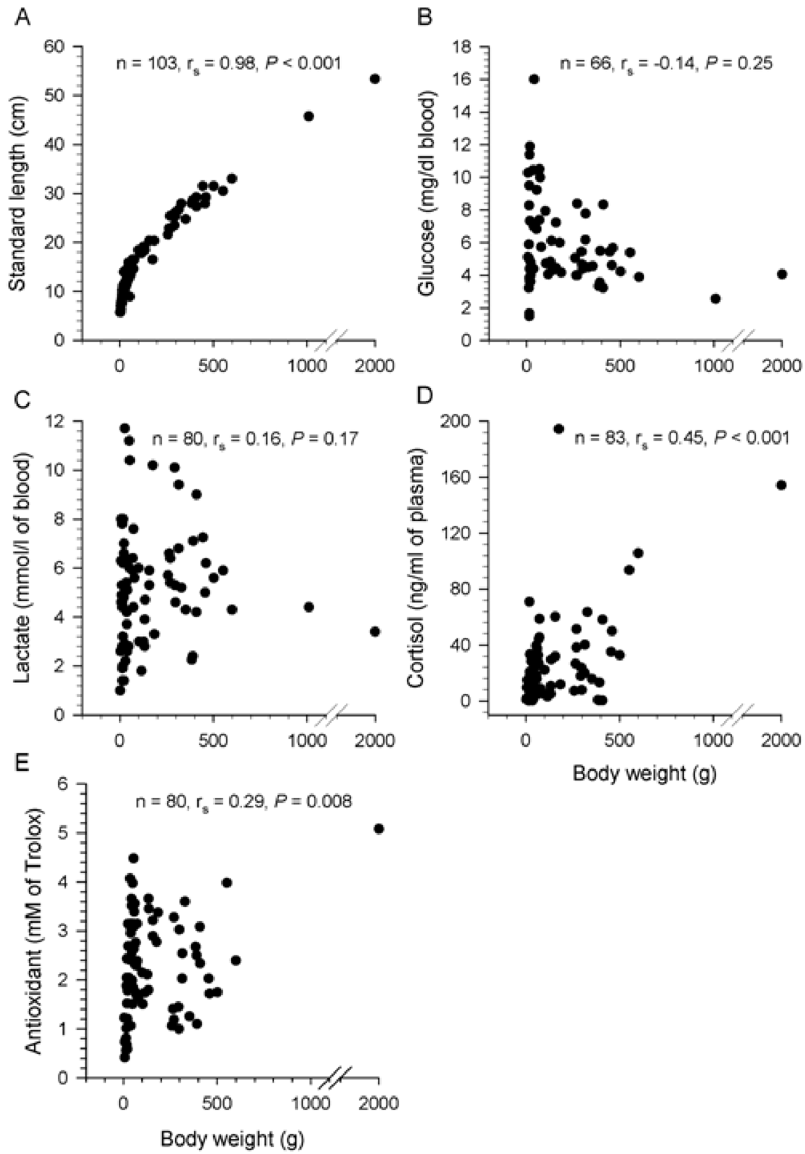

Figure 3: Graphs of; a) Standard Length (cm), b) Blood Glucose Concentrations (mmol/l), c)Lactate Concentrations (mmol/l), d) Cortisol (ng/ml) of plasma, and e) Antioxidant Enzymes mM of Trolox, for all Fish Stages Combined in all 5 Fish Farms. Spearman Rank Coefficients (rs), Number of Individuals (n) and Significance (P) are Listed forEach Relationship

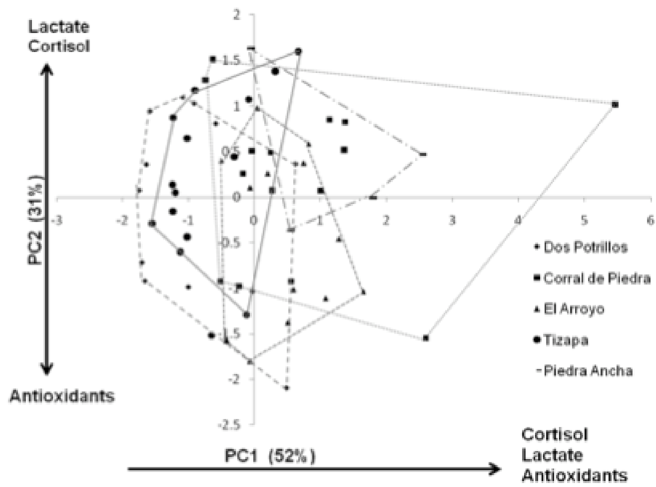

Figure 4: Principal Component Analysis (PCA) Results are Plotted for the Three Physiological Parameters (Cortisol, Lactate and Antioxidants) Significant (P<0.05) for each of the Five Fish Farms. The Representative Points of the Blood Samples for each Farm are Mapped in Space Spanned by the First to Principal Components PC1 versus PC2 Depicted by the Areas Enclosed by the Dashed Lines

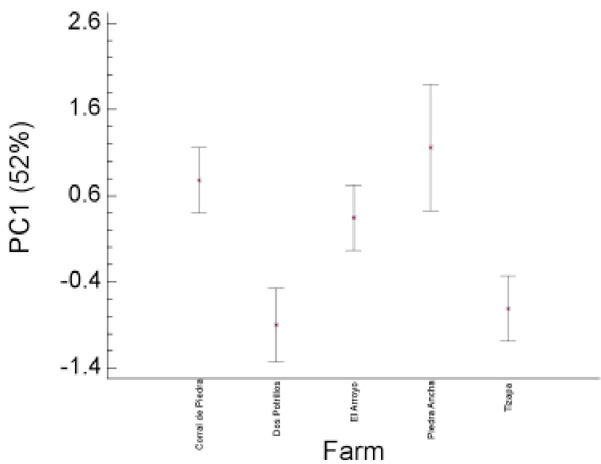

Figure 5: The First Principal Component (PC1), which has the Highest Possible Variance (that is Accounts for as much of the Variability in the Data as Possible), is Plotted for each of the Five Fish farms Showing the Relationships between Physiological Indices of Stress that were Significant (P<0.05) (Cortisol, Lactate, Antioxidants). Dos Potrillos and Tizapa had Fish with the Lowest Fish Stress

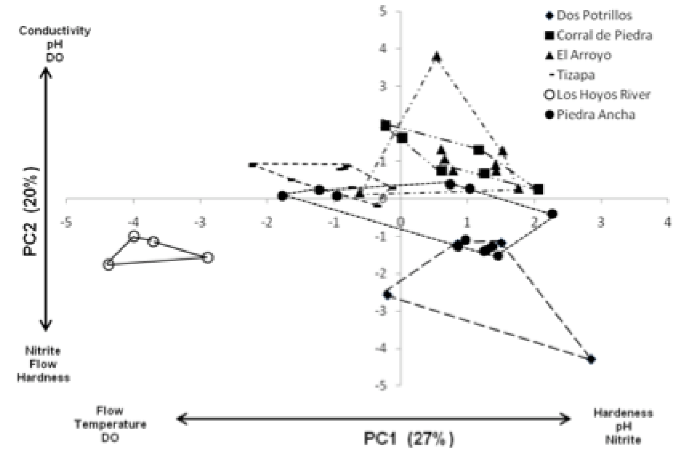

Figure 6: Principal Component Analysis (PCA) Results are Plotted for the Six Water Quality Variables (Conductivity, pH, Dissolved Oxygen Concentration (DO), Nitrite, Water Flow and Hardness), which were Significant (P<0.05) Values for each of the Five Fish Farms. The Representative Points of the Water Samples for each Farm are Mapped in Space Spanned by the First to Principal Components PC1 versus PC2 Depicted by the areas Enclosed by the Dashed Lines

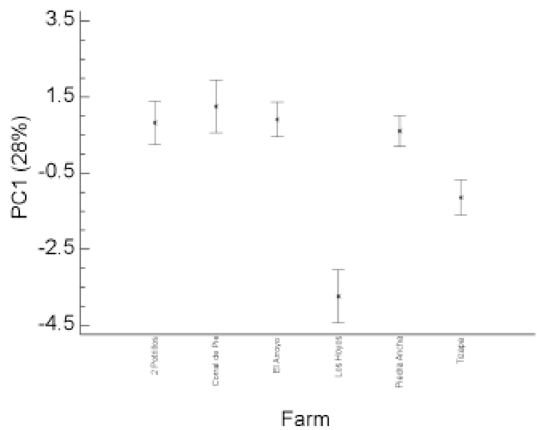

Figure 7: The First Principal Component (PC1), which has the Highest Possible Variance (that is Accounts for as much of the Variability in the Data as Possible), is Plotted for each of the Five Fish Farms Showing the Relationships between Water Quality Variables that were Significant (P<0.05) (Conductivity, pH, Dissolved Oxygen Concentration (DO), Nitrite, Water Flow and Hardness). The Los Hoyos River, had the Highest Water Quality

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