Aquaculture and Child Nutrition among the Tharu People in Rural Nepal: An Investigation of the Impact of Fish Consumption and Methylmercury in Cultured Fishes on Child Health

By

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ABSTRACT

To explore potential impact of small scale aquaculture on child nutrition in rural Nepal, a cross-sectional study was carried out among the Tharu people in the southern districts of Chitwan and Nawalparasi. Anthropometric measurements of 111 children were taken to determine growth status, and mothers in 86 families were interviewed regarding fish consumption and socioeconomic, health, and demographic characteristics. To determine if fish consumption was related to high levels of mercury in human hair, hair samples from 66 mothers and 75 children were taken and analyzed. Average fish mercury values were determined from samples taken from commonly cultured fish species. Mothers and children who lived in families that owned fish ponds were found to consume an average of 453 and 1620 more grams of fish per month, respectively, than mothers and children who lived in families without fish ponds, but children in fish farming households did not have better growth than children in non-fish farming households. Mercury values in hair (average = 0.762 μg/g) and fish samples (average range of 0.005 – 0.100 mg/kg among all species) were below harmful levels. In multivariate analyses, no variables related to aquaculture were found to be associated with child growth. Aquaculture is encouraged as a way to increase consumption of fish with low mercury levels, and suggestions are made for changing aquaculture practices to benefit child nutrition.

ACKNOWLEDGMENTS

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In Nepal, Dr. Madhav Shreshta was not only responsible for helping to organize all of the field work carried out for this study, but he and his family were the most excellent hosts a person could hope to have. I am indebted to them always for their kindness. None of this would have been possible without my field assistants/translators Rim Bahadur Thapa and Satiza Bikram Chaudhary, whom I owe many thanks for their hard work and friendship. While carrying out field work, I was welcomed over and over again by the Tharu people in the villages of Kathar, Bhandara, and Kawasoti, and I was lucky to be able to collect data in such amazing communities.

Last, I want to thank my parents, Joe and Paula, for their endless support.

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INTRODUCTION

Fish is recognized as a nutritionally beneficial food source around the world. Fish provide high quality protein and important micronutrients such as vitamin A, vitamin D, and iodine, and they can also be a source of phosphorus, fluoride, and calcium if bones are consumed (Speedy, 2003). Additionally, the benefits of consuming fish rich in omega-3 fatty acids has been widely documented in recent years (Oken and Belfort, 2010; Mahaffey et al., 2011). While certain fishes can provide all of these health benefits, there currently exists a difference in the perceived nutritional gains in developed versus developing countries: in the former health researchers are primarily concerned with omega-3 fatty acids and the protection they provide against cardiovascular disease (Domingo et al., 2007; Oken and Belfort, 2010), while in the latter the primary concern is healthy development augmented by protein and micronutrients (Aiga et al., 2009; Parajuli et al., 2012). This study is concerned with child growth and nutrition and will therefore focus on protein and micronutrients.

In Nepal, the benefits of fish consumption have been linked with such outcomes as improving protein intake (Bhujel et al., 2008) and increasing vitamin A and zinc ingestion (Parajuli et al., 2012). Little current data exists in relation to total fish production in Nepal (including cultured fish and wild-caught fish), but approximately half of all fish produced in 1994/1995 was raised in aquaculture systems (Pradhan, 2013). Additionally, the Nepali aquaculture sector has seen a marked increase in production over the last 40 years (Figure 1) (Pradhan, 2013). Given this trend and my observation made over the course of this study that nearly all fish sold in markets in Kathmandu and surrounding areas were raised in ponds, I believe the majority of fish currently consumed in Nepal is produced in aquaculture systems.

Aquaculture is the fastest growing food producing sector in the world, and it is responsible for approximately 50% of all human consumed fish (Diana, 2009). Demand for seafood has been forecasted to grow in the future (Delgado et al., 2003; Diana, 2009), while harvest from natural fish stocks is expected to decline or remain at current levels (Wijkstrom, 2003; Diana, 2009; FAO, 2010). In order to meet the demand for seafood while maintaining the health of wild stocks, aquaculture will increasingly be practiced. Such worldwide trends have been mirrored in Nepal (Figure 1) (Pradhan, 2013).

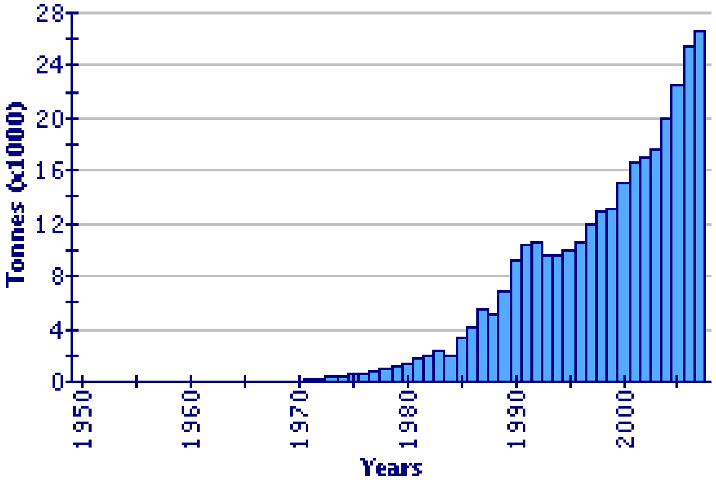


Figure 1. Food and Agriculture Organization reported aquaculture production in Nepal (Pradhan, 2013).

Although aquaculture has been practiced in Asia for thousands of years (Pradhan, 2013), it is fairly new in Nepal. It was not until the 1940s that the country began raising fish, and an additional 40 years passed before any significant progress was made in the field (Pradhan, 2013). Considering Nepal’s late start in aquaculture, it is no surprise that the county is yet to contribute substantially to the huge volume of Asian aquaculture production (Asia produced 88.8% of the world’s total aquaculture in 2008; FAO, 2010). Furthermore, Nepal is limited in land available for pond culture because of its mountainous geography. Several small aquaculture projects in the country have had positive results regarding issues such as income generation and women empowerment (Bhujel et al., 2008).

In 2000, a small scale aquaculture project was started in the village of Kathar in Chitwan, Nepal by the Aquaculture Department at Tribhuvan University's Institute of Agriculture and Animal Science (IAAS). The project was extended through multiple workshops that trained local village people – all members of the Tharu ethnic group – successful aquaculture methods. This was followed by construction of 26 fish ponds of varying sizes, and stocking ponds with tilapia and carp species. Assistance was given to farmers until May of 2002, at which point the community was largely left to manage fish ponds on its own. Since this time, villagers have expanded their aquaculture operation to the extent that nearly every family in Kathar has at least one fish pond. This practice has not been repeated anywhere in the Chitwan region, making Kathar a unique community which has had much greater access to fish compared to other Tharu villages over the past 12 years. A similar project was started by IAAS in the Tharu community of Kawasowoti, Nawalparasi in 2004. In this project, 48 ponds were constructed for individual households. Like fish ponds in Kathar, those built in Kawasowoti were unique to the region.

Small scale aquaculture in developing countries has been identified as a method to generate income (Duc, 2009), empower women (Bhujel et al., 2008), and increase food availability (Katz, 1987). Studies have also explored the effect of aquaculture on improved nutrition (Dey et al., 2006; Aiga et al., 2009; Jahan and Pemsl, 2011), but different researchers have measured nutritional outcomes in different ways. For example, Dey et al. (2006) and Jahan and Pemsl (2011) considered increased fish consumption to be synonymous with improved nutrition, while

Aiga et al. (2009) measured child nutrition through the assessment of physical growth. Other research has linked fish consumption with child nutrition, though not in relation to aquaculture (Larety et al., 1999; Gibson et al., 2003; Kongsbak et al., 2008; Lin et al., 2008). These studies used either child growth or micronutrient status to estimate nutritional outcomes. To my knowledge, no studies have been conducted in Nepal to measure the relationship between aquaculture and child nutrition.

Undernutrition is characterized by "inadequate intake of protein, energy, and micronutrients and by frequent infections and disorders that result" (WHO, 2000). Among many disorders experienced by undernourished individuals is growth retardation (Lunn, 2002; Neumann et al., 2004), which can be manifested from the fetal stage through child and pubertal development (Kramer, 1987) as low height-for-age (stunting), low weight-for-height (wasting), or low weight-for-age (underweight) (MOHP, 2012). The presence of wasting, underweight, and stunting in children as a result of dietary intake can be explained by multiple factors including mild to moderate forms of protein-energy malnutrition (PEM), which is defined by inadequate intake of protein, in addition to micronutrient deficiencies in iron, iodine, vitamin A, and zinc (Neumann et al., 2004). Wasting and stunting of children is directly related to diet and environment of the afflicted individual (Neumann et al., 2004). Animal source foods are known to contain protein and micronutrients that are lacking in diets of growth restricted children, but animal foods are often limited or unattainable for underprivileged individuals, especially in developing countries (Neumann et al., 2004). Because of the effect poor nutrition has on physical development, "child growth is internationally recognized as the best global indicator of physical well-being in children..." (Onis, 2008).

Child undernutrition in Nepal, as evidenced by growth retardation, is a serious problem. The most recent data from the Nepal Demographic and Health Survey showed that 41% of children in Nepal under the age of five were stunted, 29% were underweight, and 11% were wasted (MOHP, 2012). Similar percentages were found in the Terai, where 37.4% of children were stunted, 29.5% were underweight, and 11.2% were wasted (MOHP, 2012). The typical diet in Nepal consists primarily of rice, vegetable curry, dhal (a thick soup made from legumes), and other fruits and vegetables (Martorell et al., 1984). Meat and fish are noticeably absent, resulting in many people in the Terai having diets deficient in micronutrients such as vitamin A, iron, and zinc (Parajuli et al., 2012).

By supplementing the diet with protein and micronutrients from fish, aquaculture has the potential to affect the prevalence of child undernutrition in rural Nepal (Parajuli et al., 2012). Given the complexity of undernutrition and growth retardation, however, aquaculture cannot be considered a complete solution. For example, dietary intake and health status are immediate determinants of child undernutrition, but they are in turn affected by underlying determinants such as family income, food security, healthy living conditions, and access to health services (Smith and Haddad, 2000). Nevertheless, the potential benefit that aquaculture can have on child nutrition in rural Nepal, including increased consumption of protein and micronutrients to improve growth, clearly deserves attention.

Although fish can be nutritionally beneficial, aquatic species can also be contaminated with harmful pollutants such as mercury (Nesheim and Yaktine, 2007). Mercury is a naturally occurring element throughout the world, which cycles between terrestrial and aquatic habitats and the atmosphere. Although it occurs naturally, humans have greatly increased the amount of mercury emissions; it is estimated that anthropogenic activities are responsible for one-half to three-fourths of all mercury in the atmosphere (UNEP, 2008). Of these activities, coal combustion is the largest source, accounting for approximately half of all anthropogenic emissions (UNEP, 2008). After being emitted, mercury can be transported across the globe. Eventually, however, it is dry or wet deposited, where it can be transported to aquatic sediments (Selin, 2009). It is here that mercury is converted to methylmercury by sulfate and iron reducing bacteria (Selin, 2009). Methylmercury is lipophilic, and it biomagnifies through aquatic food chains and bioaccumulates in individual organisms like fish (Morel et al., 1998). Methylmercury is toxic, and humans that consumed large amounts of methylmercury-contaminated fish have been shown to experience adverse health outcomes (Grandjean et al., 1997; Eto et al., 2010).

The understanding that fish can both benefit and be a detriment to human health has resulted in numerous studies which have explored the potential risk and benefit of seafood consumption (Nesheim and Yaktine, 2007; Bloomingdale et al., 2010; Mahaffey et al., 2011). The consensus of such research suggests the benefits of fish consumption should not be overlooked, though effort should be made to eat fish high in omega-3 fatty acids and low in contaminants (Dovydaitis, 2008; Bloomingdale et al., 2010; Mahaffey et al., 2011). Fish species high in trophic level such as sharks and swordfish should be largely avoided (Domingo et al., 2007; EPA, 2012). Because species raised in aquaculture ponds in the Terai are low on the food chain, it is not expected that they, nor the people that consume them, will be burdened with high levels of methylmercury. However, given the global reach of mercury, its known harmful effects, and the relative ease with which quantities of the toxic metal can be determined in the human body through such methods as hair sampling (Airey, 1983; Wang et al., 2012), it is important to assess mercury concentrations in fish and in humans consuming fish.

The overall goal of this study was to explore the potential link between small scale aquaculture and child nutrition among the Tharu people in rural Nepal through assessment of growth of children between the ages of two and ten in villages which practice or do not practice fish farming. Specific objectives included determining: 1) if the presence of fish ponds in Tharu villages resulted in a higher consumption of fish than was accounted for among Tharu people without fish ponds; 2) if owning a fish pond in Tharu villages was linked to a decrease in prevalence of undernutrition among children between two and ten years old; 3) what socio-economic variables were associated with being undernourished in Tharu villages, and whether these variables were related to aquaculture; 4) if levels of methylmercury in cultured fishes in Tharu villages exceeded recommended levels; and 5) whether consumption of cultured fish led to unsafe levels of mercury in the bodies of children and adults among the Tharu population. I hypothesized that children who lived in fish farming families consumed greater amounts of fish than children in non-fish farming families, and this would results in lower prevalence of undernutrition. Because fishes raised in the Terai are low on the food chain, I further hypothesized that levels of methylmercury in fish and the people that consumed them would be below recommended levels.

METHODS

Geographically, Nepal is divided into three ecological regions: the mountain zone in the northern portion of the country, the central hill zone, and the southern Terai, or plains (MOHP, 2012). In contrast to high altitudes found in the first two regions, average elevation in the Terai ranges from 100-300m above sea level (Savada, 1991). Climate in the Terai is described as tropical and subtropical, and soils in this region are the most fertile in Nepal. The Terai holds 70% of the country's agricultural lands despite making up only 17% of Nepal's total area (Bindloss et al., 2009). Similarly, 94% of fish ponds in Nepal are located in the Terai **(**Pradhan, 2013), making this area a target for study of aquaculture in the country.

Kathar was originally planned to be the only aquaculture village surveyed, but Kawasowoti was later added to increase the number of children sampled. The village of Bhandara, Chitwan was selected for study as a control population. Bhandara was chosen because of its reputation of representing a typical Tharu community in the Terai without fish ponds.

Child nutrition studies are typically carried out through determination of growth of children between the ages of two and five years old (Martorell et al., 1984; Aiga et al., 2009; MOHP, 2012). In this study, the age range was broadened to include children between two and ten years because of limited sample size.

Within Kathar and Nawalparasi, households which included children between the ages of two and ten and owned at least one fish pond were recruited for participation through door to door visits. In Bhandara, all households with children between the ages of two and ten were recruited through door to door visits. A guide from each village identified eligible households and directed the field crew to the homes. After obtaining informed consent, mothers, who are the traditional care-givers and food preparers in Nepali culture, were specifically targeted to respond to survey questions. Interviews were conducted with the aid of a skilled Tharu/Nepali/English translator and cultural "broker" whose duties included ensuring that cultural sensitivities were considered at all times. In order to compensate survey respondents for their time, each family that participated was given US $5.00. All data for this study were collected from May-August, 2012.

The survey was designed to provide data to be used to determine rates of fish consumption among mothers and children and to test for possible variables associated with child undernutrition. The fish consumption portion of the survey was modeled after a questionnaire proven to be effective in the United States (Goodrich et al., 2011), while child nutrition queries were based on previous child nutrition studies and the USAID Nepal Demographic and Health Survey (NDHS) completed in 2011 (MOHP, 2012). Questions were asked regarding age, sex, duration of breastfeeding, introduction of first complementary food, history of child illness, socioeconomic status (in terms of income and possessions), parental education level, family number, number of children in the household, maternal health, ownership of a fish pond, and regular dietary intake (Appendix II). Survey techniques were approved by the University of Michigan Institutional Review Board for the use of human subjects (approval ID HUM00062823). A total of 86 surveys were completed for the study.

Child measurement data as well as child and maternal hair samples were collected immediately following interviews. If children were not available, return visits were made to the household at appropriate times specified by the mother. Weights and heights of children were determined following Demographic and Health Surveys (DHS) Methodology as a means for determining prevalence of undernutrition (MOHP, 2012). For weights, a digital SECA 803 scale was used. The scale was carried from house to house, and was placed on a hard, level surface. Children were weighed individually. Parents were asked to remove shoes and any heavy clothing of children before weighing. If a child was incapable of standing on the scale, the child’s mother was asked to stand on the scale while holding the child. She was then weighed without the child, and the child’s weight determined by subtraction. Child height was determined with a portable stadiometer. The stadiometer was placed on a hard, level surface. The parent was then asked to remove the child’s shoes, bring the child to the stadiometer, and to kneel in front so the child remained comfortable and cooperative. A total of 111 children were weighed and measured.

Samples were taken from mothers and children in order to determine levels of methylmercury in the body. Approximately 20-30 strands of the ends of hair were cut from the back of each subject’s head using blunt scissors. The hair was placed onto the adhesive end of a Post-It note, folded, and placed in a labelled plastic Ziploc bag. All samples were stored at room temperature until they were brought to Ann Arbor, MI, where they were stored at 4°C. A total of 141 hair samples were successfully analyzed, including 66 moms, 36 girls, and 39 boys. Hair samples were not taken from the village of Kawasowoti due to time constraints. In certain cases, families did not allow boys’ hair to be cut because of cultural beliefs.

Three fish from each carp species (*Cirrhinus cirrhosis, Hypophthalmichthys molitrix, Labeo rohita, Hypophthalmichthys nobilis, Cyprinus carpio,* and *Ctenopharyngodon idella*), Nile tilapia (*Oreochromis niloticus*), and African catfish (*Clarias gariepinus*) were purchased from Kathar fish farmers. Seven individuals from each of what are referred to as Small Indigenous Species (SIS), *Puntius sophore* and *Esomus denricus*, were collected in the same manner. All of the fish had been harvested and killed before collection. For each carp, tilapia, and catfish individual, approximately one thimble full of flesh was cut laterally from the body. For the SIS species, the entire fish was kept. All samples were dried at 60°C overnight at the IAAS research center. They were then ground into powder using a mortar and pestle, packaged, and brought to the University of Michigan for further analysis. The two SIS species were ground together after being dried.

DATA ANALYSIS

Fish Consumption

Average portion size of fish consumed at fish meals and frequency of consumption of each fish species during a month in the harvest season were determined from survey questions for mothers and eldest children from each family (Appendix II). Monthly fish consumption (g) was then calculated for individuals by multiplying portion size by the total number of times fish was eaten. Following these calculations, comparisons of fish consumption were made between mothers and children who lived in households with and without fish ponds.

Mercury

Calibrated analytical balances were used for measuring all samples (i.e., fish, hair, and Standard Reference Materials). Clean instruments were used to handle all samples. The direct mercury analyzer (DMA-80, Milestone Inc., CT http://www.milestonesci.com/mercury-dma.php) was used to determine total mercury. Nickel and ceramic boats for the DMA were freshly cleaned and sonicated prior to analysis.

Samples collected in Nepal were analyzed between September and December 2012. Fish tissue was re-dried at 60°C overnight before being evaluated. Samples were then weighed into 10 milligram portions in nickel boats before being run in the DMA. Two species of fish (*Cyprinus carpio* and *Clarias gariepinus*) were digested in acid prior to placement in the DMA because of their oily content. Approximately 0.5 g of each sample was weighed into acid-washed Teflon tubes and 0.5 mL of concentrated nitric acid was added. After 24 hours, test tubes were heated to 60°C for one hour and then 90°C for three hours. These acid digested samples were diluted with 4.5 mL Milli-Q water resulting in a solution of 7% nitric acid. A 0.3 mL aliquot of these solutions was pipetted into ceramic boats in the DMA. The concentration of fish in each sample (approximately 0.1g/mL) was multiplied by the 0.3 mL sample volume to obtain fish mass (g). Then, this value and the amount of mercury found in each sample by the DMA (ng) were converted to mg/kg.

Hair samples were placed into aluminium boats and washed once with 5 mL of acetone and three times with 5 mL of de-ionized water. While being washed with acetone and de-ionized water, each sample was agitated for 60 seconds. Samples were left to dry overnight in a fume hood. For most samples, 5 mg of hair was used. When the total sample did not amount to 5 mg, all hair available was used.

Appropriate Standard Reference Materials (SRMs) were measured each day of analysis to determine validity of calibration curves. The SRM for fish tissue was DOLT-4 (dogfish liver), and the SRM for hair samples was NIES Japan CRM #13 (human hair). An SRM was run at least once every eight samples. Empty nickel boats were also run at least once every eight samples as blanks. Thus, every ten samples included at least one SRM and one blank.

Precision (reproducibility) was measured by within day replicate analysis of SRMs. The Relative Standard Deviation (RSD) was calculated as (SD/$\bar{x}$) x 100 where SD = standard deviation and $\bar{x}$ = mean of replicate samples. RSD gave an indication of precision in sample preparation, sample aliquotting, and analysis. Replicates of an SRM on each day provided an indication of within day precision.

The amount of mercury found in blank samples was considered noise. For measurement of the concentration of mercury in fish and hair samples to be considered reliable, mean values had to exceed three times the standard deviation (i.e., above the 99% confidence level) of this noise. Noise was calculated as the mean value of mercury detected in all blanks run each day. Three times the standard deviation was referred to as the theoretical method detection limit, or TMDL. The Practical MDL (PMDL) was five times the TMDL. Any value reported between the TMDL and PMDL could not be considered accurate as Hg was present in low levels but the absolute concentration was not precise. TMDL and PMDL were calculated for fish and hair samples. All of the mercury values of the 27 fish samples or 141 hair samples were greater than the TMDL and PMDL, so none were excluded.

Average hair mercury values were compared between those from households with and without fish ponds with independent samples T-tests.

Aquaculture and Nutrition

The 2006 World Health Organization (WHO) growth standard charts were used to determine height-for-age, weight-for-height, and weight-for-age trends among the children sampled. The WHO growth charts were created from data collected globally, and serve as a standard that describes the way healthy children should grow under optimal conditions (Grummer-Strawn et al., 2010). A child two standard deviations below average weight-for-age is considered "underweight," a child two standard deviations below average height-for-age chart is considered "stunted," and a child two standard deviations below average weight-for-height is considered "wasted."

Measurements for each child measured were plotted on WHO height-for-age, weight-for-age, and weight-for-height z-score growth standard curves (Appendix III). Children that were below two standard deviations on any of the curves were recorded. Additionally, individual z-scores for each child were determined for weight-for-age (WAZ) and height-for-age (HAZ) using WHO Anthro and WHO AnthroPlus growth standard software (www.who.int/childgrowth/en) to show the exact number of standard deviations each child was from average growth.

In order to determine the effect of a household fish pond on child nutrition, children were separated into groups of those whose families had ponds and whose families did not. This was later changed, however, when I realized that 16 children lived in families with ponds that they had owned for less than two years. Since children would most likely not show a difference in growth after having a family fish pond for only one season, children were separated into groups based on household possession of fish ponds for at least five years. Five years was chosen because it was the shortest amount of time any family had operated a fish pond excluding the eight families that had ponds for less than two years. Of the total 111 children sampled, 54 children lived in families with ponds for at least 5 years and 57 children lived in families without ponds or with ponds for less than 5 years. This distinction was made for all child nutrition analyses, but not for comparisons made between groups of mothers and children for fish consumption and hair mercury. For these analyses, individuals were simply grouped into those who lived in households with and without ponds because interview questions focused on activities in the year prior to data collection.

Data from the surveys was organized into 25 variables describing demographic, dietary, health, and socioeconomic status (Appendix IV). Variables with a binary outcome (e.g., yes or no, male or female) were coded into 0 and 1, respectively.

To test if owning a fish pond in Tharu villages was linked to a decrease in the prevalence of undernutrition, comparisons were made between percentages of children from households with and without ponds that were stunted and underweight using Chi squared tests. Additionally, average z-scores for weight-for-age and height-for-age were compared between children from households with and without ponds using independent samples T-tests.

Bivariate and multivariate analyses were carried out to determine if monthly fish consumption and/or other survey variables were associated with being undernourished. First, bivariate analyses were used to determine associations between background variables (Appendix V) and being undernourished. Outcome variables included stunted or not stunted (binary), underweight or not underweight (binary), HAZ (continuous), and WAZ (continuous). For continuous predictors and binary outcomes, Independent samples T-tests were carried out when each category of the predictor was shown to be normally distributed. For non-normal distributions, Mann-Whitney U tests were used. Normality was determined through Shapiro Wilk tests. Chi squared tests were used to explore associations between binary predictors and binary outcomes. For continuous predictors and continuous outcomes, Pearson correlations were used when data was found to be normally distributed (Shapiro Wilk), and Spearman correlations were used for non-parametric testing. Regarding binary predictors and continuous outcomes, means were compared using Independent samples T-tests or Mann-Whitney U tests depending on normality (Appendix V).

Next, multivariate analysis was used to identify significant independent predictors. Multiple linear regression models were run with the continuous outcomes of WAZ and HAZ instead of carrying out logistic regression with binary outcomes (stunted yes or no, wasted yes or no) to give the models more power. Background variables were tested for multicollinearity with a correlation matrix. When two predictors were found to be highly correlated, each was removed from the model in successive simulations to determine if either was independently significant. In this way, every variable was tested in the model. Predictors were entered in the model in a stepwise process.

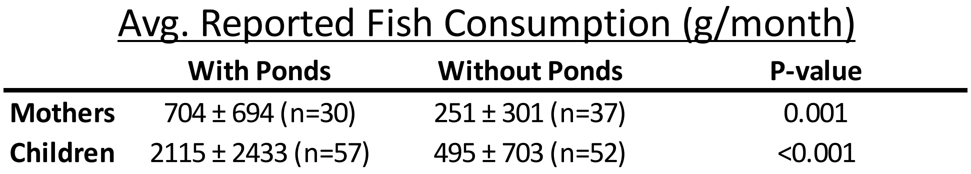
Because only five children were found to be wasted, variables were not tested against this outcome. All analyses were carried out using IBM SPSS version 20 software.

RESULTS

Fish Consumption

Although the fish consumption survey used in this study **(**Appendix II) provided clear results when used in the U.S., it was not equally successful in Nepal. Mothers surveyed found the questions to be confusing, especially in regards to the species of fish consumed. After surveying was completed, it was determined that Tharu families usually did not attempt to catch particular fish for meals. Rather, they cast a net into their ponds and consumed whatever fish they happened to catch. They did not remember how often they caught and ate particular species of fish, likely because they were not concerned with this information at the time of harvest. As a result, monthly reports of fish consumption frequency were regularly exaggerated. For example, some mothers reported to have eaten over 120 meals of fish per month, which was extremely unlikely given that Nepali people generally consume two meals a day. Nevertheless, average fish consumption values for mothers and children were estimated from these data. Mothers and children from households with ponds consumed significantly more fish than mothers and children from households without ponds (Table 1). Girls and boys were grouped together as "children" for these analyses as there were no significant differences in fish consumption between boys and girls.

Table 1. Fish consumption (g) reported (mean ± SD) for mothers and children from households with and without ponds. P-value from Mann-Whitney U test.



Avg. Reported Fish Consumption (g/month)

	With Ponds	**Without Ponds**	**P-value**
Mothers	704 ± 694 (n=30)	251 ± 301 (n=37)	0.001
Children	2115 ± 2433 (n=57)	495 ± 703 (n=52)	<0.001

In order to account for possible inaccuracy, an additional strategy was used: the percentages of mothers and children who reported consuming each fish species were estimated independent of the frequency with which they reported to consume the species. A significantly greater percentage of mothers and children with fish ponds consumed carp species and tilapia compared to mothers and children without fish ponds (Figures 2 and 3). However, this trend was not observed in SIS and catfish, for which both groups had similar levels of consumption ($p > 0.05$).

A similar trend was shown in the average amounts of fish consumed by those with and without ponds; those with ponds reported to eat far greater amounts of carp and tilapia per month than those without ponds, while the amounts of SIS and catfish consumed each month were similar between groups (Figures 4 and 5).

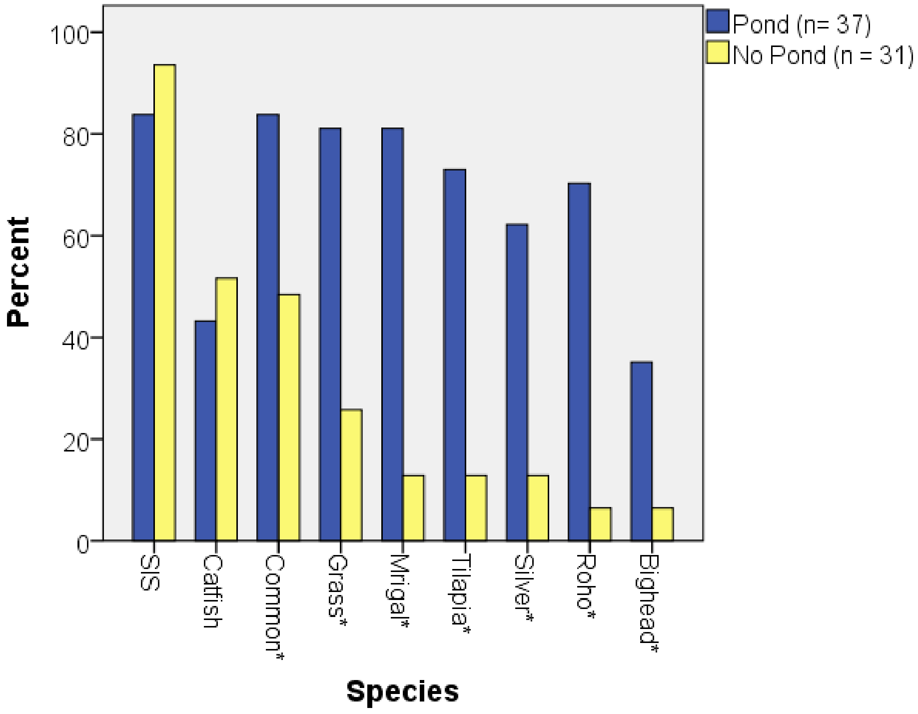


Figure 2. Average percent of mothers who consumed each fish species from households with and without ponds. Starred (*) species represent significant differences ($p < 0.05$).

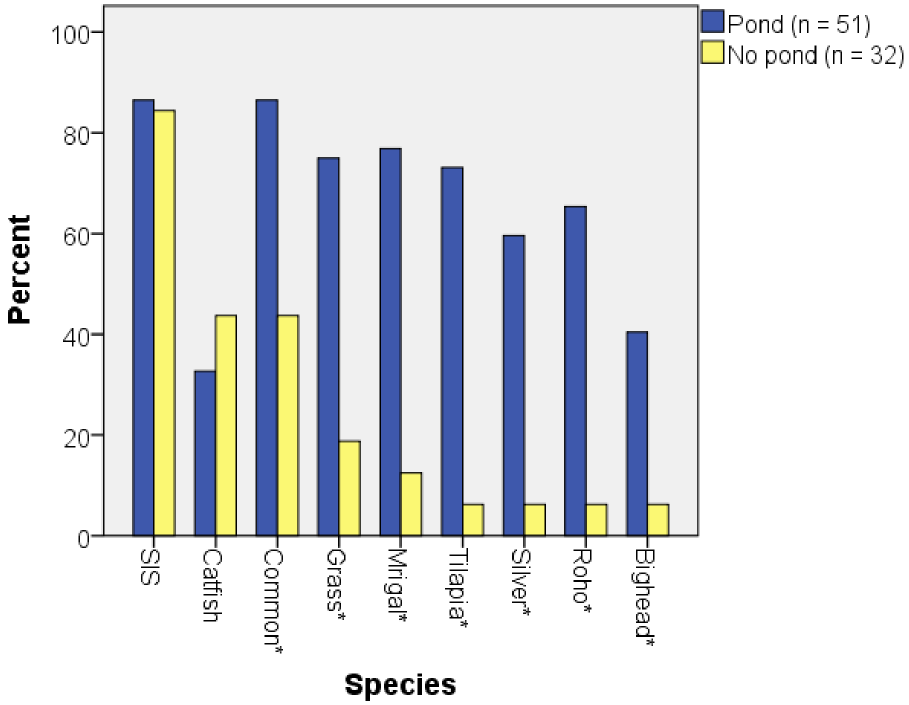


Figure 3. Average percent of children who consumed each fish species from households with and without ponds. Starred (*) species represent significant differences ($p < 0.05$).

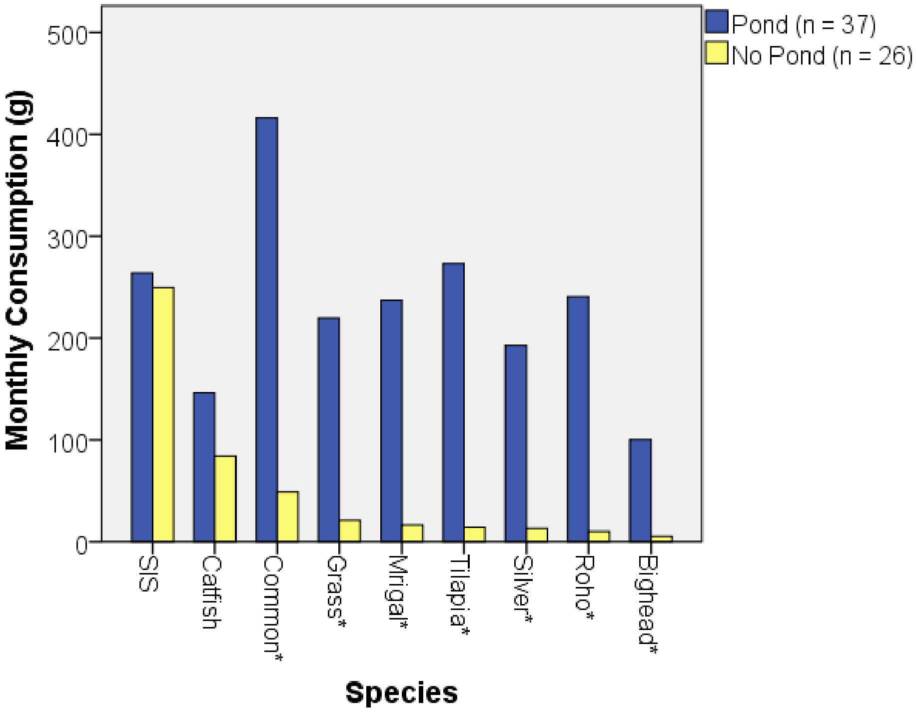


Figure 4. Average monthly consumption of each fish species by mothers from households with and without ponds. Starred (*) species represent significant differences ($p < 0.05$).

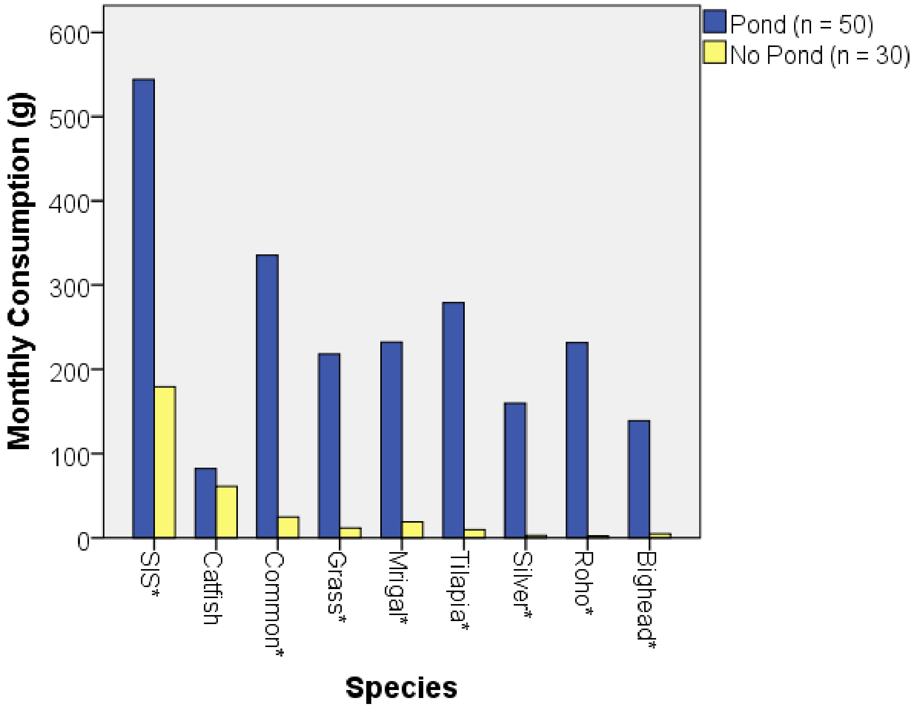


Figure 5. Average monthly consumption of each fish species by children from households with and without ponds. Starred (*) species represent significant differences ($p < 0.05$).

Mercury

Quality control results for fish and hair mercury (mercury recovery, variability, and detection limits) were all found to be within acceptable limits (Appendix VI).

Total mercury was measured in approximately 10 mg of dried fish tissue for all fishes with the exception of common carp and North African catfish, for which it was measured from an approximate 0.1g/mL concentration of dried fish tissue in a 0.3 mL nitric acid-digested sample. Three samples of each species were run in the DMA. In the case of the SIS species, each of the three samples run were a mix of *Puntius sophore* and *Esomus denricus*. Wet weight concentrations were determined by dividing average dry weight concentrations by four (assuming 25% dry matter). Wet weight mercury concentrations for all species were found to be below the US EPA standard of 0.3 mg/kg (EPA, 2001) and WHO standard of 0.5 mg/kg (WHO, 2008) for human consumption (Table 2), suggesting mercury contamination was not a concern in cultured fishes in the Terai.

Table 2. Concentration of methylmercury (mean ± SD) found in cultured fish species.

Scientific Name	Common Name	N	Dry weight concentration (mg/kg)	Wet weight Concentration (mg/kg)
Cirrhinus cirrhosus	mrigal carp	3	0.401 ± .039	0.100 ± 0.001
Hypophthalmichthys molitrix	silver carp	3	0.320 ± .039	0.080 ± 0.001
Labeo rohita	roho labeo	3	0.277 ± .026	0.069 ± 0.007
Hypophthalmichthys nobilis	bighead carp	3	0.213 ± .135	0.053 ±0.034
Puntius sophore, Esomus denricus	SIS	3	0.180 ± .077	0.045 ± 0.019
Cyprinus carpio	common carp	3	0.142 ± .013	0.036 ± 0.003
Ctenopharyngodon idella	grass carp	3	0.059 ± .011	0.015 ±0.003
Oreochromis niloticus niloticus	Nile tilapia	3	0.025 ± .016	0.006 ± 0.004
Clarias gariepinus	North African catfish	3	0.020 ± .011	0.005 ± 0.003

The average mercury found in hair samples (±SD) was 0.762 ± 0.807 µg/g. This is slightly higher than the 0.48 µg/g average found in the general U.S. population, but it is below the 1 µg/g reference dose set by the EPA (EPA, 1997). No significant difference was found in average hair mercury values between those who lived in households with and without ponds (Table 3). Because consumption of aquatic organisms such as fish is known to be the greatest source of mercury contamination in humans, and because it was determined that those in households with fish ponds consumed more fish than those without fish ponds, this relationship was not expected. These results suggest the Tharu people were exposed to mercury from a different and more largely contributing source than fish. The results for total mercury in samples can be seen in Appendix VII.

Table 3. Average mercury found in hair samples (µg/g) from mothers, girls, and boys who lived in households with and without fish ponds.

	Total		Mothers		Girls		Boys	
	Pond	No Pond	Pond	No Pond	Pond	No Pond	Pond	No Pond
n	75	66	37	29	21	15	20	19
Avg	0.7230	0.8070	0.8230	0.9240	0.5931	0.8360	0.6620	0.6260
St. Dev.	0.7780	0.8430	1.0900	1.0600	0.1890	0.9360	0.1920	0.2400
T-test p-value	0.539		0.706		0.253		0.607	

Child Nutrition

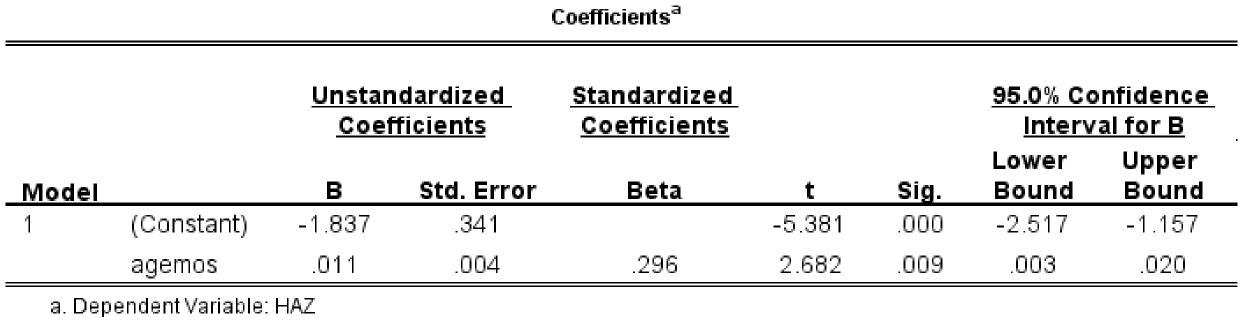
No significant difference was found between children whose families had ponds and those whose families did not have ponds for any category of stunted, underweight, HAZ, or WAZ (Table 4). This suggests owning a fish pond in Tharu villages was not linked to a decrease in the prevalence of undernutrition among children between two and ten years old.

Table 4. Percentage of undernutrition, average HAZ, and average WAZ for children aged between two and five by the NHDS (MOHP, 2012) and children aged between two and ten in this study. P-values are shown for tests of association between those from households with and without ponds. Different tests were used based on normality and sample size.

	NHDS Nepal	NHDS Central Terai	Total	Pond	No Pond	P-value	Test
N	2475	507	111	58	53	N/A	N/A
Stunted							
< 3 SD	16.2%	19.5%	4.5%	6.9%	1.9%	0.367	Fisher
< 2 SD	40.5%	40.5%	15.3%	13.8%	17.0%	0.641	X^2
Avg. HAZ	-1.7	-1.7	-0.99	-1.10	-0.91	0.922	Mann
Underweight							
< 3 SD	7.7%	10.7%	5.4%	6.9%	3.8%	0.681	Fisher
< 2 SD	28.8%	32.0%	25.2%	20.7%	30.2%	0.25	X^2
Avg. WAZ	-1.4	-1.4	-1.4	-1.4	-1.4	0.836	T

The only significant predictor of HAZ identified in multiple linear regression was child age (agemos) (p = 0.009). Every one month increase in child age was significantly associated with a 0.011 higher height-for-age z-score (95% CI: 0.007, 0.027) (Table 5).

Table 5. Multiple linear regression results for dependent variable HAZ.



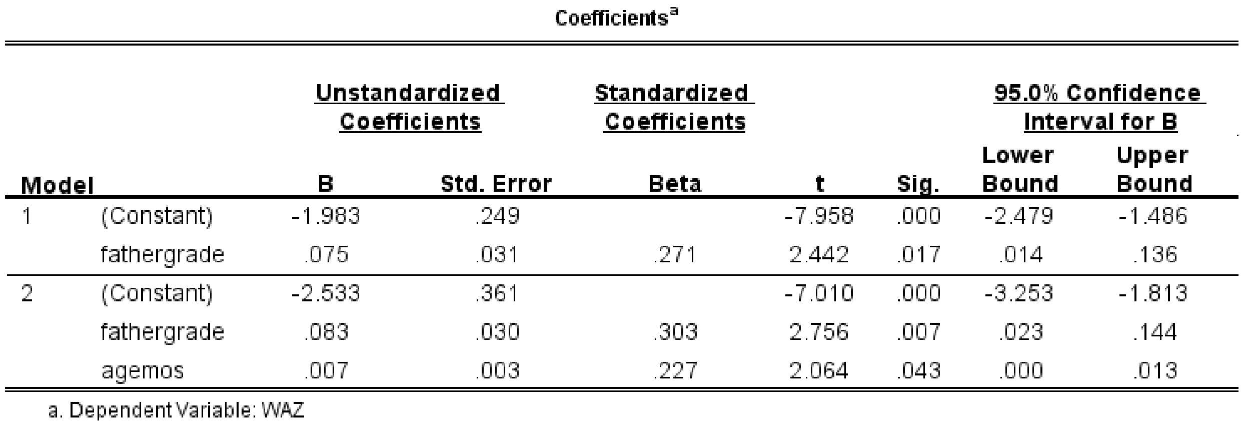
Coefficients[a]

Model		Unstandardized Coefficients B	Unstandardized Coefficients Std. Error	Standardized Coefficients Beta	t	Sig.	95.0% Confidence Interval for B Lower Bound	95.0% Confidence Interval for B Upper Bound
1	(Constant)	-1.837	.341		-5.381	.000	-2.517	-1.157
	agemos	.011	.004	.296	2.682	.009	.003	.020

a. Dependent Variable: HAZ

Significant predictors of WAZ identified in multiple linear regression included paternal grade level (fathergrade) (p = 0.007) and child age (agemos) (p = 0.043). An increase in one grade level completed by the father was shown to be significantly associated with a 0.083 higher weight-for-age z-score in children (95 % CI: 0.023, 0.144), and every one month increase in child age was significantly associated with a 0.007 higher weight-for-age z-score (95% CI: 0.000, 0.013) (Table 6).

Table 6. Multiple linear regression results for dependent variable WAZ.



Coefficients[a]

Model		Unstandardized Coefficients B	Unstandardized Coefficients Std. Error	Standardized Coefficients Beta	t	Sig.	95.0% Confidence Interval for B Lower Bound	95.0% Confidence Interval for B Upper Bound
1	(Constant)	-1.983	.249		-7.958	.000	-2.479	-1.486
	fathergrade	.075	.031	.271	2.442	.017	.014	.136
2	(Constant)	-2.533	.361		-7.010	.000	-3.253	-1.813
	fathergrade	.083	.030	.303	2.756	.007	.023	.144
	agemos	.007	.003	.227	2.064	.043	.000	.013

a. Dependent Variable: WAZ

No variables related to aquaculture were found to be associated with HAZ or WAZ, suggesting aquaculture was not related to child nutrition among the Tharu.

DISCUSSION

No conclusive evidence was found regarding the link between aquaculture and child nutrition; neither owning a household fish pond nor any variable associated with nutrition was linked to a decreased prevalence of undernutrition (Tables 4, 5, and 6). Therefore, the hypothesis that children in fish farming households would have a lower prevalence of undernutrition was not supported.

As hypothesized, mothers and children with fish ponds ate more fish than those without fish ponds. Aquaculture projects in this region have focused on raising carps and tilapia, so it was not surprising to find that a greater percentage of those with ponds ate these species than those without ponds. Similarly, because those with ponds had greater access to these species, it was expected they would consume greater amounts of these fishes than those without ponds. Because SIS were largely wild caught, those with ponds were not be expected to have greater access to them than those without ponds. However, farmers explained that SIS found their way into carp and tilapia ponds inadvertently through travel in manmade waterways, and I witnessed SIS being caught in nets in fish ponds. This perhaps provided slightly greater access to those with ponds.

African catfish were raised in the Terai before recent carp polyculture projects began recently. While those in fish pond communities largely raised carps and tilapia, some also maintained separate catfish ponds. Bhandara, the selected control village, was identified as a Tharu village without fish ponds. This village was certainly without carp and tilapia fish ponds, but it may have had a small number of catfish ponds, which were not considered to be part of a fish pond community and which were generally small and difficult to see. This potentially explains why no significant differences existed between average monthly catfish consumption in the two groups.

Studies assessing the effects of small-scale aquaculture on fish consumption of families that own fish ponds have had mixed results (Kawarazuka, 2010). For example, research in Bangladesh showed that among households practicing aquaculture, lower income households tended to sell raised fish rather than consume them, while higher income families had higher rates of consumption (Kawarazuka, 2010). In the Terai, no correlation was found between household income and fish consumption, and no difference was found between income levels of families who sold or did not sell fish. This suggests income was not an important factor in relation to fish consumption. Instead, simple presence of a fish pond was shown to increase fish consumption regardless of income. Studies in Malawi found similar outcomes, concluding the presence of fish ponds resulted in higher fish consumption (Dey et al., 2006).

The determination of whether a fish farming household consumes the fish it raises can be based on a number of factors, including income of the family, species of fish raised, and ultimate desires of the household (Kawarazuka, 2010). If the primary goal of the household is to increase income, it is likely that few fish will be kept for consumption. Nepali Tharu fish farmers generally had small ponds which served the main purpose of supplying fish for household consumption, but some families also possessed larger ponds used for raising a surplus of fish to be sold at local markets. Families with small ponds often expressed their desire to have larger ponds to raise more fish, but were usually limited by the amount of land they possessed. This suggests land ownership and pond size could be the most important factors determining whether a family sells fish in the Terai. These factors were not considered when collecting data for this study, and could have provided more detailed information regarding consumption and selling practices.

The results of the fish consumption analysis were affected by two limitations: 1) as aforementioned, respondents did not appear to completely understand the survey due to uncertainty regarding species of fish consumed, and 2) due to seasonality in fish production, subjects were asked to recall fish consumption practices from approximately eight months prior to the time of data collection. In regards to the former, analyses were carried out with the purpose of minimizing error as a result of possible confusion. Rather than determining the amount of each species consumed by each respondent, percentages of respondents that ate a particular species were determined, and the average amount of each species consumed by certain groups was estimated. In future research, improvements could be made by altering the survey to inquire into the frequency and amount of which all fish was consumed instead of asking for species-specific data. Alternatively, monitoring could be carried out by the researcher during harvest season to observe consumption practices of subjects.

In the populations sampled, fish ponds were filled and stocked between April and June, fish were fed and grown between May and October, and fish were harvested, consumed, and sold between October and February. At the end of the harvest season, ponds were drained and cleaned before being filled again for a new season. This process is related to water availability; Nepal receives nearly all of its rainfall during a monsoon period in the summer months, a time which allows the Tharu to divert water from heavy flowing rivers to fill their ponds. Due to such seasonal practices, nearly all local fish consumption takes place in the late fall and winter. Additionally, Tharu people reported not desiring to eat fish in summer months due to local beliefs that consuming fish heats the human body and leads to discomfort in hot weather. The survey was designed to inquire about fish consumption during the month prior to data collection in order to minimize recall bias. However, because fish had been almost completely absent from the diet in the month prior to data collection, subjects were instead asked to report consumption data during the harvest season, for the month in which they consumed the largest amount of fish. This was approximately six months prior to data collection. In order to reduce recall bias, it would be better to collect fish consumption data in the winter months.

As hypothesized, all of the fish species analyzed were found to contain mercury levels below recommended guidelines for safe consumption. Mercury was not found to be a harmful contaminant in fishes raised in the Terai. No other studies have assessed mercury levels in fishes in Nepal, but similar work carried out in nearby Northern India showed freshwater species to range in mercury concentration from 0.119 to 0.277 mg/kg in Lucknow (Agarwal et al., 2007) and from 0.073 to 0.94 mg/kg in West Bengal (Bhattacharyya et al., 2010). These levels are higher than those found in the Terai, but both of these studies measured mercury from fishes in highly polluted waters.

Average hair mercury values among the Tharu people were found to be below the one µg/g reference dose level considered to be potentially damaging to human health by the USEPA (EPA, 1997). Furthermore, average Tharu hair mercury values were mostly lower than those in fish-eating populations around the world in which similar studies have been carried out (Agusa et al., 2005). For example, the average Tharu values (0.762 mg/kg) were lower than those found in populations in Japan (1.57-5.0 mg/kg), Cambodia (3.1 mg/kg), Kuwait (2.62-4.18 mg/kg) and Kenya (1.44-4.50 mg/kg) (Agusa et al., 2005).

Average hair mercury values were not shown to be higher in individuals with fish ponds than in those without fish ponds. Consumption of aquatic foods is generally accepted as the major source of mercury contamination in humans (Mergler et al., 2007), and I found that those with ponds ate more fish than those without ponds, so this relationship was not expected. This association suggests that other sources of mercury were responsible for concentrations found among the Tharu. An alternative pathway of mercury into the human body is rice consumption, which is an exposure route that can be greater than that of fish consumption in some locations (Zhang et al., 2010). Rice is a staple food among Tharu people, and it was generally consumed at least two meals every day in all families surveyed. Taking this into consideration, rice consumption may serve as an important route of mercury exposure among Tharu populations, while cultured fish may contribute to a lesser degree. Further research into mercury concentrations in rice could help to clarify such relationships in the Terai.

Studies which assess hair mercury generally do so by analyzing the amount present in the two cm of hair closest to the scalp of the subject (Goodrich et al., 2011; Hsiao et al., 2011; Nyland et al., 2011). Because hair grows at approximately one centimeter per month (Nuttall, 2006), testing this fragment allows for the association of hair mercury and recent mercury ingestion. In this study, hair samples were cut from the ends of hair. Subjects had varying lengths of hair, including large differences between females and males, making a time association impossible. Nevertheless, hair mercury information was still obtained from the sample, providing insight into mercury burden in the Tharu people. Because boys had shorter hair than mothers and girls, it was expected that male hair samples would give the best indication of mercury gained from recent consumption practices. The majority of fish consumption occurred approximately six months prior to hair collection, so boys' hair most likely indicated mercury from rice instead of fish. However, hair mercury from males was not shown to be statistically different from that of other groups (Figure 8). This provides further evidence that fish is not the major pathway of mercury consumption in the Tharu people.

The aquaculture and child nutrition results could have been affected by several factors. Overall, the percentages of undernutrition among Tharu children in this study were found to be lower than those found by the NDHS for the same region and countrywide (with the exception of average WAZ) (MOHP, 2012), (Table 4). This could be due to low sample size (n=111), which may have been too small to reflect overall population trends and to detect significant differences among predictor variables. Additionally, physical growth was the sole outcome used to measure nutritional status. While growth is recognized as an effective indicator for child nutrition (Onis, 2008), it cannot be used to measure micronutrient deficiencies, which can cause serious health consequences while also being symptomless (Kawarazuka, 2010). An assessment of such deficiencies, which can be carried out using biochemical indicators, could have strengthened child nutrition analyses in this study.

Significant predictors in multiple linear regression included child age (HAZ and WAZ) and paternal education level (WAZ). Regarding age, an increase in one month was significantly associated with improved growth in relation to HAZ and WAZ (Figures 13-16). Associations with age in child nutrition studies are often found, but they generally show the opposite relationship; as age increases, there is a greater chance of being undernourished (Rahman et al., 2009; Khan and Azid, 2011; Babatunde et al., 2011). This relationship has been attributed to a number of explanations, including the possibility that older children receive less food after younger siblings have been born (Khan and Azid, 2011), that undernutrition is not experienced by children until after they have been breastfed (resulting in healthier young children) (Babatunde et al., 2011), and that older children are more exposed to disease, lowering their nutritional status (Rahman et al., 2009). It is uncertain why older children were found to be better nourished in the Terai, but similar results were found in another child nutrition study carried out in the same region; Martorell et al. (1984) found that children beyond the age of five years were less likely to be stunted than those who were younger. It is unlikely this relationship exists due to differences in dietary intake. In a study regarding household food allocation in rural Nepal, Gittelsohn (1991) determined that children under eight years of age were usually given food first at mealtime, and that they were treated equally, regardless of age or sex, in terms of the amounts and types of food served.

Educational level of parents has been linked to child undernutrition in multiple studies (Delpeuch et al., 1999; Tharakan and Suchindran, 1999; Skoufias, 1999). Among the Tharu, education level of the father was shown to be significant while that of the mother was not. Fathers are responsible for providing income in Tharu families, a factor supported by a positive relationship shown between paternal grade level and socioeconomic status. Socioeconomic factors have also been widely shown to be determinants of child nutrition level (Firestone et al., 2011; Delpeuch et al., 1999; Martorell et al., 1984).

In a study in Malawi, Aiga et al. (2009) found that children were better nourished in fish farming households, but this was not linked to fish consumption. Instead, the authors suggested that income generated by fish farming allowed households to purchase additional food items which benefitted child growth (Aiga et al., 2009). Among the 51 Tharu families surveyed in this study with fish ponds (excluding 4 families for which fish sales data was missing), 41% used the fish they raised solely for household consumption. Of the remaining 59%, fish sales were made which averaged approximately 4% of the household's yearly family income. Analyses were not carried out to determine how these households spent money generated by selling fish, but no link was found between aquaculture sales and child growth in multivariate analysis.

The nutrients available to children from fishes raised in the Terai differ among species. Carp, tilapia, and catfish species are mostly beneficial for the high value protein they provide, while SIS contain high amounts of vitamin A, calcium, and zinc (Kawarazuka and Bene, 2011). Protein and each of these micronutrients are known to affect physical growth, and in situations in which children have especially limited diets, the lack of any one of these nutrients can be responsible for retarded growth (Rivera et al., 2003). However, based on studies carried out in Peru and Mexico, it has been suggested that "relatively small increases in the intake of animal source foods may reduce the prevalence of growth stunting in populations at risk" (Rivera et al., 2003). Although families with fish ponds in the Terai were shown to eat more fish than families without ponds, the latter still consumed fish, and consumption patterns of catfish and SIS were quite similar. It is possible that children sampled in this study without fish ponds consumed enough fish and other animal source foods to provide sufficient nutrients to allow them to grow at a similar rate as those with fish ponds, and the consumption of fish by all children could be responsible for the overall better nutrition shown in this group compared to country-wide averages (Table 4). Furthermore, because those with fish ponds only consumed more fish during the harvest season, the benefit provided to them may not have been overly significant.

Children who suffer from poor nutritional status due to a limited diet can improve their health by consuming nutrient-rich foods such as fish (Kawarazuka, 2010), and aquaculture serves as an effective way to provide fish in locations where the creation and maintenance of ponds is feasible. However, child undernutrition is a complex condition with results from a combination of dietary, health, and socioeconomic factors (Smith and Haddad, 2000), and it is challenging to link improvements in nutrition from benefits provided solely by aquaculture. Amongst the Tharu no relationship was found between aquaculture and child undernutrition, but the entire sample of children, all of whom had access to fish, were shown to have better nutrition than other children in Nepal. The improvement in child diet provided by a nutritious food source like fish cannot be overlooked, and aquaculture can serve as at least one method to help fight child undernutrition.

Because of the high nutritional value of SIS and the frequency with which they are consumed, I recommend that efforts be made to help the Tharu people incorporate SIS production into carp polyculture ponds. This could help increase the availability of a highly nutritional food source, and it could help to reduce fishing pressure on wild SIS stocks. I also recommended that Tharu people explore methods of raising fish on a year-round basis. Although fish may not be highly desirable during hot weather, it could benefit growing children to have access to fish regardless of the season.

LITERATURE CITED

Aiga, H., Matsuoka, S., Kuriowa, C., and Yamamoto, S. 2009. Malnutrition among children in rural Malawian fish-farming households. Transactions of the Royal Society of Tropical Medicine and Hygiene 103: 827-833.

Agarwal, R., Kumar, R., and Behari, J.R. 2007. Mercury and lead content in fish species form the River Gomti, Lucknow, India, as biomarkers of contamination. Bulletin of Environmental Contamination and Toxicology 78: 118-122.

Airey, D. 1983. Mercury in human hair due to environment and diet: a review. Environmental Health Perspectives 52: 303-316.

Agusa, T., Kunito, T., Iwata, H., Monirith, I., Tana, T.S., Subramanian, A., and Tanabe, S. 2005. Mercury contamination in human hair and fish from Cambodia: levels, specific accumulation and risk assessment. Environmental Pollution 134: 79-86.

Babatunde, R.O., Olagnuju, F.I., Fakayode, S.B., and Sola-Ojo, F. E. 2011. Prevalence and determinants of malnutrition among under-five children of farming households in Kwara State, Nigeria. Journal of Agricultural Science 3: 173-181.

Bhattacharyya, S., Chaudhuri, P., Dutta, S., and Santra, S.C. 2010. Assessment of total mercury level in fish collected from East Calcutta Wetlands and Titagarh sewage fed aquaculture in West Bengal, India. Bulletin of Environmental Contamination and Toxicology 84: 618-622.

Bhujel, R. C., Shrestha, M. K., Pant, J., and Buranrom, S. 2008. Ethnic women in aquaculture in Nepal. Development 51: 259-264.

Bindloss, J., Holden, T., and Mayhew, B. 2009. Lonely Planet Nepal. Eighth ed. Lonely Planet Publications Propriety Limited. Toppan Security Printing Private Limited., Singapore.

Bloomingdale, A., Guthrie, L.B., Price, S., Wright, R.O., Platek, D., Haines, J., and Oken, E. 2010. A qualitative study of fish consumption during pregnancy. American Journal of Clinical Nutrition 92: 1234-40.

Delgado C.L., Wada N., Rosegrant M.W., Meijer S., Ahmed, M. 2003. Fish to 2020: Supply and Demand in Changing Global Markets. Penang (Malaysia): World Fish Center.

Delpeuch, F., Traissac, P., Martin-Prevel, Y., Massamba, J.P., and Maire, B. 1999. Economic crisis and malnutrition: socioeconomic determinants of anthropometric status of preschool children and their mothers in an African urban area. Public Health Nutrition 3: 39-47.

Dey, M. M., Kambewa, M., Prein, D., Jamu, F.J., Paraguas, E., Pemsl, E., Briones, M. 2006. Impact of development and dissemination of integrated aquaculture - agriculture (IAA) technologies in Malawi. Naga WorldFish Centre Quarterly 29 (1 & 2): 28-35.

Diana, J.S. 2009. Aquaculture production and biodiversity conservation. Bioscience 59: 27-39.

Domingo, J.L., Bocio, A., Falco, G., and Llobet, J.M. 2007. Benefits and risks of fish consumption Part I. A quantitative analysis of the intake of omega-3 fatty acids and chemical contaminants. Toxicology 230: 219-226.

Dovydaitis, T. 2008. Fish consumption during pregnancy: An overview of the risks and benefits. Journal of Midwifery and Women's Health 53: 325-330.

Duc, N.M. 2009 Economic contribution of fish culture to farm income in Southeast Vietnam. Aquaculture International 17: 15-29.

EPA (U.S. Environmental Protection Agency). 1997. Mercury study report to congress. Retrieved February 4, 2013, from http://www.epa.gov/ttnatw01/112nmerc/volume7.pdf.

EPA (U.S. Environmental Protection Agency). 2001. Water quality criterion for the protection of human health: Methylmercury. Retrieved January 9, 2013, from http://water.epa.gov/scitech/swguidance/standards/criteria/aqlife/methylmercury/upload/2009_01_15_criteria_methylmercury_mercury-criterion.pdf.

EPA (U.S. Environmental Protection Agency). 2012. Mercury fish consumption advisories. Retrieved January 10, 2013, from http://www.epa.gov/mercury/advisories.htm.

Eto, K., Marumoto, M., and Takeya, M. 2010. The pathology of methylmercury poisoning (Minamata disease). Neuropathology 30: 471-479.

FAO (Food and Agriculture Organization of the United Nations). 2010. The State of World Fisheries and Aquaculture. Rome: FAO.

Firestone, R., Punpuing, S., Peterson, K.E., Acevedo-Garcia, D., and Gortmaker, S.L. 2011. Child overweight and nutrition in Thailand: Is there an urban effect? Social Science and Medicine 72: 1420-1428.

Gibson, R.S., Yeudall, F., Drost, N., Mtitimuni, M., and Cullinan, T.R. 2003. Experiences of a community-based dietary intervention to enhance micronutrient adequacy of diets low in animal source foods and high in phytate: A case study in rural Malawian children. Journal of Nutrition 133: 3992S – 3999S.

Gittelson, J. 1991. Opening the box: intrahousehold food allocation in rural Nepal. Social Science and Medicine 33: 1141-1154.

Goodrich, J.M., Wang, Y., Gillespie, B., Werner, R., Franzblau, A., and Basu, N. 2011. Glutathione enzyme and selenoprotein polymorphisms associate with mercury biomarker levels in Michigan dental professionals. Toxicology and Applied Pharmacology 257: 301-308.

Grandjean, P., Weihe, P., White, R.F., Debes, F., Araki, S., Yokoyama, K., Murata, K., Sorensen, N., Dahl, R., and Jorgensen, P.J. 1997. Cognitive deficit in 7-year-old children with prenatal exposure to methylmercury. Neurotoxicology and Teratology 19: 417-428.

Grummer-Strawn, L.M., Reinold, C., and Krebs, N.F. 2010. Use of World Health Organization and CDC growth charts for children aged 0-59 months in the United States. Centers for Disease Control and Prevention Morbidity and Mortality Weekly Report 59: 1-15.

Hsiao, H.W., Ullrich, S.M., and Tanton, T.W. 2011. Burdens of mercury in residents of Temirtau, Kazakhstan I: Hair mercury concentrations and factors of elevated hair mercury levels. Science of the Total Environment 409: 2272-2280.

Jahan, K.M., and Pemsl, D.E. 2011. The impact of integrated aquaculture-agriculture on small-scale farm sustainability and farmers' livelihoods: Experience from Bangladesh. Agricultural Systems 104: 392-402.

Katz, A. 1987. The role of aquaculture in Nepal: Towards sustainable development. Ambio 16: 222-224.

Kawarazuka, N. 2010. The contribution of fish intake, aquaculture, and small-scale fisheries to improving nutrition: A literature review. Working Paper No.2106, The WorldFish Center, Penang, Malaysia.

Kawarazuka, N., and Bene, C. 2011. The potential role of small fish species in improving micronutrient deficiencies in developing countries: building evidence. Public Health Nutrition 14: 1927-1938.

Khan, R.E.A., and Azid, T. 2011. Malnutrition in primary school-age children: A case of urban and slum areas of Bahawalpur, Pakistan. International Journal of Social Economics 38: 748-766.

Kongsbak, K., Thilsted, S. H., and Wahed, M.A. 2008. Effect of consumption of the nutrient-dense, freshwater small fish *Amblypharyngodon mola* on biochemical indicators of vitamin A status in Bangladeshi children: a randomized, controlled study of efficacy. British Journal of Nutrition 99: 581-597.

Kramer, M.S. 1987. Intrauterine growth and gestational duration determinants. Pediatrics 80: 502-511.

Larety, A., Manu, A., Brown, K. H., Peerson, J.M., and Dewey, K.G. 1999. A randomized, community-based trial of the effects of improved, centrally processed complementary foods on growth and micronutrient status of Ghanian infants from 6 to 12 months of age. American Journal of Clinical Nutrition 70: 391-404.

Lin, C.A., Manary, M.J., Maleta, K., Briend, A., and Ashorn, P. 2008. An energy-dense complementary food is associated with a modest increase in weight gain when compared with a fortified porridge in Malawian children aged 6-18 months. The Journal of Nutrition 138: 593-598.

Lunn, P.G. 2002. Growth retardation and stunting of children in developing countries. British Journal of Nutrition 88: 109-110.

Mahaffey, K.R., Sunderland, E.M., Chan, H.M., Choi, A.L., Grandjean, P., Marlen, K., Oken, E., Sakamoto, M., Schoeny, R., Weihe, P., Yan, C.H., and Yasutake, A. 2011. Balancing the benefits of n-3 polyunsaturated fatty acids and the risks of methylmercury exposure from fish consumption. Nutrition Reviews 69: 493-508.

Martorell, R., Leslie, J., and Moock, P.R. 1984. Characteristics and determinants of child nutritional status in Nepal. The American Journal of Clinical Nutrition 39: 74-86.

Mergler, D., Anderson, H.A., Chan, L.H.M., Mahaffey, K.R., Murray, M., and Sakamoto, M. 2007. Methylmercury exposure and health effects in humans: a worldwide concern. Ambio 36: 3–11.

MOHP (Ministry of Health and Population) [Nepal], New ERA, and ICF International Inc. 2012. Nepal Demographic and Health Survey 2011. Kathmandu, Nepal: Ministry of Health and Population, New ERA, and ICF International, Calverton, Maryland.

Morel, F. M., Kraepiel, A. M., and Amyot, M. 1998. The chemical cycle and bioaccumulation of mercury. Annual Review of Ecology and Systematics 29: 543-566.

Nesheim, M.C., and Yaktine, A.L., eds. 2007. Seafood choices: Balancing benefits and risks. Institute of Medicine, National Academies Press. Washington, D.C.

Neumann, C. G., Gewa, C., and Bwibo, N. 2004. Child nutrition in developing countries. Pediatric Annals 33: 658.

Nuttal, K.L. 2006. Review: Interpreting hair mercury levels in individual patients. Annals of Clinical and Laboratory Science 36: 248-61.

Nyland, J.F., Fillion, M., Barbosa Jr., F., Shirley, D.L., Chine, C., Lemire, M., Mergler, D., and Silbergeld, E.K. 2011. Biomarkers of methylmercury exposure immunotoxicity among fish consumers in Amazonian Brazil. Environmental Health Perspectives 119: 1733-1738.

Oken, E., and Belfort, M.B. 2010. Fish, fish oil, and pregnancy. Journal of the American Medical Association 304: 1675-1683.

Onis, M.D. 2008. Child growth and development. Ch 5. Nutrition and Health: Nutrition and Health in Developing Countries 2nd ed. Eds. Semba, R.D. and Bloem, M.W. Humana Press, Totowa, NJ.

Parajuli, R.P., Umezaki, M., Watanabe, C. 2012. Diet among people in the Terai region of Nepal, an area of micronutrient deficiency. Journal of Biosocial Science 44: 401-415.

Pradhan, G. 2013. National aquaculture sector overview Nepal – national aquaculture sector overview fact sheets. Retrieved March 25, 2013 from http://www.fao.org/fishery/countrysector/FI-CP NP/en.

Rahman, A., Chowdhury, S., and Hossain, D. 2009. Acute malnutrition in Bangladeshi children: levels and determinants. Asia-Pacific Journal of Public Health 21: 294-302.

Rivera, J.A., Hotz, C., Gonzalez-Cossio, T., Neufeld, L., and Garcia-Guerra, A. 2003. The effect of micronutrient deficiencies on child growth: A review of results from community-based supplementation trials. The Journal of Nutrition 133: 4010S-4020S.

Savada, A.M. 1991 ed. Nepal: A Country Study. Washington: GPO for the Library of Congress.

Selin, N. 2009. Global biogeochemical cycling of mercury: A review. The Annual Review of Environment and Resources 34:43-63.

Skoufias, E. 1999. Parental education and child nutrition in Indonesia. Bulletin of Indonesian Economic Studies 35: 99-119.

Smith, L.C., and Haddad, L. 2000. Explaining child malnutrition in developing countries: A cross-country analysis. International Food Policy Research Institute. Washington, D.C.

Speedy, A.W. 2003. Global production and consumption of animal source foods. Journal of Nutrition 133: 4048S-4053S.

Tharakan, C.T., and Suchindran, C.M. 1999. Determinants of child malnutrition – an intervention model for Botswana. Nutrition Research 19: 843-860.

UNEP Chemicals Branch, 2008. The global atmospheric mercury assessment: Sources, emissions and transport. UNEP – Chemicals, Geneva.

Wang, Y., Goodrich, J., Werner, R., Gillespie, B., Basu, N., and Franzblau, A. 2012. An investigation of modifying effects of single nucleotide polymorphisms in metabolism-related genes on the relationship between peripheral nerve function and mercury levels in urine and hair. Science of the Total Environment 417-418: 32-38.

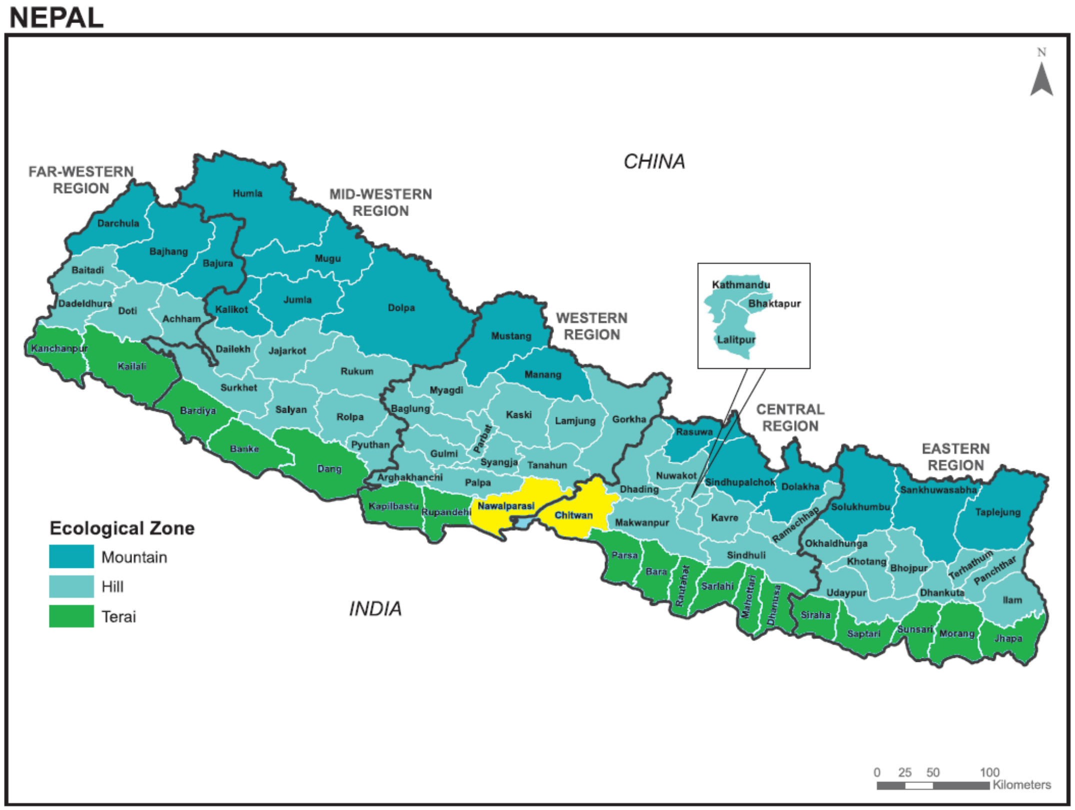
Wijkstrom, U.N. 2003. Short and long-term prospects for consumption of fish. Veterinary Research Communications 27 (suppl. 1): 461–468.

WHO (World Health Organization). 2000. Turning the tide of malnutrition: Responding to the challenge of the 21st century. Retrieved November 4, 2012, from http://www.who.int/mip2001/files/2232/NHDbrochure.pdf.

WHO (World Health Organization). 2008. Guidance for identifying populations at risk from mercury exposure. Retrieved January 15, 2013, from http://www.who.int/foodsafety/publications/chem/mercuryexposure.pdf.

Zhang, H., Feng, X., Larssen, T., Shang, L., and Li, P. 2010. Bioaccumulation of methylmercury versus inorganic mercury in rice (*Oryza sativa* L.) grain. Environmental Science and Technology 44: 4499-4504.

Appendix I: Map of Nepal showing ecoregions and the districts of Chitwan and Nawalparasi (yellow), where research was carried out. Map adapted from MOHP (2012).



Appendix II: Study Survey

Maternal Fish Consumption

Do you eat fish?

When you eat fish, estimate the average portion size for fish you eat at typical meals.

___1/4 ___1/2 ___3/4 ___1 ___1½ ___2 ___3

___ ≥4

Note: One portion = 3 ounces of grilled fish = the size of a deck of cards; two portions = a regular 6oz can of tuna

During a month in the harvest season, how many meals did you eat the following fish?

Fish sp.	Never	once	2-3 times	1 time per week	2 times per week	3-4 times per week	5-6 times per week	1 time per day	2 or more times per day
SIS									
Tilapia									
Common Carp									
Silver Carp									
Rohu									
Naini									
Bighead Carp									
Grass Carp									
Catfish									
Other									

Socioeconomic Status

How much money does your family make in one month?

How much money does your family make in one month from aquaculture?

Does your household have: (Y/N)

Electricity___ A radio___ A television___ A mobile telephone___

A non-mobile telephone___ A refrigerator___ A table___

A chair___ A bed___ A sofa___ A cupboard___

A computer___ A clock___ A fan___ A dhiki/janto___

In the past 12 months, how frequently did you worry that your household would not have enough food?

Educational Status

What was the last grade level completed in school?

Mother__________ Father_____________

Child Dietary Considerations

1) Yesterday during the day or at night, did your child eat or drink any of the following: (Y/N/DK)

Plain water___ Juice or Juice Drinks___ Soup___

Milk___ (if yes, how many times___) Infant formula like Lactogen___ (if yes, how many times___)

Any other liquids___ Yogurt___ (if yes, how many times___)

Any fortified baby food like Cerelac, Nestrum, Champion, etc.___

Roti, rice, maize, millet, noodles, porridge, or other foods made from grains___

Pumpkin, carrots, squash, or sweet potatoes that are yellow or orange inside___

White potatoes, white yams, colocasia, or any other foods made from roots___

Any dark green, leafy vegetables like spinach, amaranth leaves, mustard leaves___

Ripe mangoes, papayas, or apricot___

Any other fruits or vegetables___

Liver, kidney, heart, or other organ meats___

Any meat, such as pork, buff, lamb, goat, chicken, or duck___

Eggs___

Fresh or dried fish or shellfish___

Any foods made from beans, peas, lentils, or nuts___

Cheese or other food made from milk___

Any other solid, semi-solid, or soft food (jaulo, lito, sarbottam pitho etc)___

Never___ Rarely___ Sometimes___ Often___

2) Does your child eat fish?

At what age did your child first eat fish?

When your child eats fish, estimate the average portion size for fish he/she eats at typical meals.

___1/4 ___1/2 ___3/4 ___1 ___1½ ___2 ___3

___>4

Note: One portion = 3 ounces of grilled fish = the size of a deck of cards; two portions = a regular 6-oz. can of tuna

During a month in the harvest season, how many meals did your child eat the following fish?

Fish sp.	Never	once	2-3 times	1 time per week	2 times per week	3-4 times per week	5-6 times per week	1 time per day	2 or more times per day
SIS									
Tilapia									
Common Carp									
Silver Carp									
Rohu									
Naini									

Bighead Carp									
Grass Carp									
Catfish									
Other									

History Child Health

Did you breastfeed your child?

How long was your child breastfed?

At what age was your child first fed complementary food?

Has your child had a diarrhea related illness within the past two weeks?

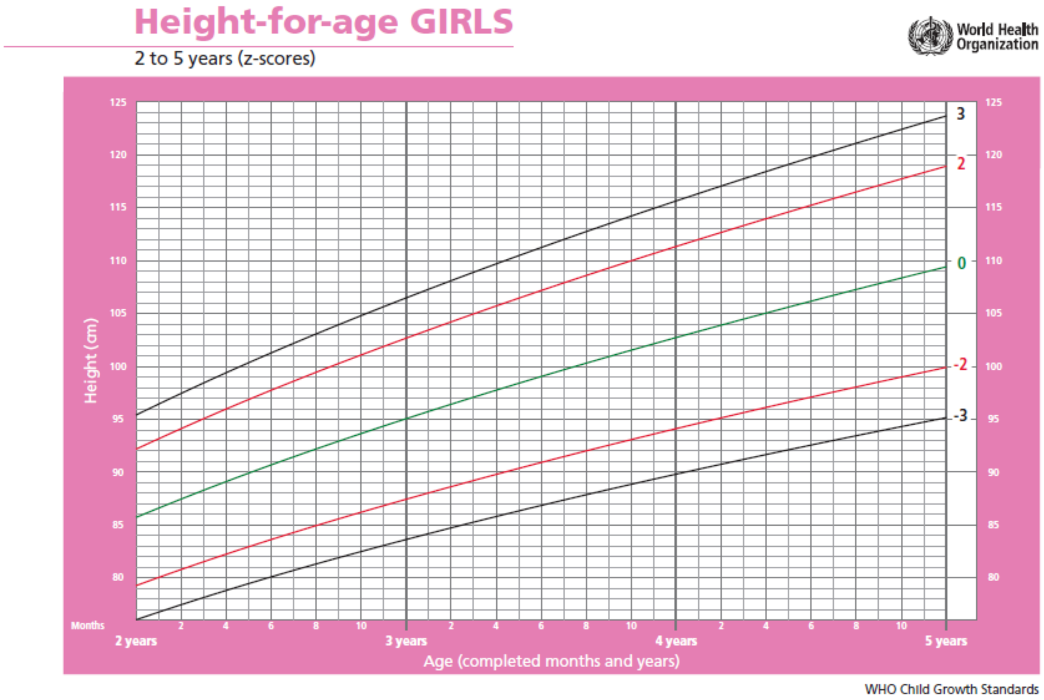
Has your child had a respiratory illness within the past two weeks?

How many children do you have?

Measurement Data

ID #__________	**ID #__________**	**ID #__________**
Age__________	**Age__________**	**Age__________**
Sex__________	**Sex__________**	**Sex__________**
Ht__________(cm)	**Ht__________(cm)**	**Ht__________(cm)**
Wt__________(kg)	**Wt__________(kg)**	**Wt__________(kg)**

Appendix III. Copy of a WHO growth chart. Downloaded from http://www.who.int/childgrowth/standards/en/



Appendix IV: Descriptive statistics for continuous variables.

Descriptive Statistics

Variable	N	Mean	Std. Dev.	Min	Max
months breastfed	87	36.59	12.81	12.00	72.00
age first food	110	6.17	1.09	4.00	12.00
age first fish	104	13.94	6.47	6.00	36.00
age (months)	111	74.47	30.57	14.00	125.00
family number	101	5.98	2.01	2.00	12.00
monthly income	109	220.08	148.91	52.08	1020.83
income/member	98	40.55	39.31	7.81	340.28
no. children	110	2.13	1.02	1.00	7.00
maternal BMI	87	20.92	2.77	16.28	29.56
% income from aqua	64	3.11	7.07	0.00	50.00
mother grade	111	4.51	3.65	0.00	12.00
father grade	111	7.49	3.19	0.00	12.00
socioeco score	111	9.31	3.24	1.00	17.00
fish/month (g)	109	1342.20	1990.62	0.00	11268.93
WAZ	111	-1.42	0.88	-4.10	0.95
HAZ	111	-0.99	1.18	-4.87	2.41

Appendix V: Bivariate analysis results. Variables found to be significant and the 0.1 level are highlighted. Different tests were used based on variable type, normality, and sample size.

Bivariate Analysis Continuous Covariates vs. Underweight (Mean ± SD)

Variable	Underweight (n=28)	Not Underweight (n=83)	Test	p-value
family no.	n=23 5.83 ± 1.749	n=78 6.03 ± 2.095	T	0.679
age (months)	n=28 69.11 ± 27.91	n=83 76.28 ± 31.38	T	0.285
no. children	n=28 2.32 ± 1.49	n=82 2.06 ± .807	Mann	0.968
monthly income ($)	n=28 209.97 ± 140.11	n=81 223.57 ± 152.52	Mann	0.694
income/person ($)	n=23 36.18 ± 29.21	n=75 41.89 ± 42.00	Mann	0.392
% income from aquaculture	n=15 .869 ± 1.488	n=49 3.80 ± 7.93	Mann	0.067
socioeco score	n=28 8.29 ± 3.26	n=83 9.65 ± 3.18	T	0.054
months breastfed	n=21 35.29 ± 14.63	n=66 37.00 ± 12.27	Mann	0.37
age first food	n=27 6.04 ± .898	n=83 6.22 ± 1.15	Mann	0.553
age first fish	n=27 12.89 ± 5.05	n=77 14.31 ± 6.89	Mann	0.524
maternal BMI	n=22 20.92 ± 2.16	n=65 20.92 ± 2.96	T	0.993
maternal education	n=28 4.18 ± 3.97	n=83 4.63 ± 3.56	T	0.577
paternal education	n=28 6.18 ± 3.53	n=83 7.93 ± 2.96	Mann	0.008
monthly fish consumption (g)	n=26 1080.72 ± 1180.75	n=83 1424.10 ± 2182.99	Mann	0.935

Bivariate Analysis Continuous Covariates vs. Stunted (Mean ± SD)

Variable	Stunted (n=18)	Not Stunted (n=93)	Test	p-value
family no.	n = 15 6.33 ± 1.95	n = 86 5.92 ± 2.03	T	0.465
age (months)	n=18 62.17 ± 29.17	n=93 76.85 ± 30.42	Mann	0.049
no. children	n=18 1.89 ± .758	n=92 2.17 ± 1.07	Mann	0.266
monthly income ($)	n=18 220.66 ± 148.32	n=91 219.96 ± 149.85	Mann	0.954
income/person ($)	n=15 37.51 ± 34.19	n=83 41.10 ± 40.33	Mann	0.436
% income from aquaculture	n=12 2.72 ± 4.23	n=52 3.20 ± 7.61	Mann	0.537
socioeco score	n=18 10.11 ± 4.03	n=93 9.15 ± 3.07	T	0.252
months breastfed	n=14 32.64 ± 11.06	n=73 37.34 ± 13.06	Mann	0.252
age first food	n=18 6.17 ± 1.54	n=92 6.17 ± .990	Mann	0.284
age first fish	n=18 12.72 ± 6.29	n=86 14.2 ± 6.52	Mann	0.254
maternal BMI	n=14 21.05 ± 1.92	n=73 20.90 ± 2.91	Mann	0.499
maternal education	n=18 5.33 ± 4.12	n=93 4.35 ± 3.56	T	0.3
paternal education	n=18 7.44 ± 2.79	n=93 7.49 ± 3.27	Mann	0.664
monthly fish consumption (g)	n=16 1221.25 ± 1625.42	n=93 1361 ± 2053.76	Mann	0.758

Bivariate Analysis Categorical Covariates vs. Underweight

Variable		Underweight (n=28)	Not Underweight (n=83)	X^2 test p-value
own pond 5 yrs	**yes**	12 (42.9%)	46 (55.4%)	0.25
	no	16 (57.1%)	37 (44.6%)	
sex	**male**	12 (42.9%)	48 (57.8%)	0.169
	female	16 (57.1%)	35 (42.2%)	
respiratory illness	**yes**	11 (39.3%)	22 (26.5%)	0.201
	no	17 (60.7%)	61 (73.5%)	
diarrhea	**yes**	1 (3.6%)	5 (6.0%)	0.62
	no	27 (96.4%)	78 (94.0%)	
ate dairy	**yes**	9 (32.1%)	32 (38.6%)	0.543
	no	19 (67.9%)	51 (61.4%)	
ate starch	**yes**	17 (60.7%)	62 (74.7%)	0.158
	no	11 (39.3%)	21 (25.3%)	
ate vegetable	**yes**	17 (60.7%)	61 (73.5%)	0.201
	no	11 (39.3%)	22 (26.5%)	
ate fruit	**yes**	26 (92.9%)	77 (92.8%)	0.988
	no	2 (7.1%)	6 (7.2%)	
ate meat	**yes**	9 (32.1%)	34 (41.0%)	0.407
	no	19 (67.9%)	49 (59.0%)	
ate fish	**yes**	13 (46.4%)	27 (32.5%)	0.185
	no	15 (53.6%)	56 (67.5%)	
ate pulse	**yes**	22 (78.6%)	64 (77.1%)	0.873
	no	6 (21.4%)	19 (22.9%)	

Bivariate Analysis Categorical Covariates vs. Stunted

Variable		**stunted (n=17)**	**not stunted (n=94)**	**X^2 test p-value**
own pond 5 yrs	**yes**	9 (52.9%)	50 (53.2%)	0.641
	no	8 (47.1%)	44 (46.8%)	
sex	**male**	11 (64.7%)	49 (52.1%)	0.338
	female	6 (35.3%)	45 (47.9%)	
respiratory illness	**yes**	4 (23.5%)	29 (30.9%)	0.543
	no	13 (76.5%)	65 (69.1%)	
diarrhea	**yes**	1 (5.9%)	5 (5.3%)	1.00*
	no	16 (94.1%)	89 (94.7%)	
ate dairy	**yes**	5 (29.4%)	36 (38.3%)	0.485
	no	12 (70.6%)	58 (61.7%)	
ate starch	**yes**	13 (76.5%)	66 (70.2%)	0.774*
	no	4 (23.5%)	13 (76.5%)	
ate vegetable	**yes**	10 (58.8%)	68 (72.3%)	0.262
	no	7 (41.2%)	26 (27.7%)	
ate fruit	**yes**	17 (100%)	86 (91.5%)	0.606*
	no	0 (0%)	8 (8.5%)	
ate meat	**yes**	7 (41.2%)	36 (38.3%)	0.823
	no	10 (58.8%)	58 (61.7%)	
ate fish	**yes**	7 (41.2%)	33 (35.1%)	0.631
	no	10 (58.8%)	61 (64.9%)	
ate pulse	**yes**	15 (88.2%)	71 (75.5%)	0.351*
	no	2 (11.8%)	23 (24.5%)	

Bivariate Analysis Continuous Covariates vs. WAZ

Variable	N	Test	Correlation Coefficient	p-value
family no.	101	Spearman	0.2	0.84
age (months)	111	Spearman	0.16	0.092
no. children	110	Spearman	-0.062	0.52
monthly income ($)	109	Spearman	0	0.996
income/person ($)	98	Spearman	0.069	0.498
% income from aqua	64	Spearman	0.9	0.481
socioeco score	111	Pearson	0.088	0.359
months breastfed	87	Spearman	0.117	0.281
age first food	110	Spearman	0.115	0.231
age first fish	104	Spearman	0	0.997
maternal BMI	87	Spearman	-0.026	0.814
maternal education	111	Spearman	0.093	0.333
paternal education	111	Spearman	0.288	0.002
monthly fish cons. (g)	111	Spearman	0.049	0.616

Bivariate Analysis Continuous Covariates vs. HAZ

Variable	N	Test	Correlation Coefficient	p-value
family no.	101	Spearman	-0.178	0.075
age (months)	111	Spearman	0.239	0.011
no. children	110	Spearman	0.18	0.06
monthly income ($)	109	Spearman	-0.063	0.512
income/person ($)	98	Spearman	0.107	0.294
% income from aqua	64	Spearman	-0.125	0.327
socioeco score	111	Pearson	-0.057	0.551
months breastfed	87	Spearman	0.209	0.052
age first food	110	Spearman	0.08	0.406
age first fish	104	Spearman	0.118	0.234
maternal BMI	87	Spearman	-0.173	0.109
maternal education	111	Spearman	-0.091	0.345
paternal education	111	Spearman	0.158	0.098
monthly fish cons. (g)	111	Spearman	0.054	0.58

Bivariate Analysis Categorical Covariates vs. WAZ

Variable		mean	n	test	pvalue
own pond 5 yrs	yes	-1.406 ± .872	58	T	0.836
	no	-1.440 ± .895	53		
sex	male	-1.355 ± .838	60	T	0.389
	female	-1.501 ± .928	51		
respiratory illness	yes	-1.622 ± .935	33	T	0.12
	no	-1.338 ± 847	78		
diarrhea	yes	-1.432 ± .682	6	T	0.978
	no	-1.423 ± .892	105		
ate dairy	yes	-1.301 ± .909	41	T	0.268
	no	-1.493 ± .860	70		
ate starch	yes	-1.356 ± .910	79	T	0.212
	no	-1.586 ± .787	32		
ate vegetable	yes	-1.355 ± .882	78	T	0.22
	no	-1.58 ± .864	33		
ate fruit	yes	-1.418 ± .902	103	T	0.845
	no	-1.481 ± .540	8		
ate meat	yes	-1.274 ± .928	43	T	0.16
	no	-1.516 ±.840	68		
ate fish	yes	-1.461 ± .919	40	T	0.727
	no	-1.400 ± .862	71		
ate pulse	yes	-1.420 ± .897	86	T	0.96
	no	-1.430 ± .831	25		

Bivariate Analysis Categorical Covariates vs. HAZ

Variable		**mean**	**n**	**test**	**pvalue**
own pond 5 yrs	yes	-1.065 ± 1.218	58	Mann	0.922
	no	-.906 ± 1.135	53		
sex	male	-1.081 ± 1.190	60	T	0.377
	female	-0.882 ± 1.164	51		
respiratory illness	yes	-1.065 ± 1.177	33	Mann	0.476
	no	-.9573 ± 1.182	78		
diarrhea	yes	-.903 ± .759	6	Mann	0.754
	no	-.994 ± 1.198	105		
ate dairy	yes	-.935 ± 1.183	41	Mann	0.425
	no	-1.021 ± 1.18	70		
ate starch	yes	-.966 ± 1.275	79	T	0.742
	no	-1.048 ± .906	32		
ate vegetable	yes	-.896 ± 1.207	78	Mann	0.109
	no	-1.210 ± 1.089	33		
ate fruit	yes	-1.00 ± 1.210	103	T	0.632
	no	-.796 ± .632	8		
ate meat	yes	-.923 ± 1.327	43	Mann	0.527
	no	-1.032 ± 1.079	68		
ate fish	yes	-.897 ± 1.099	40	Mann	0.931
	no	-1.041 ± 1.223	71		
ate pulse	yes	-.940 ± 1.220	86	Mann	0.294
	no	-1.159 ± 1.017	25		

Appendix VI: Quality control results for mercury analyses. All results were found to be within acceptable limits.

Fish Mercury

The average recovery of mercury from the SRMs for both days was 90.9 ± 8.80 % (n = two days) (Table 1). Expected total mercury for DOLT-4 was 2.58 ± 0.22 µg/g dw and observed mercury was 2.39 ± 0.22 µg/g dw.

Table 1. Daily averages (± SD) of total mercury recovery for DOLT-4 SRMs.

Day	N	% Recovery DOLT-4 SRM
1 (10/5/2012)	4	97.1 ± 0.20
2 (12/13/2012)	2	84.7 ± 0.001
Average		90.9 ± 8.80

Variability, as measured by the %RSD of within-day analyses of DOLT – 4 (n = two to four samples), ranged from 0.05 to 7.80 % RSD (n = two days) (Table 2).

Table 2. Variability as measured by average %RSD of within-day replicates of DOLT – 4 SRMs.

Day	N	% RSD DOLT-4 SRM
1 (10/5/2012)	4	7.80
2 (12/13/2012)	2	0.05
Average		3.93 ± 5.48

Most samples were analyzed one time, but at least one of every ten samples was duplicated (n = two) to measure precision of sample analysis. The within-day precision for Day 1 was 4.891 ± 0.017 % RSD (Table 3). Day two of analysis included acid digested samples, and no duplicates were run this day.

Table 3. Variability as measured by average %RSD of within-day replicates (2) of fish samples.

Day	N*	Within Day Precision (% RSD)
1 (10/5/2012)	3	4.891 ± 0.017
2 (12/13/2012)	N/A	N/A

Several blanks were run on Day 1, and two blanks were run on Day 2. The daily TMDL ranged from 0.0087 to 0.0899 ng Hg, with an average of 0.0493 ± 0.057 ng Hg. The daily PMDL ranged from 0.0145 to 0.1498 ng Hg, with an average of 0.0822 ± 0.096 ng Hg (Table 4). Blanks measured immediately after an SRM were excluded from the calculations (purge blanks). Using the calculated TMDL and PMDL, all samples were above the TMDL (0.0493 ng) and PMDL (0.0822 ng).

Table 4. Theoretical and practical detection limits for each day of analysis.

Day	**TMDL (ng)**	**PMDL (ng)**
1 (10/5/2012)	0.0899	0.1498
2 (12/13/2012)	0.0087	0.0145
Average	0.0493 ± 0.057	0.0822 ± 0.096

Hair Mercury

The average recovery of mercury from the SRMS for all days was 98.37 ± 5.63 % (n = 5 days) for the NIES hair SRM and 97.95 ± 2.01 % (n = 5 days) for DOLT – 4. Each day, the NIES hair and DOLT – 4 SRMs were analyzed one (n = 1) to seven (n = 7) times to determine daily average recovery (Table 5). Expected total mercury in the NIES hair SRM is 4.42 ± 0.20 µg/g dry weight (dw) and the mean observed Hg was 4.35 ± 0.25 µg/g dw. As aforementioned, expected Hg for DOLT – 4 is 2.58 ± 0.22 µg//g. The mean observed Hg was 2.53 ± 0.05 µg/g dw.

Table 5. Daily averages (± SD) of total mercury recovery for NIES hair and DOLT – 4 SRMs.

Day	N	% Recovery NIES #13 Hair SRM	N	% Recovery DOLT-4 SRM
1 (11/9/2012)	1	107.21	4	98.51
2 (11/26/2012)	2	101.73	4	100.93
3 (12/10/2012)	2	99.00	4	98.01
4 (12/13/2012)	2	97.14	4	98.86
5 (12/14/2012)	2	92.76	4	95.46
5 (12/14/2012)	2	92.40	4	95.96
Average		98.37 ± 5.63		97.95 ± 2.01

Variability, as measured by the %RSD of within-day analyses (n = one to two samples) of the NIES hair SRM ranged from 0.12 to 4.79 % RSD (n = five days). Within-day analyses of DOLT – 4 (n = four samples) ranged from 1.67 to 5.03 % RSD (n = five days) (Table 6).

Table 6. Variability, as measured by average %RSD of within-day replicates of the NIES hair and DOLT – 4 SRMs.

Day	N	% RSD NIES # 13 Hair SRM	N	% RSD DOLT-4 SRM
1 (11/9/2012)	1	N/A	4	3.32
2 (11/26/2012)	2	2.37	4	5.03
3 (12/10/2012)	2	0.12	4	1.67
4 (12/13/2012)	2	4.79	4	1.93
5 (12/14/2012)	2	4.26	4	3.41
5 (12/14/2012)	2	0.89	4	2.88
Average		2.49 ± 2.04		3.04 ± 1.21

Most samples were only analyzed one time, but at least one every tem samples was duplicated (n = two) to measure precision of sample analysis. The average % RSD for within-day replicates was 5.42 ± 2.74 % RSD (n = five days) (Table 7).

Table 7. Variability as measured by average % RSD of within-day replicates of human hair samples.

Day	N*	Within Day Precision (% RSD)
1 (11/9/2012)	3	7.00
2 (11/26/2012)	2	9.32
3 (12/10/2012)	2	1.88
4 (12/13/2012)	2	6.15
5 (12/14/2012)	2	2.84
5 (12/14/2012)	1	5.33
Average		5.42 ± 2.74

*Each sample indicated was replicated twice.

The average daily results for each SRM were used to determine the between-day average (n = five days). Between-day variability for the NIES hair SRM was 5.72 % RSD. Between-day variability for the DOLT-4 SRM was 2.05 % RSD. The average daily values for the NIES hair and DOLT-4 SRMs were 4.35 ± 0.25 µg/g and 2.53 ± 0.05 µg/g, respectively. The total average for between-day % RSD for both SRMs was 3.89 ± 1.84 % RSD (Table 8).

Table 8. Variability as measured by % RSD of between-day replicates of NIES hair SRM and DOLT – 4 SRM.

Sample	# Days Replicated	Between Day % RSD
Hair SRM	5	5.72
DOLT SRM	5	2.05
Average		3.89 ± 1.84

Several blanks were run each day. The daily TMDL ranged from 0.018 to 0.166 ng Hg, with an average of 0.071 ± 0.053 ng Hg. The daily PMDL ranged from 0.030 to 0.276 ng Hg, with an average of 0.118 ± 0.088 ng Hg (Table 9). Blanks measured immediately after an SRM were excluded from the calculations (purge blanks). Using the calculated TMDL and PMDL, all samples were above the TMDL (0.071 ng) and PMDL (0.118 ng).

Table 9. Theoretical and practical detection limits for each day of analysis.

Day	TMDL (ng)	PMDL (ng)
1 (11/9/2012)	0.018	0.030
2 (11/26/2012)	0.028	0.046
3 (12/10/2012)	0.081	0.135
4 (12/13/2012)	0.073	0.122
5 (12/14/2012)	0.166	0.276
5 (12/14/2012)	0.058	0.097
Average	0.071 ± 0.053	0.118 ± 0.088

Total mercury was measured in approximate five mg portions for 117 of the 141 (83%) total samples analyzed. In cases where the total sample weighed less than five mg, the total sample was analyzed. Of these samples, 9 weighed between 0.0009 and 0.002 g (6.4%) and 15 weighed between 0.002 and 0.004 g (10.6%). Any sample that did not have the recommended five mg of hair cannot be held as accurate.

Appendix VII: Hair Mercury Results. A small (a) denotes a washed hair weight from 3-5 mg. A small (b) denotes a washed hair weight below 3 mg. The % RSD is indicated for duplicate samples.

Total Mercury in Hair: Mothers

ID	µg/g	%RSD	ID	µg/g	%RSD	ID	µg/g	%RSD
001M	1.5690		023M	0.5804		046M	0.5986	
002M	0.4545		024M	0.5523		047M	0.4440	
003M	0.4729	4.460145	025M	1.3190		048M	0.5573	
003M(2)	0.5037		026M	0.7011		049M	0.6350	3.49698
004M	0.5185		027M	0.2375		049M(2)	0.6672	
005M	0.5853	5.391907	028M	0.3352		050M	0.6808	2.228557
005M(2)	0.6317		029M	0.4082		050M(2)	0.7026	
006M	0.5653	0.672249	030M	0.4174	2.184711	051M	0.4264	
006M(2)	0.5707		030M(2)	0.4047		052M	0.7922	1.536229
008M	0.7365		031M	0.0449		052M(2)	0.8096	
009M	0.9714		032M	0.4112		053M[b]	0.3522	
010M	0.6887		033M	0.5988		054M	0.4497	
011M	0.6539		034M	0.5479		057M	0.4447	
012M	0.9519		035M	0.9166		058M	0.5544	
013M	1.3791		036M	0.8417		059M[a]	0.5167	
014M	7.0139		037M	0.6246		060M	0.694	
015M	0.5612		038M	0.4725	9.271468	061M	0.7174	
016M	0.9042		038M(2)	0.5388		062M	0.8272	
017M	0.3704		039M	5.9525		063M	0.027	
018M	0.6526	14.17528	040M	0.6361		064M	0.3205	
018M(2)	0.7980		041M	0.9002		065M	0.6602	
019M	0.1979		042M	1.6039		066M	1.0136	
020M	0.6887		043M	1.2204		067M[b]	2.2099	
021M	0.7562		044M	1.6619		068M	0.4783	
022M	0.6067		045M[a]	0.7744		070M[a]	0.6254	

Total Mercury in Hair: Girls and Boys

Girls			Boys		
ID	µg/g	%RSD	ID	µg/g	%RSD
001C1f	0.6414		005C2m	0.5341	
002C1f	0.9021		008C1m	1.0901	
003C1f	0.5487		009C1m	0.9662	
004C1f	0.5308		010C1m[b]	0.8476	
005C1f	0.4122		011C2m	0.8427	
006C1f	0.4883		012Cm	0.7703	0.395829
011C1f	0.4806		012Cm(2)	0.766	
013C1f	0.8040		016C1m	0.5818	
014C1f	0.8413		016C2m[a]	0.7059	
015C2f	0.7191	19.94241	019C2m[a]	0.477	
015C2f(2)	0.9552		021C1m[b]	0.5251	
017C2f	0.2607		023C1m	0.7072	
018C1f	0.4120		024C1m	0.7422	
019C1f[b]	0.7142		025C1m	0.6559	
020C1f	0.8456		029C1m	0.3535	
022C1f	0.4162		031C1m	0.4548	
026C1f	0.8066		032C1m	0.8039	
026C2f[b]	0.5045		033C1m	0.7244	
027C1f	0.5681		034C1m[b]	0.4212	
028C1f	0.4539		037C1m[b]	0.5116	
028C2f	0.3344	5.33128	038C1m	0.5193	
028C2f(2)	0.3606		040C1m	0.9430	
030C1f	0.6388		042C1m	0.6239	
039C1f	0.8786		045C1m[b]	0.4177	
041C1f	1.0494		046C1m[b]	0.6020	
041C2f[a]	0.9802		048C1m	0.6250	
043C1f	0.7742		049C2m	0.5438	
044C1f	0.9645		052C2m[a]	1.3467	
047C1f[a]	0.3224		054C1m	0.9254	
049C1f	0.4933		058C1m[b]	0.4750	
051C1f	0.2834		062C1m	0.7642	
052C1f	0.6201	3.024161	063C1m	0.687	
052C1f(2)	0.6472		063C2m	0.4639	
057C1f[b]	0.04945		064C1m	0.4577	
061C1f	0.5124		065C1m	0.5487	
061C2f	0.6457		065C2m	0.5777	
062C2f	0.5688		066C1m	0.5703	
067C1f	4.0571		068C2m[b]	0.2676	
068C1f[a]	0.3317		069C1m[b]	0.4322	
			070C1m[b]	0.6132	