Effects of mercury contamination on the immune system and on parasitism in painted turtles (*Chrysemys picta*)

By
Guillaume Slevan-Tremblay
6121378

Supervisor: Dr. Gabriel Blouin-Demers

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Abstract

Mercury (Hg) is one of the most common heavy metals in aquatic systems in the world. The consequences of Hg contamination on freshwater turtles are poorly known. Since turtles are on top on the food chain, they are vulnerable to bioaccumulation of Hg. We know that Hg can have various deleterious effects on the immune system and, thus, potentially on the level of parasitism. I tested the hypothesis that variation in parasite load in painted turtles can be explained by Hg contamination since Hg affects the immune system negatively. I measured Total Hg concentrations from blood and scutes of free-ranging painted turtles of Upper Rideau Lake in the Rideau Canal, Ontario, Canada. I analyzed blood smears to count lymphocytes and evaluate the intra-erythrocyte prevalence of *Haemogragarina balli* parasites. No correlations were found between parasite abundance and measures of Hg and white blood cell counts. Although it was only marginally significant, I observed a negative correlation between Hg and lymphocyte count. I conclude that Hg concentrations found in painted turtles are probably not high enough to induce immunosuppression and affect parasite prevalence.

Introduction

Mercury (Hg) is considered as one of the most widely spread toxic heavy metal in the environment, particularly in North American aquatic systems, explaining why Hg contamination has been extensively studied (Ullrich et al. 2001). In fact, from all bioavailable heavy metals present in the environment, Hg was identified as the most toxic (Lawrence et McCabe, 2002). Mercury in his inorganic form is released in the atmosphere mainly by anthropogenic sources like incinerators and coal operated generators and then, once deposited on the landscape, transformed by bacterial and decomposition processes into a more bioavailable form, methylmercury (MeHg) (Wiener et al. 2006). While ionic Hg does not present serious health risks for living organisms, MeHg bioaccumulates easily in organic tissues and biomagnifies through the food chain, explaining why it is important to quantify the consequences it has on wildlife (Driscoll et al. 2007). This high rate of accumulation of MeHg in the tissues is due to its uncommon stability and lipid solubility (Ullrich et al. 2001). MeHg contamination has deleterious physiological and morphological effects. It can damage the nervous system and increase the chances of heart disease in mammals (Jarup 2003) as well as reduce the growth and development of fishes and birds (Zelikoff et al. 1994).

Generally, the presence of toxic heavy metals in the body can decrease immune system function and eventually affect various health parameters like lymphocyte production which can ultimately cause severe infections or cancers (Zelikoff et al. 1994). Of all the heavy metals, MeHg is known to induce the most important immunosuppressive activities (Lawrence, 1981). Immunosuppression can inhibit the

production of lymphocytes whose functions are to recognize and eliminate unknown agents that have the potential to harm the host (Lawrence et McCabe 2002). More precisely, MeHg decreases humoral immunity by compromising DNA and RNA synthesis, which stops the production of B-lymphocytes (Daum et al. 2003). The relation between mercury and the immune system has been investigated in birds (Sagerup et al. 2009) and in seals (Lalancette et al. 2003) revealing that the presence of mercury in an individual affects its immune system response by reducing the capacities of lymphocytes to perform phagocytosis on parasites. In addition, a negative correlation between mercury and the number of lymphocyte cells was found in loggerhead sea turtles meaning that these turtles may be more vulnerable to certain diseases as mercury levels increase (Day et al. 2007).

My study focuses on freshwater turtles as they are more vulnerable to mercury bioaccumulation given they have a long life span, they live in aquatic habitats, and occupy high trophic levels (Day et al. 2007). Animals at higher trophic levels are also more likely to contain high levels of MeHg due to its high biomagnification potential (Morel et al. 1998). For instance, snapping turtles from New York State accumulate high concentrations of mercury in their body over the years (Turnquist et al. 2011).

Various health parameters can be negatively affected by a weakened immune system. One of them is the capacity to resist parasite intrusions that can eventually lead to the establishment, development, and survival of parasites in the host body (Lloyd et al. 1995). In various species of fish, heavy metals tend to facilitate establishment of

parasites (Lafferty 1997). Some studies on birds revealed positive relationships between mercury concentration and infection with parasites (Sagerup et al. 2009, Wayland et al. 2001). However, very few studies have investigated the consequences of immunosuppression by mercury on parasitism in turtles.

Like fish, turtles spend most of their life in water, thus increasing their vulnerability to parasite infections. In fact, freshwater turtles are commonly hosts to various parasites (Davis & Sterrett 2011). One of the better-known internal parasites that infect freshwater turtles are haemogregarines (McAuliffe 1977) that are generally found free-ranging in the blood or inside erythrocytes of the host (Davis & Sterrett 2011). Turtles in North America are known as intermediate hosts for internal parasites and Siddall and Desser (2001) found that external parasites, in this case leeches, were the vectors by which haemogregarines are transmitted between painted and snapping turtles. Painted turtles (*Chrysemys picta*) often have leeches on them (Readel et al. 2008) and therefore have a high probability of being infected with haemogregarines.

Concentrations of various heavy metals in sediments, including Hg, are above federal environmental quality guidelines in lakes of the Rideau Canal (Leblond 2009). In this contaminated system, I will test the hypothesis that variation in parasite infection by *Haemogregarina balli* in painted turtles can be explained by mercury contamination since mercury has immunosuppressive capacities. By counting lymphocytes in the blood, I will determine whether mercury burden affects the immune system response. I predict that high burdens of mercury will inhibit production of lymphocytes. Parasitism level

should increase with lower counts of lymphocytes. Finally, I predict that number of parasites will be higher in painted turtles that have higher level of mercury because their lymphocyte counts should be lower.

Materials and Methods

Study site

The study took place in eastern Ontario (Canada) on the Rideau Canal, a waterway connecting Ottawa to Kingston through 14 lakes and the Rideau River. I captured turtles in Upper Rideau Lake (44.682°N 76.336°W) situated near the village of Westport, 120 kilometers southwest of Ottawa. We trapped turtles at two sites. The first site had a rocky bottom and was situated in McNallys Bay. The second site had a muddy bottom and was situated in Duck Bay.

Turtle capture

I captured painted turtles in late May and early June 2012 with two sets of paired fyke nets. Each net was 3.5 m long and composed of seven 0.9 m diameter steel rings. There were two throats at the second and fourth rings in each net to prevent escape. Each net was equipped with two 4.6 m wings and a 10.7 m long lead. Nets, throats and leads were built with a 5.08 cm knotted nylon mesh. The nets were emptied every day to avoid turtle deaths by asphyxia or predation. We brought the turtles captured back to Queen's University Biological Station to take morphological measurements and blood and scute samples. We marked turtles with a notch on the carapace to avoid recapture.

We kept turtles overnight at QUBS and released them at the same site where they were captured.

Blood sampling

We collected 0.6 ml of blood from 26 painted turtles by subcarapacial vein puncture (Dyer et Cervasio 2008) using 1 ml un-heparinized syringes fitted with a 25 gauge, 38 mm needle. The first 0.5 ml of blood was transferred to 1.5 ml microcentrifuge tubes and immediately frozen at -20^{o}C for mercury analysis. The blood left was used to make two replicates of thin blood smears on glass slides. Blood smears were fixed and stained using Wright-Giemsa Sure Stain.

Parasites and lymphocytes counts

I examined each blood smear under a 40x objective with an Olympus CX41RF light microscope. An estimate of the number of lymphocytes circulating in the blood was calculated per 4,000 erythrocytes for each individual (Davis et al. 2011). I examined 10,000 erythrocytes in each slide to count intra-erythrocyte parasites *Haemogregarina balli* (Manwell 1977). I considered two intra-erythrocyte stages of the life cycle of this parasite: the meronts and the gamonts. Discrimination between different kinds of leukocytes and stages of parasites was done according to Thrall et al. (2004).

Mercury analyses

Blood was dried and decomposed. Scutes were freeze-dried with dry ice and then crushed into powder with a mortar and pestle. Then, total mercury (THg) concentrations in the blood and the scutes were determined by thermal decomposition,

amalgamation and atomic absorption spectrophotometry. I measured THg in all 26 samples, and both THg and MeHg in 6 samples. The analyses of both forms of Hg on the 6 samples indicated that 82% of the THg was made of MeHg. Therefore, THg is a good approximation of MeHg in my samples.

Statistical analyses

I performed statistical analyses with JMP 10.0 (SAS Institute Inc.). To control for the effects of individual characteristics and site on immunological characteristics and parasitism, I included morphological traits, sex, and sites in my analyses. I derived an index of body condition from the residuals of a regression of log mass on log carapace length. The continuous variables were log-transformed for normalization.

Due to my modest sample size (n=26), I used preliminary univariate analyses, linear regressions for continuous variables and paired t tests for categorical variables, to determine which variables to use in the final models. The independent variables were sex, site, carapace length, body condition, lymphocytes count, blood total mercury, and scute total mercury. The dependent variables were the percentage of erythrocytes infected by parasites and the count of lymphocytes per 4,000 erythrocytes in the blood. Only independent variables with nearly significant univariate relationships ($p < 0.15$) with the dependent variables were retained for the final ANCOVA models: one for lymphocyte count and one for parasitism.

Results

Blood smears examination revealed the presence of intra-erythrocyte parasites in 20 of the 26 blood samples, with an average of 4.0 (±0.6) parasites per 10,000 erythrocytes. Average levels of total mercury were 459.6 ppb (±160.7) in scutes and 11.8 ppb (±1.8) in blood, indicating that painted turtles are little contaminated. For scute total mercury (THg) (n=12), no predictor nearly significantly explained the variation in lymphocytes or parasites (Table 1). For blood THg (n=26), ratios of lymphocytes and parasites in the blood were nearly significantly explained by several predictors (Table 2). I kept sex and blood THg in the lymphocyte count model and I kept site, carapace length, and body condition in the parasitism model (Table 2).

The ANCOVA model with sex and blood THg for the lymphocyte count was marginally significant (R^2 = 0.22, $F_{[2,25]}$ = 3.22, p = 0.058): blood THg had a marginally significant effect on the number of lymphocytes (r^2 partial = 0.13, $F_{[1,25]}$ = 3.81, p = 0.063, Figure 1) while sex did not have an effect (r^2 partial = 0.09, $F_{[1,25]}$ = 2.52, p = 0.126).

The ANCOVA model for parasitism was statistically significant (R^2 = 0.48, $F_{[3,25]}$ = 6.90, p = 0.002). The three variables included in this model, length of the carapace (r^2 partial = 0.12, $F_{[1,25]}$ = 4.98, p = 0.036), body condition (r^2 partial = 0.12, $F_{[1,25]}$ = 5.21, p = 0.033), and site (r^2 partial = 0.15, $F_{[1,25]}$ = 6.40, p = 0.019) all had a statistically significant effect on the number of parasites. Of note, small turtles and those in worse body condition tended to have more parasites.

I noticed a potential masking effect by site on the possible relationship between blood THg and parasitism as I observed a significant difference in parasitism between the two sites (R^2 = 0.25, $F_{[1,25]}$ = 7.81, p = 0.01). A marginally significant difference in blood THg was also found between the two sites (R^2 = 0.12, $F_{[1,25]}$ = 3.13, p = 0.09). However, the absence of a statistically significant relationship between parasitism and blood THg (Table 1) means that the masking effect is unlikely.

Discussion

The capacity of an individual to resist parasite infections is possibly dictated by the quality of the response of his immune system (Day et al. 2010, Sagerup et al. 2009). Thus, I used lymphocyte counts to see whether Hg can act as an immunosuppressor that could lead to more intra-erythrocyte parasites (Lawrence et McCabe 2002). I found a marginally significant negative trend between lymphocyte counts and blood THg, which is in accordance with results from marine turtles (Day et al. 2007). This suggests that Hg exposure may be responsible for a reduced number of lymphocytes circulating in the blood.

My main goal was to relate the level of parasitism to the total burden of mercury in the scutes and the blood of painted turtles. However, I detected no significant relationship between mercury load and level of parasitism. In total, 77% of the painted turtles in my study were infected by *Haemogregarina balli*, and the number of infected erythrocytes rarely exceeded 4 per 10,000. Previous studies found prevalence of haemoparasites varying between 22% and 41% in populations of painted turtles from

Algonquin Park in Ontario, Canada, and Northeast Georgia, United States (Siddal et Desser 1992, Davis et Sterrett 2011). The intensities in both studies rarely exceeded one haemoparasite per 10,000 erythrocytes. The prevalence and abundance of *Haemogregarina balli* parasites in my study therefore seem higher than previously found. Still, the abundance of parasites may not be high enough to observe an effect of MeHg on levels of parasitism. Levels of parasitism could be related to the prevalence of leeches (Siddall et Desser 2001), but this is a variable I did not quantify.

The reason why mercury levels do not change the susceptibility of painted turtles to be infected by *Hemogregarina balli* parasites could be because the levels of mercury were not high enough to change individual capacity to fight off parasites. This explanation would be consistent with previous findings about contaminated loggerhead sea turtle in which no effect of mercury on internal parasitism was found (Day et al. 2010). The same was true in several species of marine birds feeding on fishes and mollusks (Robinson et al. 2009, Sagerup et al. 2009, Wayland et al. 2001). The capacity of *Haemogregarina balli* parasites to infect the host may therefore not be related to MeHg contamination, at least when contamination levels are relatively low.

I did not find any relationship between measures of the immune system and blood parasite load. There are two potential explanations for this result. First, the immune system may not play an important role in the fight against blood parasites. Second, the effect of mercury on the immune system may not have been strong enough to cause an increase in blood parasites. MacKenzie et al. (1999) proposed that low levels

of infection by parasites can be a sign of heavy metal pollution. This could also explain the lack of correlation between mercury and parasites. More research is required on the effect of heavy metals on the diversity and abundance of parasites.

In conclusion, contrary to my predictions, parasitism in painted turtles was not affected by either total mercury in the blood or by a weakened immune system despite mercury seemingly having a deleterious effect on the immune system. It is probable that mercury levels at my study sites were too low to induce severe enough changes in the immune system of the host to affect parasite load. Hence, parasitism and immune system comparisons in more contaminated sites are warranted; further studies should be conducted in sites varying in mercury contamination to determine whether an effect of contamination on rates of parasitism is present at high concentrations.

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References

Daum, J. R., Sheperd, D. M. & Noelle, R. J. (1993). Immunotoxicology of cadmium and mercury on B-lymphocytes — I. Effects on lymphocyte function. *Internationnal Journal of Immumopharmacology*, **15**(3), 383-394.

Davis, A. K., Ruyle, L. E. & Maerz, J. C. (2011). Effect of trapping method on leukocyte profiles of black-chested spiny-tailed iguanas (*Ctenosaura melanosterna*): Implications for zoologists in the field. *International Scholarly Research Network Zoology*, **2011**, 1-8.

Davis, A. K., & Sterrett, S. (2011). Prevalence of Haemogregarine Parasites in Three Freshwater Turtle Species in a Population in Northeast Georgia, USA. *International Journal of Zoological Research*, **7**, 156-163.

Day, R. D., Segars, A. L., Arendt, M. D., Lee, A. M. & Peden-Adams, M. M. (2007). Relationship of blood mercury levels to health parameters in the loggerhead sea turtle (Caretta caretta). *Environmental Health Perspectives*, **115**(10), 1421-1428.

Day, R. D., Keller, J. M., Harms, C. A., Segars, A. L., Cluse, W. M., Godfrey, M. H., Lee, A. M., Peden-Adams, M., Thorvalson, K., Dodd, M. & Norton, T. (2010). Comparison of mercury burdens in chronically debilitated and healthy loggerhead sea turtles (*Caretta caretta*). *Journal of Wildlife Diseases*, **46**(1), 111-117.

Driscoll, C. T., Han, Y. J., Chen, C. Y., Evers, D. C., Lambert, K. F., Holsen, T. M., Kamman, N. C. & Munson, R. K. (2007). Mercury contamination in forest and freshwater ecosystem in the northeastern United States. *BioScience*, **57**(1), 17-28.

Dyer, S. M. & Cervasio, E. L. (2008). An Overview of Restraint and Blood Collection Techniques in Exotic Pet Practice. *Veterinary Clinics of North America: Exotic Animal Practice*, **11**(3), 423-443.

Jarup, L. (2003). Hazards of heavy metal contamination. *British Medical Bulletin*, **68**, 167-182.

Lafferty, K. D. (1997). Environmental parasitology: What can parasites tell us about human impacts on the environement?. *Parasitology Today*, **13**(7), 251-255.

Lalancette, A., Morin, Y., Measures, L. & Fournier, M. (2003). Contrasting changes of sensitivity by lymphocytes and neutrophils to mercury in developing grey seals. *Developmental and Comparative Immunology*, **27**, 735–747

Lawrence, D. A. (1981). Heavy metal modulation of lymphocyte activities. *Toxicology And Applied Pharmacology*, **57**, 439-451.

Lawrence, D. A. & McCabe, M. J. (2002). Immunomodulation by metals. *International Immunopharmacology*, **2**, 293-302.

Leblond S. S. (2009). Examination of metal contamination within the UNESCO designated rideau river waterway. *Unpublished manuscript*.

Lloyd, S. (1995). *Environmental influences on host immunity: Ecology of Infectious Diseases in Natural Populations*. Cambridge University Press. Cambridge, UK. pp. 327-361.

MacKenzie, K. (1999). Parasites as pollution indicators in marine ecosystems: a proposed early warning system. *Marine Pollution Bulletin*, **38**(11), 955-959.

Manwell, R.D. (1977). *Gregarines and haemogregarines. In Parasitic protozoa. Vol. III. Gregarines, haemogregarines, coccidia, plasmodia, haemoproteids*. Academic Press, New York. pp. 17–31.

McAuliffe, J. R. (1977). An hypothesis explaining variations of hemogregarine parasitemia in different acquatic turtle species. *The Journal of parasitology*, 63(3), 580-581.

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

Readel, A. M., Phillips, C. A., & Wetzel, M. J. (2008). Leech parasitism in a turtle assemblage: Effects of host and environmental characteristics. *Copeia*, **1**, 227-233.

Robinson, S. A., Forbes, M. R. & Hebert, C. E. (2009). Parasitism, mercury contamination, and stable isotopes in fish-eating double-crested cormorants: no support for the co-ingestion hypothesis. *Canadian Journal of Zoology*, **87**, 740-747.

Sagerup, K., Savinov, V., Savinova, T., Kuklin, V., Muir, D. C. G. & Gabrielsen, G. W. (2009). Persistent organic pollutants, heavy metals and parasites in the glaucous gull (*Larus hyperboreus*) on Spitsbergen. *Environmental Pollution*, **157**, 2282-2290.

Siddall, M. E. & Desser, S. S. (1992). Prevalence and intensity of Haemogregarina balli in three turtles from Ontario. *Canadian Journal of Zoology*, **70**, 123-128.

Siddall, M. E., & Desser, S. S. (2001). Transmission of *Haemogregarina balli* from painted turtles to snapping turtles through the leech *Placobdella ornata*. *Journal of Parasitology*, **87**(5), 1217-1218.

Thrall, M. A., Dale, T., Baker, E. & Lassen, E. D. (2004). *Hematology of Reptiles : Veterinary hematology and clinical chemistry*. Lippincott Williams & Wilkins. Pennsylvania, USA. 260-276.

Turnquist, M. A., Driscoll, C. T., Schulz, K. L., & Schlaepfer, M. A. (2011). Mercury concentrations in snapping turtles (*Chelydra serpentina*) correlate with environmental and landscape characteristics. *Ecotoxicology*, **20**(7), 1599-1608.

Ullrich, S. M., Tanton, W. T. & Abdrashitova, S. A. (2001). Mercury in the aquatic environment: A review of factors affecting methylation. *Critical Reviews in Environmental Science and Technology*, **31**(3), 241-293.

Wayland, M., Gilchrist, H. G., Dickson, D. L., Bollinger, T., James, C., Carreno, R. A. & Keating, J. (2001). Trace elements in king eiders and common eiders in the Canadian Arctic. *Archives of Environmental Contamination and Toxicology*, **41**, 491-500.

Wiener, J. G., Knights, B. C., Sansheinrich, M. B., Jeremiason, J. D., Brigham, M. E., Engtrom, D. R., Woodruff, L. G., Cannon, W. F. & Balogh, S. J. (2006). Mercury in soils, lakes, and fish in Voyageurs National Park: Importance of atmospheric deposition and ecosystem factors. *Environmental Science & Technology*, **40**(20), 6261-6268.

Zelikoff, J. T., Smialowicz, R., Bigazzi, P. E., Goyer, R. A., Lawrence, D. A., Maibach, H. I. & Gardner, D. (1994). Immunomodulation by metals. *Fundamental And Applied Toxicology*, **22**, 1-7.

Appendix

Table 1. Summary of paired t tests (sex and site) and univariate linear regressions (carapace length, body condition and scute total mercury) for the potential models including scute THg (n = 12). P-values in bold and italic are either marginally significant (*) or significant (**).

	Sex	Site	Carapace length	Body condition	Scute THg
Lymphocytes count	R^2 = 0.206 ***P = 0.14****	R^2 = 0.006 P = 0.81	R^2 = 0.029 P = 0.60	R^2 = 0.061 P = 0.44	R^2 = 0.111 P = 0.29
Parasitism	R^2 = 0.076 P = 0.39	R^2 = 0.632 ***P = 0.002***	R^2 = 0.368 P = 0.55	R^2 = 0.116 P = 0.278	R^2 = 0.164 P = 0.19

Table 2. Summary of paired t tests (sex and site) and univariate linear regressions (carapace length, body condition and blood total mercury) for the potential models including blood THg (n=26). P-values in bold and italic are either marginally significant (*) or significant (**).

	Sex	Site	Carapace length	Body condition	Blood THg
Lymphocytes count	R^2 = 0.090 **P = 0.14***	R^2 = 0.004 P = 0.75	R^2 = 0.036 P = 0.36	R^2 = 0.019 P = 0.50	R^2 = 0.133 **P = 0.07****
Parasitism	R^2 = 0.074 P = 0.18	R^2 = 0.246 **P = 0.01****	R^2 = 0.215 **P = 0.02****	R^2 = 0.120 **P = 0.08***	R^2 = 0.058 P = 0.24

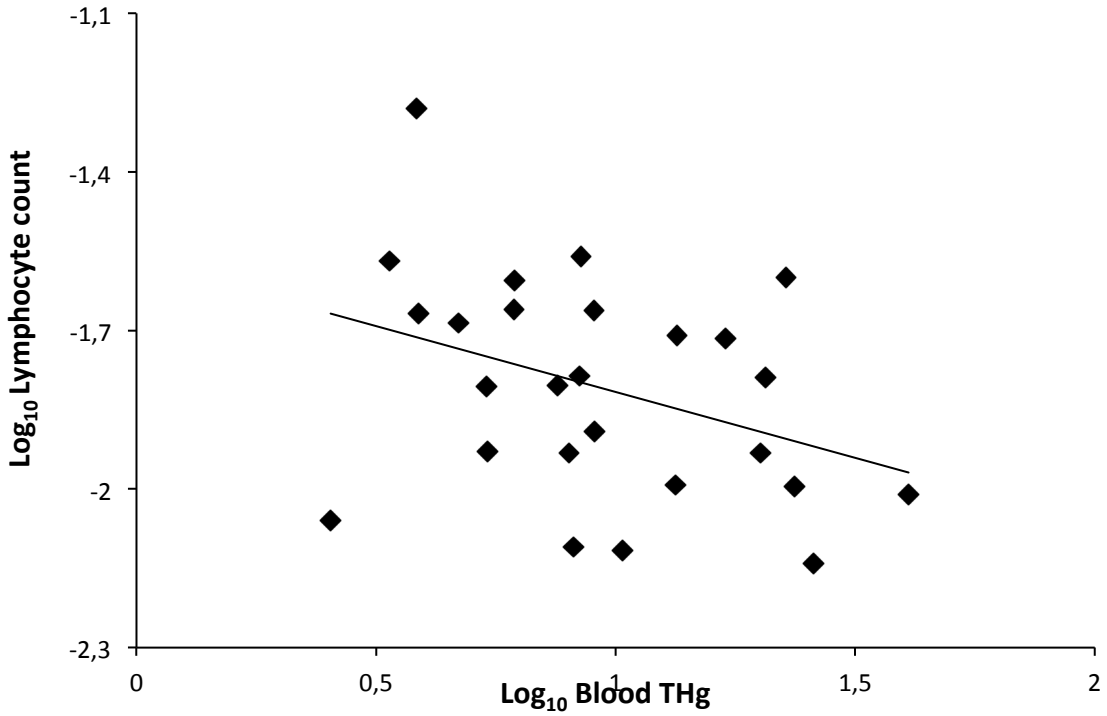

Figure 1. Lymphocyte count as a function of blood total mercury (THg) (n=26) in painted turtles (*Chrysemys picta*) from Upper Rideau Lake, Ontario, Canada.