MERCURY IN TURTLES FROM THE ASIAN FOOD TRADE

by

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(Under the Direction of J. Whitfield Gibbons and Christopher Romanek)

ABSTRACT

Mercury contamination threatens many ecosystems worldwide. Methyl mercury bioaccumulates at each trophic level, and biomagnifies within individuals over time. Long-lived turtles often occupy high trophic positions and are likely to accumulate mercury in contaminated habitats. Millions of turtles worldwide are sold in Asia for human consumption. Consumers may be at risk if turtles contain high levels of mercury. We dissected 71 turtles from 14 food trade species and analyzed their tissues (liver, kidneys, muscle, claws, and scutes) for total mercury content. Mercury was generally highest in carnivores, and lowest in herbivores. Liver and scutes had the highest concentrations. We compared mercury concentrations with consumption limits developed by the US EPA and FDA to evaluate mercury in fish tissue. Several samples exceeded the recommended 1900 ppb consumption threshold, indicating that consumers who eat certain turtle species frequently may be at risk for mercury-related health problems.

INDEX WORDS: Human health, Mercury, Methylmercury, Turtles, Wildlife trade

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B.S., Rutgers University, 2004

A Thesis Submitted to the Graduate Faculty of The University of Georgia in Partial Fulfillment of the Requirements for the Degree

MASTER OF SCIENCE

ATHENS, GEORGIA

2007

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December 2007

DEDICATION

To my parents, Kenneth Green and Karyn Karriem, who always encouraged me to aim high.

ACKNOWLEDGEMENTS

This project would not have been possible without the contributions of several colleagues at the Savannah River Ecology Laboratory in Aiken, SC. Cris Hagen assisted in identifying and choosing study specimens. Kurt Buhlmann created the species distribution maps. Eric Peters and Judy Greene-McLeod advised in turtle dissection methods. The Turtle Survival Alliance and the Tewksbury Institute of Herpetology provided the turtles. I thank Judy Greene-McLeod, Jason Unrine, Paul Bertsch, Tracey Tuberville, Sean Poppy, and Noelle Garvin for providing access to various lab supplies and equipment. I also thank Teresa Carroll, Lindy Steadman, Patsy Pittman, Cherie Summer, and Melissa Mottley for administrative support. I give special thanks to Cris Hagen and Kurt Buhlmann for contributing valuable first-hand knowledge and expertise of exotic turtles. I am especially grateful to Heather Brant for her assistance in mercury and stable isotope analysis and her sage advice. My committee members-- J. Whitfield Gibbons, Christopher Romanek, Chuck Jagoe, and Kurt Buhlmann—provided priceless guidance and support throughout my graduate studies. Lastly, I thank my family for their unconditional love and moral support, and my boyfriend, Marcus J. Ross, for the hugs, late night pots of coffee, and words of encouragement that helped me through this process.

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INTRODUCTION

Mercury (Hg) contamination is a widespread environmental problem threatening aquatic and terrestrial ecosystems worldwide. Coal-fired utility plants, gold and mercury mining operations, municipal and medical waste incineration, and discharges from chlor-alkali and cement production facilities are responsible for most of the anthropogenic load of several hundred tons of Hg emitted globally each year (Wang *et al.*, 2004). Almost all Hg emitted into the atmosphere is in inorganic forms, and these are eventually deposited into lakes, rivers, estuaries, and other bodies of water. Inorganic Hg is converted to methylmercury (MeHg) by bacterial methylation, which adheres to sediment particles and partitions into bacteria and plankton. From there it enters the food chain, where it biomagnifies at each successive trophic level and bioaccumulates within organisms over time (Wang *et al.*, 2004).

Environmental Mercury Contamination in Southeast Asia

Southeast Asia is rich in natural resources, including coal, oil, silver, gold, and other minerals. Mining, coal production and other industrial activities, and rapid urban development has resulted in contamination of many water systems with Hg and other pollutants.

The Guizhou Province in southern China is a major Hg and coal production center, producing 12% of total global Hg emissions. A 2002 study found elevated Hg levels in soil, rice, and fish in areas of the province near Hg or coal mines and industrial wastewater outputs (Finkelman *et al.*, 1999; Horvat *et al.*, 2002). Xiao *et al.* (1998) measured Hg levels in soil and moss on the Fanjing Mountain Nature Preserve (FMNP), a 419 km^2 protected area in eastern Guizhou Province. The area is surrounded by six large-scale Hg production centers at distances

of 25-200 km. Researchers found soil and moss samples at FMNP contained Hg several hundred times higher than background levels.

Many major river systems in Southeast Asia, particularly those near crowded cities, receive pollutant inputs from rapid urban development in addition to industrial inputs. Poor sanitation and sewage systems in densely-populated urban centers of developing countries result in untreated waste deposited directly into surrounding bodies of water. Zingde and Desai (1981) estimated that India's Thana Creek receives 56 million L/day of industrial wastewater, as well as large quantities of domestic wastewater from nearby Mumbai. Mercury measured in water, sediment, and zooplankton was substantially elevated compared to values measured in non-contaminated creeks.

In the 1960s and 1970s, Thailand experienced a dramatic increase in industrial activity and consequently, the amount of pollutants in water, soil, and seafood increased substantiallly. Suckcharoen and Nuorteva (1978) measured Hg in fish, aquatic birds, and human hair samples near several rivers and canals in pristine and polluted areas of Thailand. They found that Hg concentrations in most sampled areas were among the lowest reported anywhere in the world, with an average of 0.07 ppm (70 ppb). However, fish from the Chao Phraya estuary had highly elevated Hg concentrations, ranging from 320 to 3,600 ppb and averaging 1,480 ppb. The authors attribute this finding to the nearby Thai Asashi Caustic Soda Co Ltd (TACSCO) plant. Mercury levels in wastewater from the plant averaged 900 ppb. Menasveta *et al.* (1985) also documented high Hg concentrations in bivalves collected from the same area.

Gold mining is another major industry in Southeast Asia that is well known to cause extensive regional Hg pollution. A study of the population of the Diwalwal region of Mindanao Island, Philippines, concluded that residents had substantially elevated Hg levels in urine, blood, and hair samples (Drasch *et al.*, 2001). Diwalwal is home to a small-scale gold mining

operation, which is responsible for high amounts of Hg emitted into the local atmosphere. Residents were exposed to atmospheric Hg and ingested Hg from contaminated fish and locally-grown grains. Gold mines are also abundant throughout Malaysia, Papua New Guinea, and Indonesia. These high levels of Hg in the environment are likely to negatively impact human and wildlife populations.

Dietary Mercury Exposure

After consumption of Hg-contaminated food, Hg enters the bloodstream from the digestive tract and is distributed to the organs within hours (Blanvillain *et al*., 2007). Thus, Hg in blood represents a transient pool originating from recently-ingested food items. Total Hg levels are usually highest in the liver, and second highest in the kidneys (Gordon *et al*., 1998; Linder and Grillitsch, 2000; Sakai, 2000; Burger, 2001; Golet and Haines, 2001; Storelli and Marcotrigiano, 2003). However, different forms of Hg accumulate to different extents in various tissues. Inorganic Hg tends to accumulate in the kidneys (National Research Council, 2000), and MeHg in the liver and muscles (Linder and Grillitsch, 2000; Day *et al*., 2005). Some studies suggest that Hg is converted from MeHg to an inorganic form in the livers of higher vertebrates (Albers *et al*., 1986; Day *et al.*, 2005; Blanvillain *et al*., 2007). Therefore, proportions of inorganic Hg and MeHg in the liver may depend to some extent on time since last meal, individual metabolic rate, and body condition, among other factors. MeHg has a high affinity for sulfhydryl (–SH) groups such as those present on some amino acids, and so accumulates in protein-rich tissues like liver and muscle. Golet and Haines (2001) found that Hg levels in muscle from the front shoulder, hind leg, and tail of snapping turtles from Connecticut were highly correlated, indicating that Hg is evenly distributed among muscle tissues.

Keratin proteins, such as those present in scutes, are also rich in –SH functional groups, and so tend to accumulate Hg. Various studies have used keratinous tissue, such as fur, hair,

claws, scutes, and feathers, to monitor Hg exposure and accumulation (Meyers-Schone and Walton, 1994; Linder and Grillitsch, 2000). Since scutes consist of multiple layers of non-living tissue deposited over time, they can accumulate high levels of Hg over the life of a turtle. Several studies have reported that Hg concentrations in scutes are much higher than those in liver and kidneys of the same animal (Sakai, 1995, 2000; Day, 2005; Blanvillain *et al*., 2007). However, since most scutes are eventually shed, allocating contaminants to scutes may be an effective mode of depuration.

Physiological Effects of Mercury on Wildlife

As awareness of the destructive impact of environmental Hg contamination has grown, the detrimental effects of Hg have been reported in a number of studies that examined reproduction, behavior, and physiology of birds, fish, reptiles, amphibians, and mammals. Methylmercury's chemical properties allow it to persist in biological tissues as well as in the environment. Its tendency to bioaccumulate over time and biomagnify with trophic position places long-lived predatory species at highest risk for Hg exposure (Wolfe *et al.*, 1998; Linder and Grillitsch, 2000). The methyl groups of the organometallic species bind readily to --SH groups, such as those present in the amino acid cystiene. This causes MeHg to accumulate in protein-rich tissue, such as liver, skeletal muscle, hair, feathers, and scutes.

Mercury damages the nervous system (National Research Council, 2000; Linder and Grillitsch, 2000). Experiments with chronically-high dietary Hg levels in birds yielded neurologic lesions, loss of muscle coordination, spinal cord degeneration, and general nervous system dysfunction (Woebeser *et al.*, 1976; Scheuhammer, 1988). Studies with lower to moderate exposure levels comparable to those encountered in the field found detrimental behavioral changes as well. Heinz (1979) observed behavioral changes in three generations of mallards (*Anas platyrhynchos*) exposed to dietary Hg. Specimens were fed 500 ppb Hg in feed,

offered *ad libitum*, from nine days of age until they were euthanized as adults one year later. Heinz reported that hens fed Hg laid a significantly greater percent (5.4% - 8.2%) of eggs outside of the nestbox. Ducklings also had muted responses to maternal calls and were hypersensitive to fright stimuli compared to controls. Spalding *et al.* (2000) reported a marked decrease in grooming behavior in great egret nestlings (*Ardea alba*) that consumed 500 ppb Hg in fish *ad libitum* for 14 weeks. Webber and Haines (2002) conducted a study in which golden shiners (*Notemigonus crysoleucas*) were fed 500 ppb and 1,000 ppb Hg daily for 90 days. The fish displayed drastic differences in schooling behavior and decreased predator avoidance behaviors. These behavioral effects may make individuals more susceptible to predation, causing mercury to pass through each trophic level at a faster rate.

Numerous studies have demonstrated reproductive effects of dietary Hg, many of which focused on effects on birds. Common loons (*Gavia immer*) are large, long-lived birds that feed almost exclusively on fish, and so can accumulate substantial tissue Hg concentrations. As a result, they have been identified as the most important high-trophic level indicator species for Hg pollution in North American lakes (Biodiversity Research Institute, 2005). Numerous studies have shown that MeHg exposure has a negative impact on reproduction in loons. In a study by Barr (1986), exposed loons laid significantly fewer eggs than loons fed non-contaminated prey. Evers *et al.* (2003) conducted a field study in which 577 common loon eggs were collected from eight states and analyzed for Hg. They found a strong inverse relationship between egg Hg content and egg volume, suggesting that maternally-transferred Hg interferes with egg development. Meyer *et al.* (1998) found similar results when they collected and tracked adult loons and chicks from 45 lakes in Wisconsin. Reproductive success was markedly lower at lakes where chick blood mercury levels were elevated.

Heinz (1979) found that mallards produced fewer viable eggs when fed 500 ppb Hg *ad libitum* for one year. Shells of viable eggs were also significantly thinner than those from unexposed hens. Chicks of exposed hens also gained significantly less weight during the first week of life than control chicks, suggesting a developmental effect of maternally-transferred Hg.

Negative reproductive consequences of dietary Hg have also been documented in fish. For example, Friedman *et al.* (1996) found that juvenile male walleye (*Sander vitreus*) fed catfish containing 137 - 987 ppb of Hg three times per week for six months had significantly impaired growth and testicular atrophy. Female juvenile fathead minnows (*Pimephales promelas*) fed 80 - 850 ppb Hg from hatching to sexual maturity also displayed reduced gonadal development, which led to a decrease in reproductive effort and spawning success (Hammerschmidt *et al*., 2002).

Effects of Environmental Mercury Contamination on Turtles

Turtles have been used extensively as bioindicators of environmental contamination (Meyers-Schone *et al*., 1993; Ashpole *et al*., 1994; Meyers-Schone and Walton, 1994; Golet and Haines, 2001; Day *et al.*, 2005; Bergeron *et al.*, 2006; Blanvillain *et al*., 2007). Turtles have relatively long life spans, making them well-suited for monitoring bioaccumulative pollutants like Hg, polychlorinated biphenyls (PCBs), and organochlorine pesticides. Most freshwater and terrestrial turtle species display site fidelity and remain within a specific home range for most of their lives, which means contaminant levels in their tissues should closely reflect those of their environment. Also, blood, scutes, and eggs can be sampled for contaminant analysis without harming individual specimens or populations.

Commonly studied species:

Much research on contaminants in turtles has focused on sea turtles, common snapping turtles (*Chelydra serpentina*), diamondback terrapins (*Malaclemys terrapin*) and sliders

(*Trachemys scripta*); (Meyers-Schone and Walton, 1994). Table 1 summarizes results of prior studies on Hg in these and other turtle species.

Common snapping turtles (*C. serpentina*) are large, carnivorous turtles that inhabit freshwater and brackish environments from southern Canada to Ecuador. Their wide geographic distribution, presence in a variety of habitats, large size, and ease of capture make them an ideal species for monitoring contaminants in wetlands. Because of their large size, high trophic position, and relatively long life span, they are generally expected to accumulate large amounts of tissue Hg. Meyers-Schone *et al.* (1993) measured radionuclides and mercury in *C. serpentina* and *T. scripta* from Tennessee. *C. serpentina* specimens had significantly higher Hg in kidneys and muscle tissue than *T. scripta* specimens. Albers *et al.* (1986) compared Hg levels in snapping turtles from contaminated wetlands in NJ with those from uncontaminated sites in MD. They found that, although sediment Hg at the NJ sites was highly elevated, Hg in livers and kidneys of snappers from these sites was relatively low. A previous study [Galuzzi (1981), as cited by Albers *et al.*, 1986] found a similar pattern in birds and mammals from the same areas, suggesting that although sediment Hg concentrations were higher at the NJ sites, bioavailability of this material was relatively low. Nonetheless, snapping turtles seem to accumulate substantially less Hg than fish despite their size, high trophic position, and longevity, snapping turtles seem to have substantially less Hg than fish. This may reflect an ability to allocate and sequester contaminants in their scutes.

Diamondback terrapins (*Malaclemys terrapin*) are the only native US turtle that exclusively inhabits estuarine environments. They are abundant in salt marshes and tidal creeks along the Atlantic Coast from Massachusetts to Florida and along the Gulf Coast to Texas. This has generated interest in using this species to monitor contamination in coastal wetlands. Burger (2001) investigated heavy metals in tissues of terrapins from New Jersey. She found that liver

Hg concentrations were over 6 times higher than those in muscle tissue, averaging 1139 ppb Hg wet wt. She concluded that consumers of terrapin livers with Hg levels similar to those measured may be at risk due to Hg toxicity. Blanvillain *et al.* (2007) found that Hg concentrations in terrapins from South Carolina differed with season. Blood Hg was significantly lower in August than in April, June, or October.

Trophic level effects:

Diet is a major factor affecting Hg accumulation. Because Hg biomagnifies, or increases in concentration with trophic level, turtles with more carnivorous diets tend to have higher tissue Hg concentrations (Linder and Grillitsch, 2000; Hopkins, 2006). Anan *et al.* (2001) found that adult hawksbill sea turtles (*Eretmochelys imbricata*), which are omnivores, had higher Hg in livers and kidneys than herbivorous adult green turtles (*Chelonia mydas*) occupying the same habitat off the coast of the Yaeyama Islands in Okinawa, Japan. Godley *et al.* (1999) measured heavy metals in stranded loggerhead sea turtles (*Caretta caretta*) and green sea turtles from the Mediterranean Sea. As might be expected, the carnivorous loggerhead specimens had significantly higher Hg than the green turtles in all tissues sampled.

Ontogenetic effects:

A few studies have reported changes in tissue Hg concentrations with age. Day *et al.* (2005) measured Hg in blood and scutes from 40 loggerheads captured along the coasts of South Carolina, Georgia, and Florida. Using body mass as a proxy for age, they found a significant increase in blood and scute Hg with mass, and suggested that there is a stable component to blood Hg that reflects accumulated Hg from long-term exposure and which is in equilibrium with organ Hg levels. Anan *et al.* (2001) measured heavy metals in 26 specimens of *C. mydas* and 22 specimens of *E. imbricata* from Okinawa, Japan. They used standard carapace length (SCL) as a proxy for age, and found a significant positive correlation between SCL and Hg in liver and

kidneys. However, they also found a significant negative correlation between SCL and Hg in muscle tissue. Similarly, Sakai *et al.* (2000) discovered that younger *C. mydas* from the same region had higher muscle Hg than older turtles. This may be due to the dietary shift from omnivory to herbivory that green turtles make as they age.

Sex effects:

Some studies have also reported differences in Hg distribution and accumulation between sexes. The physiological demands of egg production might explain some variation in tissue Hg between reproductive males and females. However, turtle eggs contain relatively small amounts of Hg, so allocating Hg to eggs does not seem to be a major route of elimination in turtles (Sakai *et al*., 1995). In contrast, Godley *et al.* (1999) argue that although Hg levels in individual sea turtle eggs are very low, clutch sizes are very large and multiple clutches are laid within a season, so the overall amount of Hg eliminated through egg laying in a single season may be significant. Mercury concentrations in eggs may also increase with contaminant exposure. Ashpole *et al.* (2004) measured contaminants in *C. serpentina* eggs from the St. Lawrence River basin. Although egg Hg was relatively low at most sites (50 - 250 ppb dry wt.), eggs from the more contaminated sites (Raquette and Turtle Rivers) averaged 720 ppb. Blanvillan (2005) investigated the use of diamondback terrapins (*Malaclemys terrapin*) as biomonitors for Hg in southeastern US estuaries. In this species, sexual dimorphism results in different dietary preferences for males and females. Tucker *et al.* (1995) investigated foraging ecology of terrapins on Kiawah Island, South Carolina, and found that the diets of terrapins with relatively small heads (males and small- and medium-sized females) consisted of a higher proportion of small snails than did those of terrapins with larger heads (i.e., larger females). Terrapins with relatively large heads consumed nearly equal amounts of snails of all sizes. Larger prey items would be expected to have correspondingly higher levels of contaminants than smaller

conspecifics in the same habitat. Since larger snails (with presumably higher Hg) are consumed almost exclusively by large females, it is possible that this group of terrapins is exposed to higher dietary mercury concentrations than males and smaller females.

Physiological effects:

It is difficult to isolate one specific contaminant (e.g., Hg) as the cause of any physiological response seen in the field, because contaminated environments usually contain more than one pollutant. Environmental toxicants can interact with one another, and individuals can experience a combination of contaminant stresses. Nevertheless, a few studies have attempted to examine physiological effects of mercury contamination on turtles. Blanvillain *et al.* (2007) found a significant negative correlation between blood Hg and plasma lysozyme activity (a common measurement of immunity) in terrapins, suggesting elevated Hg levels in blood can result in decreased immune function. Meyers-Schone *et al.* (1993) documented a higher incidence of DNA strand breakage in liver tissue of snapping turtles and sliders from a highly contaminated site that contained Hg compared to turtles from a reference site. However, the contaminated location they studied also had elevated levels of radionuclides, so it is unclear what proportion of the strand breakage can be attributed to Hg. Albers *et al.* (1986) reported that male snapping turtles at contaminated sites were significantly smaller than males of the same age from uncontaminated sites. Blanvillain *et al.* (2007) also reported that female terrapins from a contaminated site weighed significantly less than females collected from other sites, suggesting that contaminant stress may contribute to decreased growth rates.

Population effects:

Effects of habitat contamination on entire populations have been observed in several species, but have rarely been quantified. It seems that certain species may be more tolerant of contamination than others. Luiselli *et al.* (2006) examined habitat use in four species of native

freshwater turtles (*Pelusios castaneus*, *Pelusios niger*, *Pelomedusa subrufra*, and *Trionyx triunguis*) from the Niger River delta of southern Nigeria, an area greatly altered by oil industry-related pollution. They found that numbers of all four species decreased drastically in polluted areas over the eight-year study despite an equal search effort. Conversely, several researchers have reported healthy abundances of turtles in contaminated areas. Carr (1952) noted that midland painted turtles (*Chrysemys picta marginata*) are "remarkably tolerant" of polluted habitats. Red-eared sliders are known to occupy and even thrive in extremely contaminated sites such as sewage treatment ponds (Selcer, 2006). Blanvillain *et al.* (2007) commented that terrapins were very abundant in a highly-polluted tidal creek receiving inputs from nearby urban areas and several local industrial facilities.

There is evidence that unexpectedly high numbers of turtles in polluted habitats may be associated with changes in the diet due to the presence of increased nutrients. Gibbons (1967) compared growth rates and stomach contents among three populations of painted turtles (*Chrysemys picta*) in southwestern Michigan. Turtles were captured from three locations: 1.) Sherriff's Marsh, a pristine area of open water surrounded by grassland and filled with aquatic plants in summer, 2.) Wintergreen Lake, a highly eutrophic lake with abundant plants at its periphery, and 3.) a polluted stretch of the Kalamazoo River with sparse vegetation. He discovered that the marsh turtles were mostly herbivorous, the lake turtles primarily consumed plants but also invertebrates, and the river turtles were carnivorous, eating mostly invertebrates. Consequently, specimens from the Kalamazoo River had the highest growth rates and highest maximum body size, despite the pollutants present in this habitat. Specimens from Sherriff's Marsh had the smallest maximum body size, even though this study site was the least polluted. In a subsequent study, Gibbons and Tinkle (1969) investigated reproduction of female *C. picta* from the same three populations. Body mass and clutch size were highest in river females and

lowest in marsh females. They concluded that differences in food quality between the three locations was likely responsible for the observed differences in growth rate, and possibly for the corresponding disparity in clutch size among the three populations. Although it was not discussed in either study, it is probable that the differences in *C. picta*'s prey base between the three locations resulted from high levels of nutrient pollution in the Kalamazoo River, and to a lesser extent in Wintergreen Lake.

Asian Turtle Trade

The largest and most urgent threat to turtle populations worldwide is the unregulated food trade based in China and Southeast Asia (Altherr and Freyer, 2000; Turtle Conservation Fund, 2002). Annually, more than 10 million turtles from around the world are sold in markets for consumption as food or medicine. This unsustainable trade has resulted in the dramatic decline of turtle populations worldwide, and particularly in Asia. Turtles have been used for food and medicine in China for centuries as it has been long believed that consuming them contributes to longevity and wisdom (Williams, 1999). Historically, the turtle trade was relatively small and led by locals who hunted for subsistence or trade to local restaurants. In 1989, Chinese currency became convertible, allowing direct access to foreign markets. This led to dramatically increased exportation of turtles from Southeast Asian countries to China (Behler, 1997; Lovich *et al.*, 2000).

Before this change in economic policy, the majority of turtles traded in Chinese markets were native species. Today, as many of China's turtles have been hunted to extinction, the markets are dominated by species from Southeast Asia, India, Africa, and North and South America. In 2000, experts estimated that 80% of turtles sold in Chinese markets were from other countries (McCord, 2000). This proportion is likely larger today. Several native U.S. species are also exported to Asian markets, including common snapping turtles (*Chelydra serpentina*),

alligator snapping turtles (*Macrochelys temminckii*), softshell turtles (*Apalone spp*.), desert and gopher tortoises (*Gopherus spp.*), map turtles (*Graptemys spp.*), sliders (*Trachemys* spp.), and diamondback terrapins (*Malaclemys terrapin*) (Behler, 1997; Williams, 1999; Altherr and Freyer, 2000). Several endangered species are also traded, but are usually hidden from view to avoid conflict with authorities. These are commonly smuggled across international borders in packages labeled as seafood (Williams, 1999; Haitao, 2000).

As turtle populations in Southeast Asia dwindle and individuals become more difficult to find in the wild, certain species become rarer in markets, fueling demand and raising prices. The Chinese three-striped box turtle (*Cuora trifasciata*), one of the most prized species on the market for its perceived cancer-curing properties, was reported to sell for up to $3000US each in 2000 (Behler, 1997; Altherr and Freyer, 2000). Several other *Cuora spp.* sold for $2000US each in 2000 (McCord, 2000). Softshell turtles are a very popular luxury food, sometimes selling for prices six times that of lamb or chicken (TRAFFIC, 2001). Meat from rare turtles has become a delicacy in many East Asian countries and is now consumed primarily by the elite. In a way, this economic situation drives the trade. Many collectors in Southeast Asian "source" countries, such as Indonesia, Malaysia, Myanmar, Vietnam, Bangladesh, Laos, and Cambodia, sell turtles they capture to traders because they are poor and turtles are profitable. These are then exported to more developed "consumer" countries, such as China and Japan, where they are sold to expensive restaurants and wealthy customers at high prices (Asian Turtle Working Group, 1999; Behler, 1997; Haitao, 2004).

Possible Risks to Human Consumers

The trade in wild turtles for food and medicine is not regulated by any government agency. These turtles are not subject to rules restricting sale of food items that contain contaminant levels above a threshold considered safe for human consumption. It is reasonable to

assume that turtles from Hg-contaminated habitats are sold on the market. As a result, people who consume turtles may be at risk of health consequences associated with elevated Hg exposure. Frequent consumers and pregnant women would be expected to be at greatest risk.

Acute and chronic Hg exposure can cause a myriad of health problems in humans. In the US, consumption of contaminated fish is the major source of human exposure to Hg (National Research Council, 2000). In 2000, the National Research Council concluded that although the risk of adverse effects from consuming tainted fish is low for the majority of the US population, sensitive subgroups, like frequent seafood consumers, may be at greater risk. The population at highest risk is children of women who ate large amounts of fish during pregnancy. The developing fetus is most sensitive to mercury's adverse effects at much lower doses than in adults (Linder and Grillitsch, 2000; National Research Council, 2000; Schober *et al.*, 2003).

The developing human nervous system is sensitive to Hg, which interferes with growth and migration of neurons, creating the potential for irreversible central nervous system damage. Chronic, low-level prenatal exposure to Hg in the maternal diet has been associated with subtle endpoints of neurotoxicity, such as poor performance on tests of attention, fine motor function, language, visual-spatial abilities, and verbal memory. Kjellstrom *et al.* (1986) studied a coastal New Zealand population with a high rate of fish consumption. They administered tests of mental development, motor development, and cognitive skills to children four to six years of age whose mothers reported eating fish during pregnancy. Children exposed to moderate to high Hg levels in utero (defined as maternal hair concentrations >6 ppm) performed significantly poorer on tests than did children exposed to lower levels. Grandjean *et al.* (1997) found similar results in children from the Faroe Islands of Denmark, where fish and whales are an important part of the diet. Researchers followed a cohort of children born in 1986 and 1987. They characterized exposure by measuring Hg in umbilical cord blood, maternal hair samples at partuition, and in

child hair samples at 12 months and seven years of age. Neuropsychological tests of seven year-olds revealed numerous dose-related dysfunctions, most notably in language, attention, and memory.

Risk-Based Consumption Limits

As environmental mercury contamination has become a larger and more widespread problem in the United States in recent years, the US Environmental Protection Agency (EPA) and Food and Drug Administration (FDA) have collaborated to develop fish consumption advisories for the general public. Since children and pregnant and nursing women are most susceptible to the harmful effects of dietary mercury, the EPA and FDA have recommended that these sensitive subgroups completely avoid fish with tissue concentrations above 1 ppm (1000 ppb). This is also the FDA threshold above which fish are ineligible for interstate commerce.

The EPA has determined a reference dose for mercury of $1x10^{-4}$ mg/kg/day (ppm/day). A reference dose is defined as "an estimate (with uncertainty spanning perhaps an order of magnitude) of a daily exposure to a human population (including sensitive subgroups) that is likely to be without an appreciable risk of deleterious health effects during a lifetime" (EPA, 2001). To avoid exceeding this reference dose, the EPA has recommended that humans (other than the sensitive subgroups discussed above) do not consume fish with tissue Hg concentrations above 1900 ppb.

Monthly consumption limits have been set for tissues with concentrations below the 1900 ppb threshold. These limits are based on tissue Hg concentration and standard estimates of meal size and consumer body weight. Daily consumption limits are calculated using equation 1:

(1) $$CR_{lim} = (R_fD \times BW)/C_m$$

Where CR_{lim} = Maximum allowable consumption rate (kg/day)

R_fD = Reference dose ($1x10^{-4}$ mg/kg/day for Hg)

BW = Consumer body weight (kg)

C_m = Hg tissue concentration (mg/kg)

From this, the monthly consumption limit can be calculated using equation 2:

(2) $$CR_{mm} = (CR_{lim} \times T_{ap})/MS$$

Where CR_{mm} = Maximum allowable consumption rate (meals/month)

T_{ap} = Time averaging period (365.25 days/12 months = 30.44 days/month)

MS = Meal size (kg)

Although these terms were developed for evaluating fish tissue, they will be used here to understand potential human health risks from the consumption of Hg-contaminated turtle meat.

Purpose of Study

The primary goals of this study are to measure concentrations of Hg found in several turtle species sold for human consumption in Asian food markets, compare Hg distribution and accumulation patterns observed with those of more frequently studied turtle species, and examine the relationship between tissue stable carbon and nitrogen isotope values and tissue Hg levels to better understand the potential sources of Hg that are acquired from the diet. From this information, species at risk may be identified, and patterns of Hg exposure may be related to various aspects of life history characteristics and ecology, such as habitat, body size, and trophic position. Specimens were acquired from seized shipments of turtles destined for food or pet markets. Seventy-one deceased individuals, representing 14 species and six families from four continents, were dissected and their tissues analyzed for mercury content and stable carbon and nitrogen isotope content. It is hypothesized that the highest mercury levels will be measured in turtles that are larger, older, and more carnivorous than smaller herbivorous species.

Tissue Hg levels will also be compared with Hg thresholds and guidelines established by the EPA and the FDA to determine if Hg concentrations measured in some turtles are high

enough to put consumers at risk for health problems related to dietary Hg exposure. If turtles traded in food markets contain Hg concentrations high enough to cause risk to consumers, education and awareness of the issue may devalue certain species, relaxing pressure on populations at risk.

Table 1: Mercury concentrations in turtles from prior studies. For comparison, all concentrations reported in wet weight have been converted to dry weight. (BDL= below detection limit).

			Hg, ppb dry wt.	
species	**location**	**tissue**	**mean (range)**	**reference**
Caretta caretta	South Adriatic Sea, Italy	liver	1680 (350 - 3720)	Storelli *et al.*, 1998a
			206 (109 - 324)	Storelli *et al.,* 1998b
		muscle	690 (170 - 1810)	Storelli *et al.,* 1998a
			53 (17.5 - 107.8)	Storelli *et al.,* 1998b
		kidney	650 (300 - 1530)	Storelli *et al.,* 1998a
	Moreton Bay, Queensland, Australia	liver	4.4 (0 - 9.4)	Gordon *et al.,* 1998
		kidney	14 (10.2 - 20.8)	
	South Carolina and Florida, USA	blood	live captures: 29 (5 - 188) strandings: 99 (40 - 306)	Day *et al.,* 2005
		scute	live captures: 461 (62 - 2837)	
			strandings: 941 (368 - 2326)	
		liver	175 (102 - 394)	
		kidney	66.4 (41 - 135.4)	
		muscle	38.8 (12.3 - 125)	
	Cape Ashizuri, Japan	liver	445.4 (74.6 - 2404)	Sakai *et al.,* 1995
		kidney	76.7 (12.4 - 137)	
		muscle	27 (13.3 - 47.3)	
		whole egg	1.5 (1.0 - 2.0)	
		yolk	3.3 (2.2 - 4.3)	
		albumin	0.1375 (0.0275 - 0.22)	

Caretta caretta	Mediterranean Sea, northern Cyprus	liver	2410 (820 - 7500)	Godley *et al.,* 1999
		kidney	470 (130 - 800)	
		muscle	480 (BDL - 1780)	
		hatchling	20 (BDL - 750)	
		embryo	10 (BDL - 220)	
		yolk + albumin	190 (160 - 570)	
Chelonia mydas	Mediterranean Sea, northern Cyprus	liver	550 (270 - 1370)	Godley *et al.,* 1999
		kidney	BDL	
		muscle	480 (BDL - 1780)	
		hatchling	20 (BDL - 750)	
		embryo	10 (BDL - 220)	
		egg contents	190 (160 - 570)	
	Moreton Bay, Queensland, Australia	liver	4.3 (0 - 10.6)	Gordon *et al.,* 1998
		kidney	2.94 (0 - 7.2)	
	Yaeyama Islands, Okinawa, Japan	liver	58.6 (10.8 - 130.6)	Sakai *et al.,* 2000a
		kidney	19.4 (4.3 - 36.5)	
		muscle	4.2 (0.22 - 26.2)	
	Yaeyama Islands, Okinawa, Japan	liver	420	Anan *et al.,* 2001
		kidney	300	
		muscle	40	
Dermochelys coriacea	Irish Sea, Wales, UK	liver	390	Davenport and Wrench, 1990
		pectoral muscle	120	
		blubber	110	
Eretmochelys imbricata	Yaeyama Islands, Okinawa, Japan	liver	870	Anan *et al.,* 2001
		kidney	1300	
		muscle	400	

Chelydra serpentina	Bearden Creek, Tennessee	kidney	85	Meyers-Schone *et al.,* 1993
		muscle	25	
	White Oak Lake, Tennessee, USA	kidney	325	
		muscle	42.5	
	southeastern Connecticut, USA	muscle	12.5 - 125	Golet and Haines, 2001
		scute	125-825	
	St. Lawrence River, Canada	whole egg	12.5 - 45	Bonin *et al.,* 1995
	Raquette River/Turtle Creek, Canada	whole egg	180	Ashpole *et al.,* 2004
	St. Lawrence River basin, Canada	whole egg	12.5 - 62.5	
	Minnesota, USA	leg muscle	6 (5 - 10)	Helwig and Hora, 1983
		fat	36.25 (12.5 - 75)	
	New Jersey, USA (brackish site)	liver	males: 281.6	Albers *et al.,* 1986
			females: 293.37	
		kidney	males: 89.1	
			females: 62.32	
	New Jersey, USA (freshwater site)	liver	males only; 149.4	
		kidney	males only; 66.3	
	Maryland, USA	liver	males: 219.6	
			females: 124.2	
		kidney	males: 70.84	
			females: 87.92	

Emys obicularis	Ali Bairamly, Azerbaijan	liver	1590	Swartz *et al.,* 2003
	Sumgayit, Azerbaijan	liver	19900	
Kinosternon flavescens	Wharton County, Texas, USA	whole body (w/o shell)	30	Flickinger and King, 1972
Malaclemys terrapin	South Carolina, USA	blood	43	Blanvillain *et al*., 2007
		scute	235	
	Purvis Creek, Georgia, USA	blood	742	
		scute	2891	
	New Jersey, USA	whole egg	8.75	Burger, 2001
		liver	284.75	
		muscle	43	
Trachemys scripta	Bearden Creek, Tennessee, USA	kidney	30	Meyers-Schone *et al.,* 1993
		muscle	7.5	
	White Oak Lake, Tennessee, USA	kidney	160	
		muscle	25	
	Savannah River Site, South Carolina, USA	egg contents	40±15	Burger and Gibbons, 1998
	Wharton County, Texas, USA	whole body (w/o shell)	20	Flickinger and King, 1972
Xerobates agassizi	California, USA	liver	sick: 1312	Jacobsen *et al.,* 1991
			healthy: 115	

METHODS

Study species

The fourteen species used in this study are described below. All status evaluations are reported from the 2000 IUCN Red List. Figure 1 shows the geographical distribution of each species. A summary the following information can be found in Table 2, which lists the diet, habitat, geographical range, and conservation status of each species.

Reeve's turtle (*Chinemys reevesi*) (n=4):

The Reeve's turtle occurs in eastern China, Japan, Korea, Taiwan, and Hong Kong. This small pond turtle's carapace length (CL) does not exceed 120 mm in males and 235 mm in females. It occupies small, shallow ponds and marshes, and can sometimes be found in large, slow-moving rivers, basking for most of the day (Bonin *et al*., 2006). It has also been observed to forage nocturnally during rains (SREL, unpubl. data). It is an omnivore, feeding on various invertebrates, algae, and aquatic plants. This turtle was once quite popular in the food trade, but it has become more rare as wild populations have declined in recent years. The majority of Reeve's turtles sold in markets today are raised on farms. This species is listed as endangered on the IUCN's Red List.

Malayan box turtle (*Cuora amboinensis*) (n=8):

The Malayan box turtle has a wide but discontinuous distribution throughout Southeast Asia, occurring in Bangladesh, India's Nicobar Islands, the Kaziranga National Park in Assam, southern Myanmar, Cambodia, Malaysia, the Philippines, Sumatra, Java, and east to the Mollucas in Indonesia. Its carapace length does not exceed 250 mm (Bonin *et al.*, 2000). This aquatic turtle can be found in swamps, rice paddies, and small bodies of slow or stagnant water.

Juveniles are more aquatic than the adults, who often spend the night on land (Iskandar, 2000). In water, this species eats aquatic plants, mollusks, and small crustaceans, and on land it consumes mushrooms, earthworms, and certain plants. This is the most frequently consumed turtle in Chinese restaurants (Bonin *et al.*, 2006). It is considered endangered in Bangladesh, Cambodia, Laos, and Vietnam and vulnerable in India, Indonesia, Malaysia, and Thailand (IUCN/SSC Tortoise and Freshwater Turtle Specialist Group and Asian Turtle Trade Working Group, 2000).

Indian star tortoise (*Geochelone elegans*) (n=6):

The Indian star tortoise occurs in Pakistan, northwest India, and south of the Ganges Delta in India, and Sri Lanka. Its carapace length ranges up to 380 mm. This species prefers arid areas with abundant plants, but has also been found in prairies. During the dry season, it is active in the morning and remains in the shade for most of the day. In the wet season, the species mates and feeds heavily during rainstorms (Bonin *et al.*, 2006). The species is herbivorous, consuming fruits and vegetables when available, and dead leaves and spiny vegetation during the dry season. Its attractive, colorful shell pattern has popularized this turtle in the pet trade. Its ground shell is also used in traditional Chinese medicine. It is considered a species of least concern by the IUCN, but is in CITES Appendix II because of the extent of its exportation to pet markets.

Black-breasted leaf turtle (*Geoemyda spengleri*) (n=4):

The black-breasted leaf turtle has a narrow range in southern China and north and central Vietnam. It is found in wooded, mountainous areas near wetlands and creeks, and feeds on insects, earthworms, other small invertebrates, and fruit. This species is prized in the pet trade for its small size (maximum CL is 130 mm) and unique, spiny carapace. Its limited range combined with its popularity in the pet trade contributes to the species' endangerment.

Spiny turtle (*Heosemys spinosa*) (n=6):

The spiny turtle ranges throughout southern Myanmar, Thailand, the Malay Peninsula, Singapore, and as far east as Sumatra and Borneo in Indonesia and Sulu and Mindanao in the Philippines. Its carapace length does not exceed 220 mm (Bonin *et al*., 2006). This turtle inhabits humid, montane forests and can be found near clear, shallow creeks. Primarily herbivorous, it consumes plants, vegetative debris, and fallen fruit, but sometimes eats insects, earthworms, and carrion. The spiny turtle is most active in the early morning and late afternoon, often hiding under leaf litter to avoid the midday heat (Bonin *et al.*, 2006). This species suffers from overharvesting for the food and pet trades. It is critically endangered in Indonesia, endangered in Thailand, and vulnerable in Borneo and Peninsular Malaysia (IUCN/SSC Tortoise and Freshwater Turtle Specialist Group and Asian Turtle Trade Working Group, 2000).

Sulawesi forest turtle (*Leucocephalon yuwonoi*) (n=6):

The Sulawesi forest turtle is endemic to the northern and central part of the island of Sulawesi in Indonesia. It has a maximum carapace length of 300 mm (Bonin *et al*., 2006). It is semiaquatic and lives in rocky, heavily vegetated areas near swamps and streams where it eats figs and other fruits and leaves (Hagen and Ching, 2005). This turtle began appearing in food markets less than a year after it was first described in 1995 (Lovich *et al*., 2000). Its populations are threatened by deforestation and collection for the food trade. It is listed as critically endangered on the IUCN's Red List.

Cantor's giant softshell turtle (*Pelochelys cantorii*) (n=2):

The Cantor's giant softshell turtle is widely distributed throughout southeast Asia, living in estuaries and river deltas along the coasts of southern India, Bangladesh, Thailand, Myanmar, Cambodia, Vietnam, Laos, and China as far north as the Fuzhou region (Bonin *et al*., 2006). It is also present in Borneo, eastern Sumatra, and northern Java in Indonesia, and on the island of

Luzon in the Philippines. It is carnivorous and feeds on fish, crustaceans, and mollusks. Softshell turtles are highly desired food items throughout Asia, and this species' large size (CL ranges up to 1300 mm) has contributed to its popularity in the food trade. As unsustainable harvesting has caused its populations to decline, the species is now less common in food markets in some parts of its range (Bonin *et al*., 2006). It is listed as endangered on the IUCN's Red List.

Chinese softshell turtle (*Pelodiscus sinensis*) (n=4):

The Chinese softshell turtle is native to eastern and central China and Taiwan, and it has been introduced to Japan, Thailand, and Hawaii. Its carapace length can reach up to 350 mm (Bonin *et al.*, 2006). It can be found in a variety of aquatic habitats, including slow-moving rivers, canals, lakes, swamps, and even rice paddies. It often basks on riverbanks and buries itself under water in mud or clay substrates. This turtle is mostly carnivorous, eating fish, crustaceans, mollusks, insects, worms, various larvae, and sometimes leaves and seeds. As this species has nearly disappeared from the wild, several million specimens are farmed annually to meet the high demand for softshell meat in Chinese markets (Bonin *et al*., 2006; IUCN/SSC Tortoise and Freshwater Turtle Specialist Group and Asian Turtle Trade Working Group, 2000) The Chinese softshell turtle is vulnerable in China.

Keeled box turtle (*Pyxidea mouhotii*) (n=6):

The keeled box turtle has a fragmented distribution in Southeast Asia, occurring in parts of India, southeastern Bangladesh, Myanmar, northern Thailand, Laos, Cambodia, the Malay peninsula, western Vietnam, and southeastern China, including Hainan. This species is small, reaching a maximum carapace length of 180 mm (Bonin *et al*, 2006). This primarily terrestrial box turtle prefers humid forested areas and foothills. It is an opportunistic omnivore and in captivity is particularly fond of earthworms (K. Buhlmann, pers. comm.). It is threatened

primarily by habitat destruction and collection for the food trade. It is sometimes collected for the pet trade, but does poorly in captivity. It is listed as endangered on the IUCN's Red List.

Black mud turtle (*Siebenrockiella crassicollis*) (n=6):

The black mud turtle has a maximum carapace length of 200 mm. This species inhabits marshes, ponds, and muddy, slow-moving rivers in the southern areas of Thailand, Myanmar, and Vietnam, peninsular Malaysia, Singapore, and western Borneo, eastern Sumatra, and Java in Indonesia. (Iskandar, 2000). Its omnivorous diet includes fruits, leaves, shrimp, amphibians, mollusks, and fish. This turtle spends most of its time in the water, but has also been known to travel long distances over land. Although this species is sometimes sold in food markets, its meat is not in high demand most likely because of its musky odor (Bonin *et al*., 2006). The species is endangered in Cambodia and Vietnam due to overexploitation and is vulnerable in Malaysia, Indonesia, and Thailand due to overexploitation and habitat loss (IUCN Red List, 2000).

Parker's snake-necked turtle (*Chelodina parkeri*) (n=2):

The Parker's snake-necked turtle is endemic to a small region on the island of New Guinea. Maximum carapace length is 150 mm in males and 267 mm in females. It is present in estuaries and in large lakes and rivers with abundant vegetation and shade, including Lake Murray and the Aramia, Fly, and Lorentz Rivers (Iskandar, 2000; Bonin *et al.*, 2006). It is also found in temporary wetlands and may travel on land to reach permanent water. It is believed to be mostly carnivorous. It is unknown what impact the food and pet trades have had on wild populations, but this species is listed as vulnerable on the IUCN's Red List.

Yellow mud turtle (*Kinosternon flavescens*) (n=6):

The yellow mud turtle (CL = 75 - 128 mm) ranges throughout the central and southwestern United States and northern Mexico (Carr, 1952; Ernst and Barbour, 1972). It

occurs in slow-moving rivers, swamps, and marshes with abundant aquatic plants and muddy or sandy bottoms. Like most mud turtles, it is an omnivore and feeds on insects, crustaceans, mollusks, amphibians, carrion, and aquatic plants. It is not sought in the food trade, but is occasionally found in pet markets. It is threatened by urban development, habitat alteration, and vehicles on roads, and is considered endangered in Illinois, Iowa, and Missouri (Bonin *et al*., 2006).

Diamondback terrapin (*Malaclemys terrapin*) (n=6):

The diamondback terrapin (CL = 100 - 140 mm in males and 150 - 230 mm in females) is exclusive to estuarine wetlands and brackish tidal creeks along the Atlantic coast of the US from Massachusetts to Florida and along the Gulf coast from Florida to Texas (Ernst and Barbour, 1972). Largely carnivorous, this turtle eats snails, crabs, shrimp, mollusks, other invertebrates, small fish, carrion, and aquatic plants (Tucker *et al*., 1995). Its populations declined dramatically due to overharvesting during the early 20th century when it was a popular delicacy in the United States. Terrapin numbers have since rebounded, but the species is still threatened by habitat destruction and alteration, road mortality of nesting females, and drowning in crab traps (Gibbons *et al*., 2001) This species is still harvested in the United States and shipped to food markets in Asia. It is protected in some eastern US states, but is considered a species of least concern by the IUCN.

Central American wood turtle (*Rhinoclemmys pulcherrima manni*) (n=5):

The Central American wood turtle's range extends from western Mexico to Costa Rica. This subspecies occurs from southwestern Nicaragua to northern and west central Costa Rica. Carapace length ranges up to 180 mm in males and up to 214 mm in females (Bonin *et al.*, 2006). It is largely terrestrial, occupying humid forested areas near streams. It is especially active after rainfall, and may be seen swimming at pond surfaces during the dry season. Its

omnivorous diet consists mainly of vegetables, fruit, earthworms, insects, snails, and slugs. Habitat destruction and automobile collisions are among the major threats to this species. This species is generally not sought in the food trade, but is often exported to the US through the pet trade. The IUCN considers this turtle to be a species of least concern.

Turtle acquisition

Cuora amboinensis, *H. spinosa*, and *S. crassicollis* specimens were obtained from a large shipment of turtles that likely originated in Malaysia and was destined for food markets in China. Customs officials in Hong Kong seized the shipment on December 11, 2001, and the Turtle Survival Alliance (TSA) handled the distribution of live specimens to zoos, organizations, and private individuals around the world (Hudson and Buhlmann, 2000). Several turtles were kept at the Savannah River Ecology Laboratory (SREL) in Aiken, SC. Sick animals that subsequently died were made available for this study. All other specimens were obtained from various locations, including food and pet markets, and were donated to SREL by the Tewksbury Institute of Herpetology (R. Ogust, pers. comm.) as deceased frozen specimens.

Dissection

Specimens were thawed and dissected. Portions of the liver, kidneys, pectoral muscle, hind leg muscle, scutes, and claws were removed for analysis. In addition, follicles were removed from female specimens when present. The dissected samples were stored individually in sterile polyethylene Whirl-Pak® bags (NASCO) and frozen at –10 ºC until processed. Tools were cleaned with 10% nitric acid between specimens to prevent cross-contamination.

Sample Preparation

Samples were weighed, lyophilized (Labconco), reweighed to a constant dry weight, and then lipid extracted. Analysis for $^{13}C/^{12}C$ and $^{15}N/^{14}N$ ratios required removal of lipids (Post, 2007) through a 24 hour extraction within a 2:1 chloroform: methanol mixture followed by

rinsing in methanol until the decanted liquid became clear. After air drying, the extracted tissue samples were homogenized in coffee grinders and/or a liquid nitrogen mill (Spex Sample Prep 6750 freezer mill, Metuchen, NJ, USA). Coffee grinders and freezer mill vials and stoppers were cleaned with a metal free detergent and 10% nitric acid between samples. Aliquots of lyophilized, homogenized, and lipid extracted tissue were then assayed for total mercury and stable isotope content as described below.

Analyses - Total Mercury

Tissues were analyzed for Hg following EPA method 7473 (USEPA, 1998), using a DMA80 Direct Mercury Analyzer (Milestone, Inc, Monroe, CT, USA). This method utilizes thermal decomposition, gold amalgamation, thermal desorption and atomic absorption detection. Samples were analyzed in batches of ten, with each batch including a blank, a sample replicate, and a tissue standard certified for Hg concentration (DORM-2, dogfish muscle, DOLT-2 dogfish liver, or TORT-2, lobster hepatopancreas, purchased from the National Research Council of Canada (NRCC), Ottawa, Canada). Standard recovery ranged from 85% to 116% with an average of 101% (n=58). The average difference between sample replicates was 2% (n=57). Based on a 0.98 g sample and an average blank of 0.12 ng Hg (n=27), the method detection limit (MDL) was 0.65 ppb. All samples were determined to be above the MDL. Mercury values are based on dried and lipid-extracted tissues.

Analyses – Stable Isotopes

Elemental analysis isotope-ratio mass spectrometry (EA-IRMS) was employed to measure the total carbon and nitrogen content, and the $^{13}C/^{12}C$ and $^{15}N/^{14}N$ ratios of individual samples (Barrie and Prosser, 1996). Prior to isotopic analysis, approximately 1.0 - 1.5 mg of tissue was loaded into a pre-cleaned tin capsule for weighing to ±1 μg using an ultra-microbalance (Sartorius, Edgewood, NY, USA). Capsules were then loaded with ground

samples, sealed, weighed and placed into a dessicator until analyzed on a Carlo Erba Elemental Analyzer (NC2500, Milan, Italy) attached to a continuous flow isotope ratio mass spectrometer (Finnigan Delta ^{plus} XL; Finnigan-MAT, San Jose, CA, USA). Samples were combusted to N_2 and CO_2 in oxidation/reduction furnaces, separated by gas chromatography and then measured for $^{13}C/^{12}C$ and $^{15}N/^{14}N$ ratios on the mass spectrometer. An internal N_2(g) working standard was admitted prior to the introduction of each sample and a CO_2(g) standard was admitted at the conclusion of each combustion for calibration to the AIR (nitrogen) and V-PDB (carbon) international standards (Mariotti 1983; Coplen 1996). Stable isotope ratios are reported in per mil units (‰) using standard delta (δ) notation (Craig 1957). External working standards of bovine muscle, avian feather and acetanilide were analyzed to determine external precision; these standards were reproducible to better than ±0.15‰ (1σSD) for both $\delta^{13}C$ and $\delta^{15}N$ values.

Calculation of Risk-Based Consumption Limits

To determine the maximum Hg tissue concentration that could be safely consumed in a given time period, equation 2 was rearranged and calculated for a monthly consumption rate of 0.5, 1, 2, 3, and 4 meals per month as follows:

$$CR_{lim} = (CR_{mm} \times MS)/ T_{ap}$$

This maximum allowable consumption rate was then substituted into equation 1, which was rearranged as follows:

$$C_m = (R_fD \times BW)/C_{lim}$$

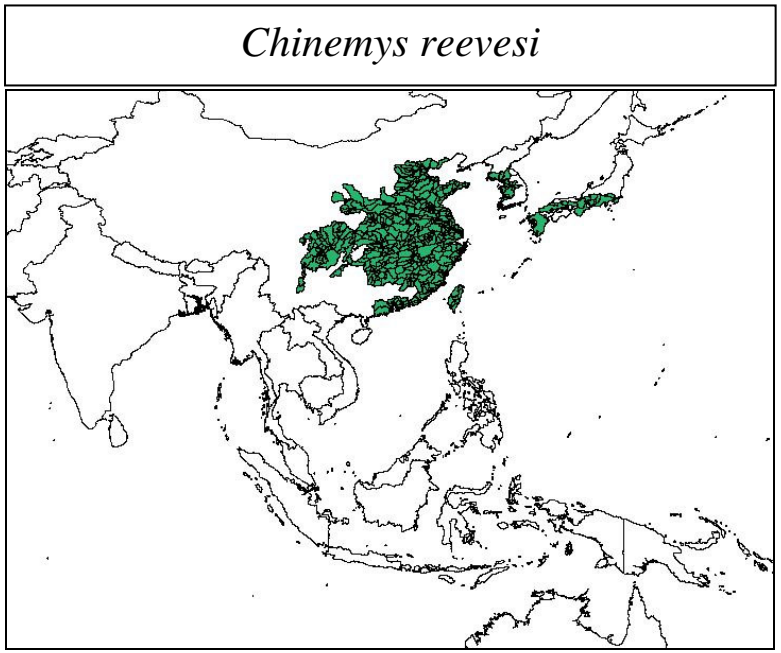
This gives the maximum Hg tissue concentration that can be safely consumed in one meal every two months (1877 ppb), one meal per month (938 ppb), two meals per month (470 ppb), three meals per month (313 ppb), and four meals per month (235 ppb). These limits were calculated using an average US adult consumer body mass of 70 kg and an average meal size of 8 oz (0.277 kg) (EPA, 2001). Table 3 lists the range of Hg concentrations associated with each consumption

limit category. These limits are based on Hg concentrations measured in fresh tissue. Since all samples in this study were lyophilized, Hg concentrations have been reported on a dry weight basis. In order to compare these Hg concentrations to the EPA's risk-based consumption limits, they have been converted to wet weights based on the average moisture content for each tissue type (liver: 76%, kidney: 82%, leg muscle: 78%, and pectoral muscle: 81%). The above equations can be modified to determine consumption limits for a specific consumer body mass and meal size, but we have used the standard values here for simplicity. Table 3.1 lists these consumption limits with their associated ranges of tissue Hg concentrations.

Statistical Analysis

Data were entered into Excel spreadsheets and analyzed using SAS v.8.1 software (SAS Institute, Cary, NC). The number of turtles from an individual species varied from 2 to 8, with most species represented by 4 to 6 specimens. Data for individual species were generally non-normal by a Shapiro-Wilk test. Plots of Hg values showed that data were often highly skewed, and there were many outliers. Variance and range of data also varied considerably among species, and no single transformation was adequate to normalize the data and homogenize the variances. Data were analyzed using both parametric (ANOVA, Pearson's correlations) and nonparametric methods (Kruskal-Wallis tests, Spearman's correlations). Because the assumptions of parametric statistics could not always be met, non-parametric methods were primarily used. Both types of analyses generally led to the same conclusions regarding relationships among variables and differences among tissues and species, suggesting that unmet assumptions about normality and homogeneity of variance were not important factors influencing the parametric analyses.

a



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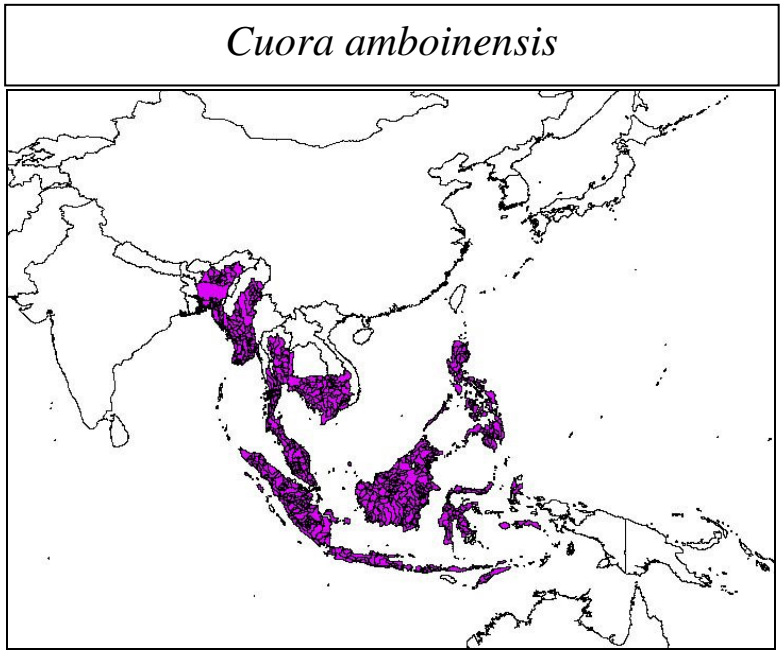
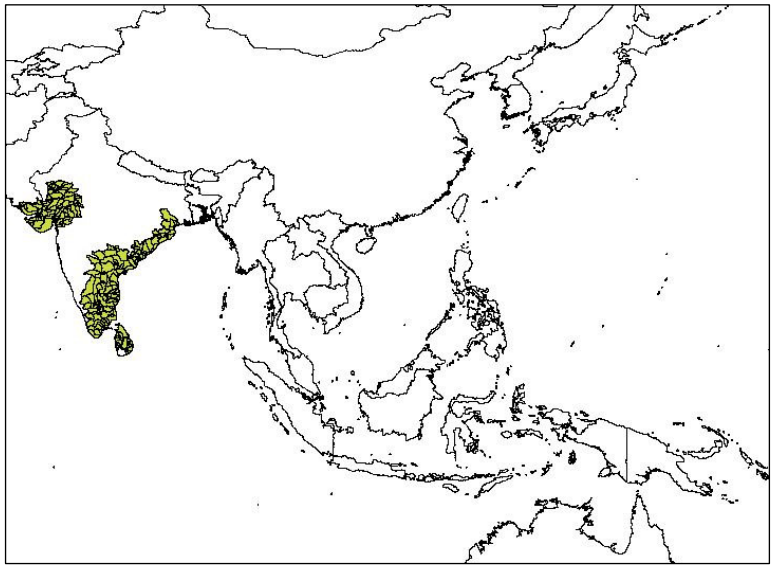


Figure 1: Geographic distribution maps. (a): *Chinemys reevesi*, (b): *Cuora amboinensis*, (c): *Geochelone elegans*, (d): *Geoemyda spengleri*, (e): *Heosemys spinosa*, (f): *Leucocephalon yuwonoi*, (g): *Pelochelys cantori*, (h): *Pelodiscus sinensis*, (i): *Pyxidea mohoutii*, (j): *Siebenrockiella crassicollis*, (k): *Chelodina parkeri*, (l): *Kinosternon flavescens*, (m): *Malaclemys terrapin*, (n): *Rhinoclemmys pulcherrima*

c

Geochelone elegans



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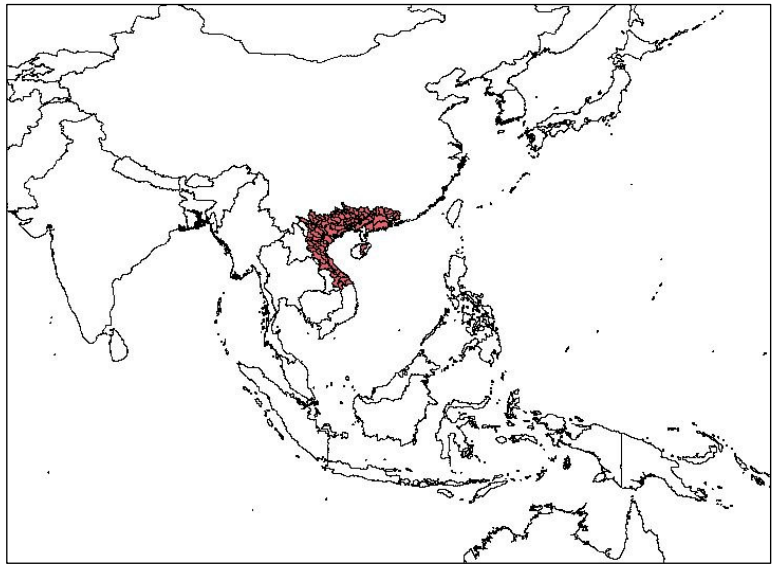
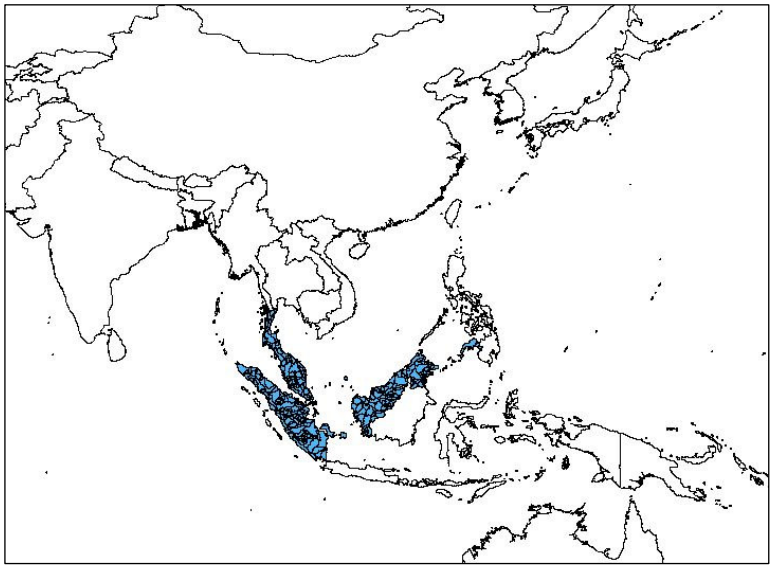
Geoemyda spengleri



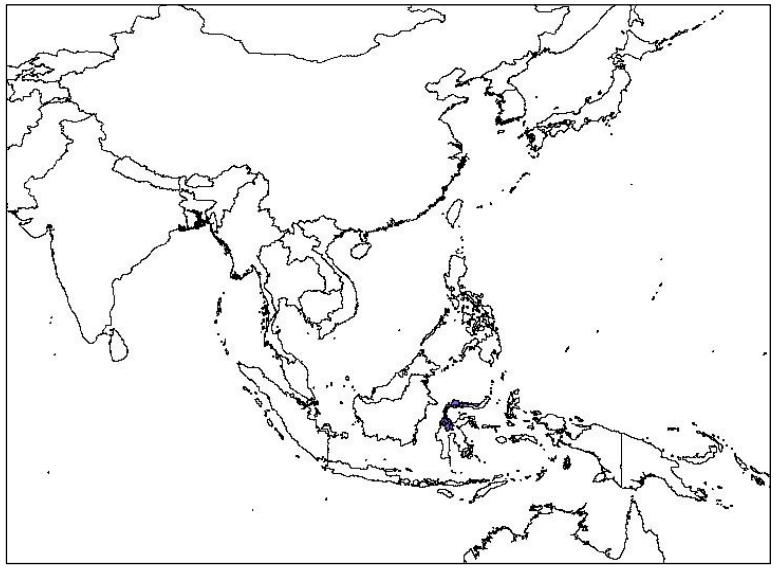
e

Heosemys spinosa



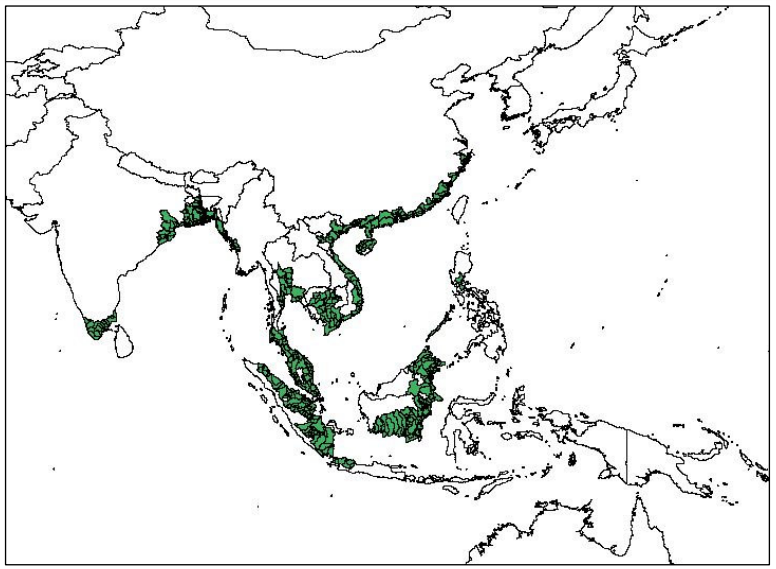
f

Leucocephalon yuwonoi



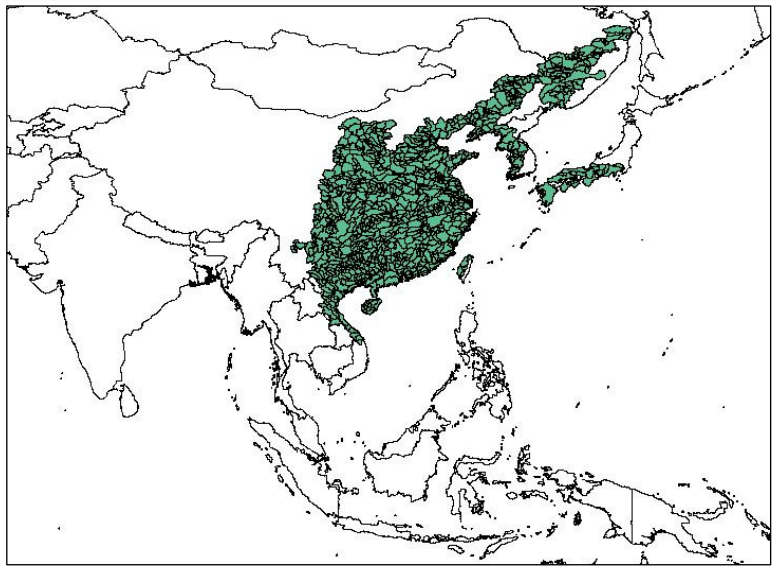
g

Pelochelys cantorii



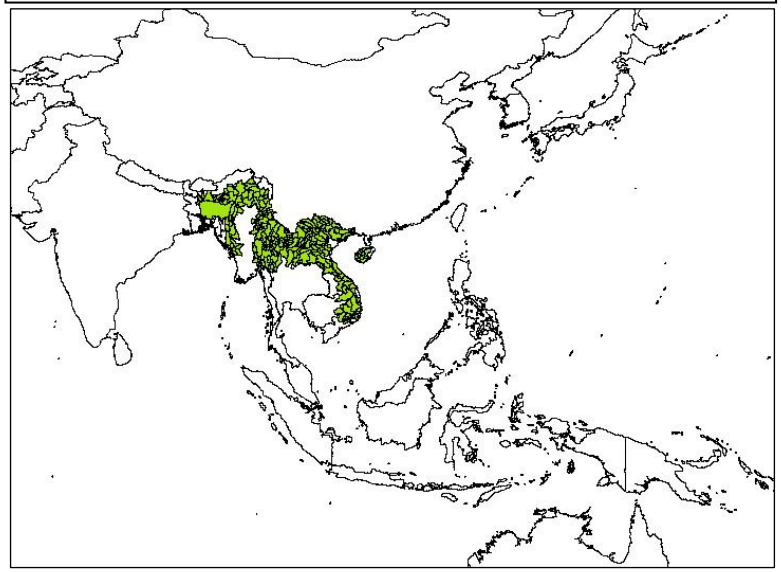
h

Pelodiscus sinensis



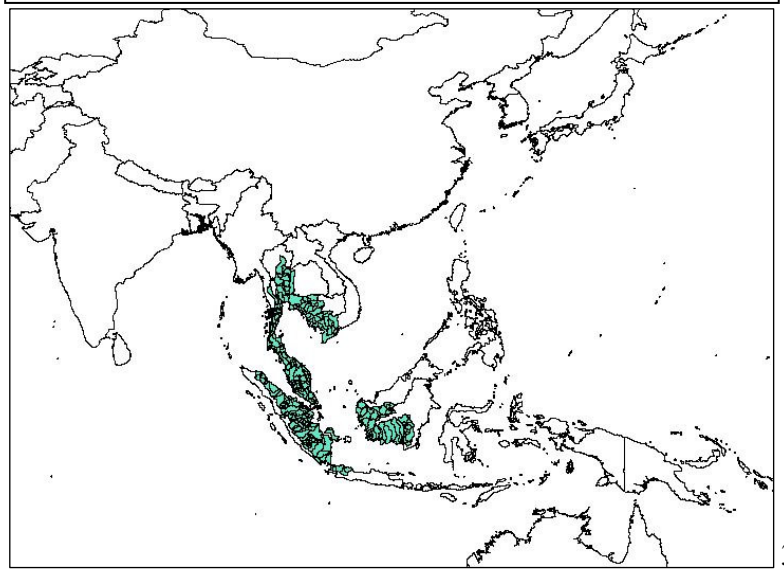
i

Pyxidea mohoutii



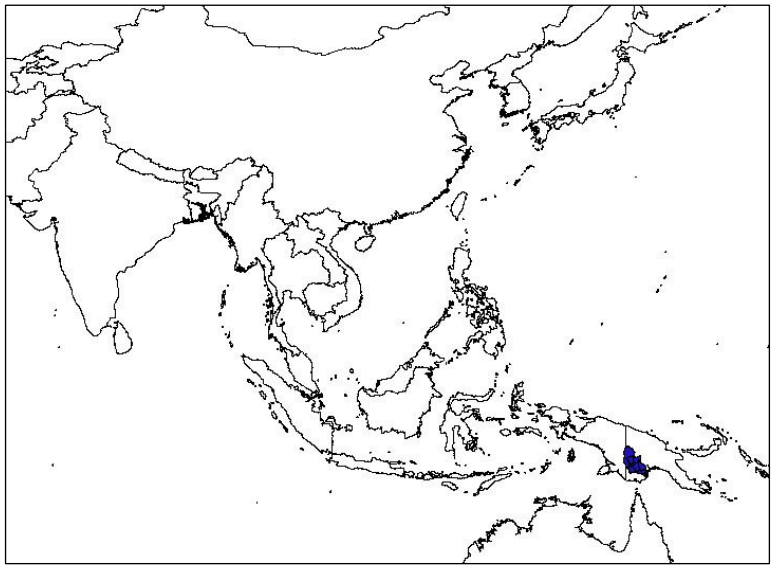
j

Siebenrockiella crassicollis



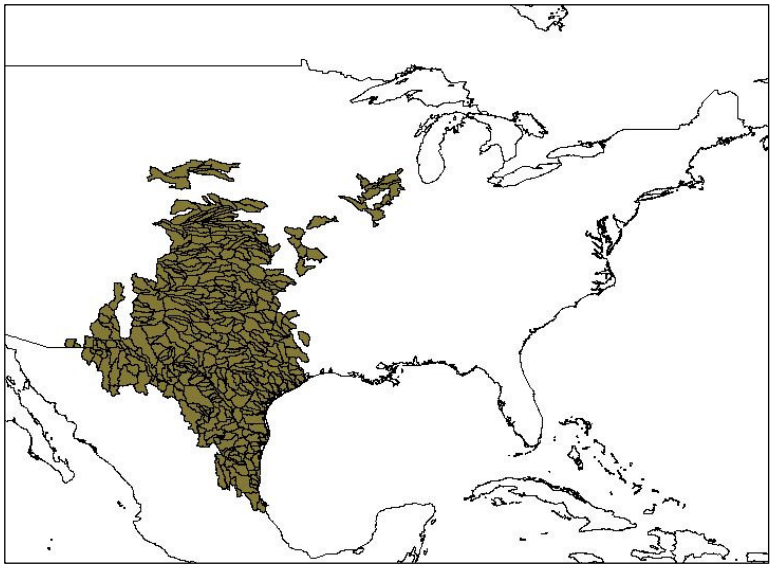
k

Chelodina parkeri



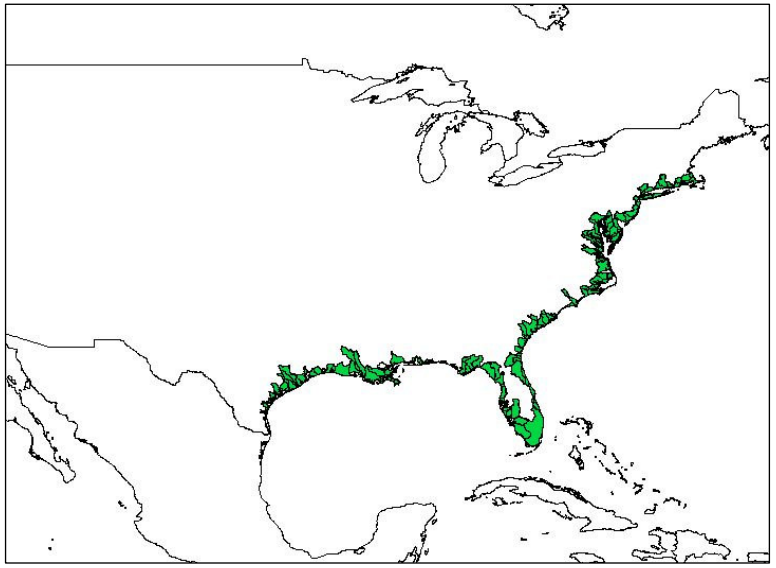
l

Kinosternon flavescens



m

Malaclemys terrapin



n

Rhinoclemmys pulcherrima

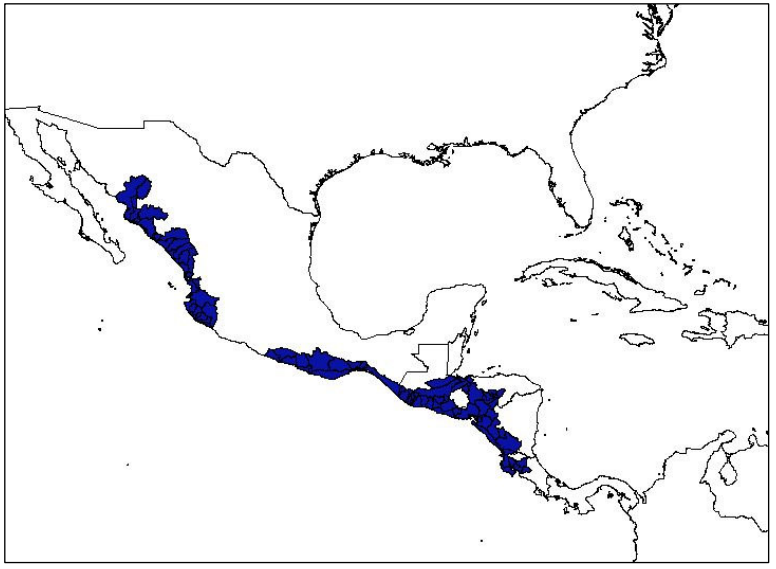


Table 2: Life history characteristics of study species. The four-letter species code is created by combining the first two letters of the genus name with the first two letters of the species name.

Species Code	Species Name	Common Name	Geographic Range	Habitat	Diet	IUCN Status
CHRE	*Chinemys reevesi*	Reeve's turtle	Central/Eastern China (native), Japan and Korea (introduced)	large, slow-moving rivers	invertebrates, algae, aquatic plants	endangered
CUAM	*Cuora amboinensis*	Malayan box turtle	Bangladesh, India, Cambodia, Malaysia, Indonesia, Philippines, Thailand, Myanmar	swamps, rice paddies, small bodies of slow or stagnant water	aquatic plants, mollusks, small crustaceans, mushrooms, earthworms, plants	endangered
GEEL	*Geochelone elegans*	Indian star tortoise	India, Pakistan, Sri Lanka	arid areas with abundant plants, prairies	fruits, vegetables, dead leaves, spiny vegetation	least concern
GESP	*Geoemyda spengleri*	Black-breasted leaf turtle	Hainan Island, southern China, Vietnam	wooded, mountainous areas near wetlands and creeks	insects, earthworms, small invertebrates, fruit	endangered

HESP	*Heosemys spinosa*	Spiny turtle	southern Myanmar, Thailand, peninsular Malaysia, Indonesia, Singapore, Philippines	humid, montane forests	plants, vegetative debris, fallen fruit, insects, earthworms, carrion	endangered
LEYU	*Leucocephalon yuwonoi*	Sulawesi forest turtle	Sulawesi (Indonesia)	semiaquatic; rocky, heavily vegetated areas near swamps and streams	fruits and leaves	critically endangered
PECA	*Pelochelys cantorii*	Cantor's giant softshell turtle	southern India, Bangladesh, Thailand, Myanmar, Cambodia, Vietnam, Laos, China, Borneo, eastern Sumatra, northern Java, Luzon (Phillipines)	estuaries, river deltas, freshwater streams, deep, slow-moving rivers	fish, crustaceans, mollusks,	endangered
PESI	*Pelodiscus sinensis*	Chinese softshell turtle	Eastern and central China, Taiwan (native), Japan, Thailand, Hawaii (introduced)	slow-moving rivers, canals, lakes, swamps, rice paddies	fish, crustaceans, mollusks, insects, worms, various larvae, leaves, seeds	vulnerable

PYMO	*Pyxidea mouhotii*	Keeled box turtle	Hainan Island, China, Vietnam, Thailand, Myanmar, India, Bangladesh, peninsular Malaysia, Laos, Cambodia	humid forested areas, foothills, rocky slopes	earthworms, mollusks, snails, fruit	endangered
SICR	*Siebenrockiella crassicollis*	Black mud turtle	Thailand, Vietnam, Java, Sumatra, Borneo, peninsular Malayasia, Myanmar, Singapore	marshes, ponds, slow-moving rivers, shallow warm waters	fruits, leaves, shrimp, amphibians, mollusks, fish	endangered
CHPA	*Chelodina parkeri*	Parker's snake-necked turtle	southern Papua New Guinea near Irian Jaya border	large lakes and rivers, temporary wetlands, estaries	fish, shrimp	vulnerable
KIFL	*Kinosternon flavescens*	Yellow mud turtle	Central and southwestern United States, northern Mexico	slow-moving rivers, swamps, and marshes	insects, crustaceans, mollusks, amphibian, carrion, aquatic inverts	least concern

MATE	*Malaclemys terrapin*	Diamondback terrapin	Atlantic and Gulf coasts of United States	tidal creeks, salt marshes, estuaries	snails, crabs, shrimp, mollusks, small fish, aquatic plants	least concern
RHPM	*Rhinoclemmys pulcherrima manni*	Central Amercian wood turtle	southern Nicaragua, northern Costa Rica	humid forested areas near streams	vegetables, fruit, earthworms, insects, snails, slugs.	least concern

Table 3: Monthly consumption limits for Hg-contaminated tissue. Modified from EPA, 2001

meals/month	**tissue concentrations (ppb Hg, wet weight)**
none	>1900
0.5	>940-1900
1	>470-940
2	>310-470
3	>230-310
4	>120-230
>4	0-120

RESULTS

Mercury

Tissue distribution:

Figure 2 shows the pattern of tissue Hg distribution for each species. Tissue distribution of Hg followed a general pattern in which liver Hg was the highest, followed by kidney and scutes. Muscle and follicles were generally lowest in Hg. Exceptions to this general pattern include *K. flavescens*, for which most scute samples were similar to or higher than liver samples in Hg; and *G. spengleri*, for which scute Hg greatly exceeded liver Hg. Kidney samples varied greatly in Hg concentration relative to other tissues. *Chelodina reevesi* and *L. yuwonoi* both had greater amounts of Hg in follicles than in kidney samples. *Malaclemys terrapin* and *C. reevesi* had scute Hg levels exceeding kidney Hg.

Differences in Hg among species:

Table 4 lists the maximum, minimum, average, and median Hg concentrations of each tissue for each species. Mercury concentrations in most tissues differed significantly among species. For kidney, a Kruskal-Wallis test indicated a marginally significant difference among species, but ANOVA indicated no such difference. As stated previously, kidney was the most variable tissue in terms of Hg content.

Four species (*P. sinensis*, *L. yuwonoi*, *G. elegans*, and *R. pulcherrima manni*) had the lowest range of Hg concentrations in the study, with most tissues <1000 ppb Hg. One *G. elegans* liver sample had 1912 ppb Hg and one *R. pulcherrima manni* liver had 21234 ppb Hg. Five species (*C. parkeri*, *C. amboinensis*, *G. spengleri*, *H. spinosa*, and *P. cantorii*), had relatively moderate Hg levels, with all tissues <5000 ppb. For the five remaining species (*C.*

reevesi, *K. flavescens*, *M. terrapin*, *P. mohoutii*, and *S. crassicollis*), most tissues were <5000 ppb as well, but some tissue samples exceeded this value.

Differences in Hg among tissues:

In most species, there were significant differences in Hg among tissue types. Exceptions were *C. reevesi* and *G. elegans*, where the Kruskal-Wallis tests indicated significant differences among tissues while ANOVA did not. No significant difference in Hg was found between tissues for *P. mouhotii*, *C. parkeri*, or *R. pulcherrima manni*.

Correlations among tissue Hg concentrations:

For *P. mouhotii*, claw Hg was strongly correlated with Hg in pectoral muscle (n=6, r_s=0.94286, p=0.0048) and scutes (n=6, r_s=0.94286, p=0.0048). Scute Hg was also correlated with pectoral muscle Hg (n=6, r_s=1, p<0.0001). In *C. amboinensis*, liver Hg and kidney Hg were highly correlated (n=7, r_s=0.89286, p=0.0068).

Range of Hg values:

Species differed greatly in the range of Hg tissue concentrations among individuals (Figure 2). Three species, *G. spengleri*, *G. elegans*, and *L. yuwonoi*, displayed the lowest amount of variation between individuals in all tissues. *G. spengleri* had low variability in all tissue types except for scutes. Although the range in scute Hg for this species was large, the minimum scute Hg concentration was 1073 ppb, by far the highest minimum scute Hg concentration of any species analyzed in this study. In fact, this minimum value was greater than the maximum scute Hg measurement of all but four species (*C. reevesi*, *K. flavescens*, *H. spinosa*, and *P. mohoutii*). For some species, the large range of Hg values was due to one individual with Hg concentrations that were substantially higher than in its conspecifics. *G. elegans* specimens displayed low variability in Hg concentrations in all tissues except liver, which was heavily influenced by a single specimen. One turtle had a liver Hg concentration of

1912 ppb, while the average liver Hg of this species was 134 ppb when this individual was excluded. This was also true for *L. yuwonoi*, where one individual had much higher Hg in its liver than others sampled from this species. For *C. amboinensis*, two individuals had substantially higher Hg in all tissues (except scute and claw) than the other six turtles in this species.

In the remaining species, ranges of Hg values were relatively large for most tissues sampled. However, in four species (*H. spinosa*, *K. flavescens*, *R. pulcherrima manni*, and *S. crassicollis*), there was relatively little variability in Hg concentrations in muscle tissue.

Relationship of Hg to body size:

S. crassicollis and *M. terrapin* were the only species for which tissue Hg was significantly related to body size (mass or standard carapace length [SCL]). For *S. crassicollis*, kidney Hg (n=6, r_s= -0.81168, p=0.0499) and scute Hg (n=6, r_s=0.81168, p=0.0499) were both significantly correlated with SCL. Liver Hg (n=6, r_s= -0.82857, p=0.0416) was significantly negatively correlated with mass. For *M. terrapin*, leg muscle Hg (n=5, r_s=0.9, p=0.0374) was significantly correlated with mass.

Differences in tissue Hg among diet groups:

To examine the influence diet has on tissue Hg, species were divided into three groups: herbivores (*G. elegans*, *H. spinosa*, and *L. yuwonoi*), omnivores (*R. pulcherrima manni*, *S. crassicollis*, *C. reevesi*, *C. amboinensis*, *G. spengleri*, and *P. mohoutii*), and carnivores (*M. terrapin*, *K. flavescens*, *P. sinensis*, *P. cantorii*, and *C. parkeri*). Figure 3 shows the median Hg values of each tissue type for each diet group. Diet groups differed significantly in Hg for all tissues except kidney and liver (claw: n=68, χ^2=36.053, df=2, p<0.0001; follicle: n=30, χ^2=6.2418, df=2, p=0.0441, leg muscle: n=64, χ^2=30.1427, df=2, p<0.0001, pectoral muscle: n=59, χ^2=26.8445, df=2, p<0.0001, scute: n=58, χ^2=13.1058, df=2, p=0.0014). As shown in

figure 3, the general pattern of Hg in carnivores>Hg in omnivores>Hg in herbivores was displayed in liver and kidney samples, even though diet groups did not differ significantly in Hg for these two tissue types.

Differences in tissue Hg between sexes:

We tested for differences between sexes by pooling all tissues within each species. *P. mouhotii* was the only species for which tissue Hg differed between sexes; males had significantly less Hg than females (n=35, χ^2=7.5044, p=0.0062).

Stable Isotopes

There was no significant correlation between δ^{15}N values and Hg when all species were pooled. There was, however, a strong positive correlation between δ^{13}C and δ^{15}N values for each tissue type when all species were pooled (claw: n=57, r_s=0.76763, p<0.0001; follicle: n=27, r_s=0.6854, p<0.0001; kidney: n=38, 0.72404, p<0.0001; leg muscle: n=60, r_s=0.70036, p<0.0001; liver: n=56, r_s=0.75783, p<0.0001; pectoral muscle: n=54, r_s=0.82159, p<0.0001; scute: n=55, r_s=0.58624, p<0.0001). This relationship is displayed in Figure 4. There was also a strong positive correlation between δ^{15}N and δ^{13}C values for four species when all tissues were pooled (*C. reevesi*: n=22, r_s=0.69735, p=0.0003; *H. spinosa*: n=40, r_s=0.75474, p<0.0001; *L. yuwonoi*: n=37, r_s=0.76268, p<0.0001; *M. terrapin*: n=24, r_s=0.6687, p=0.0004). This relationship is displayed in Figure 5.

For each species, δ^{13}C and δ^{15}N values were plotted by tissue type (liver, kidney, pectoral muscle, leg muscle, claw, scute, and follicle) for each individual in figures 6 and 7, respectively. Species differed greatly in both the variance in δ^{13}C and δ^{15}N values among individuals of a species and the variance in values among tissues in an individual. Some species, such as *G. spengleri*, had relatively small ranges of δ^{13}C and δ^{15}N values among individuals, while others, such as *S. crassicollis*, had larger ranges. For some species, such as *C. amboinenis*, δ^{13}C and

$\delta^{15}N$ values differed little between tissue types. Other species, such as *P. sinensis*, had larger differences in $\delta^{13}C$ and $\delta^{15}N$ values between tissues.

Figures 8 and 9 show the ranges of $\delta^{13}C$ and $\delta^{15}N$ values, respectively, measured in liver samples of each species. Values for liver samples were used because, due to its high metabolic rate, the stable isotope composition of the liver closely resembles that of the diet (Tieszen *et al.*, 1983). Species are grouped into categories based on habitat type in Figure 8. Stable carbon isotope values for aquatic species ranged from -17 to -30‰, while values for terrestrial species defined a slightly smaller range, from -21 to -27‰. In Figure 9, species are grouped into categories based on habitat and diet. Stable nitrogen isotope values ranged from 8 to 12‰ for aquatic carnivores, 7.7-10.3‰ for terrestrial omnivores, 5.6-10.3‰ for aquatic omnivores, and 6.5-9.5‰ for terrestrial herbivores.

Comparison of Hg Levels to Risk-Based Consumption Limits

All edible samples (liver, kidney, and muscle) were divided into seven categories based on the CR_{mm}: do not eat, 0.5 meals/month, 1 meal/month, 2 meals/month, 3 meals/month, 4 meals/month, and >4 meals/month. Figure 10 shows the proportion of samples in each consumption category for each tissue type.

Of all edible tissues, Hg was highest in liver for all species, followed by kidney and muscle. Nine of 62 (14%) liver samples analyzed exceeded the 1900 ppb consumption threshold recommended for all adults, including those of *C. reevesi*, *S. crassicollis*, *M. terrapin*, *H. spinosa*, and *P. mohoutii*. Twenty-one of 62 (34%) liver samples had Hg levels within or above the 470-940 ppb range recommended for one meal per month. The highest Hg concentrations measured in this study were in livers from a single *H. spinosa* specimen (4,768 ppb), a *R. pulcherrima manni* specimen (5,017 ppb), and two *C. reevesi* specimens (7,443 ppb and 16,561 ppb).

Nine of 42 (10%) kidney samples had levels of Hg above the range recommended for one meal per month. These were samples collected from an individual *H. spinosa*, a *C. parkeri* specimen, and two *P. mohoutii* individuals. One hundred nineteen of 125 (95%) muscle samples had Hg concentrations below the level recommended for four meals per month. Only 6 of 125 (5%) of muscle samples contained enough Hg to require consumption limitations, including muscles from *C. parkeri*, *C. reevesi*, *P. cantorii*, and *P. mohoutii*.

Figure 11 shows the proportion of samples in each consumption category for each species. For many species that are popular in the food trade, a considerable proportion of edible samples were within ranges recommended for limited consumption. Three of 18 (17%) *M. terrapin* samples had Hg levels within or above the range recommended for no more than one meal per month, and one liver sample was above the 1900 ppb consumption threshold. Four of 14 (28%) *C. reevesi* samples were within or above the range of Hg concentrations recommended for no more than one meal per month, and two liver samples were above the 1900 ppb consumption threshold. Three of 19 (15%) of *S. crassicollis* samples had Hg levels within or above the range recommended for no more than one meal every two months, and two liver samples were above the 1900 ppb consumption threshold. Two of eight (25%) of *P. cantorii* samples were within the range of Hg concentrations recommended for no more than one meal every two months. Three of seven (43%) of *C. parkeri* samples had Hg levels within or above the range recommended for no more than one meal per month.

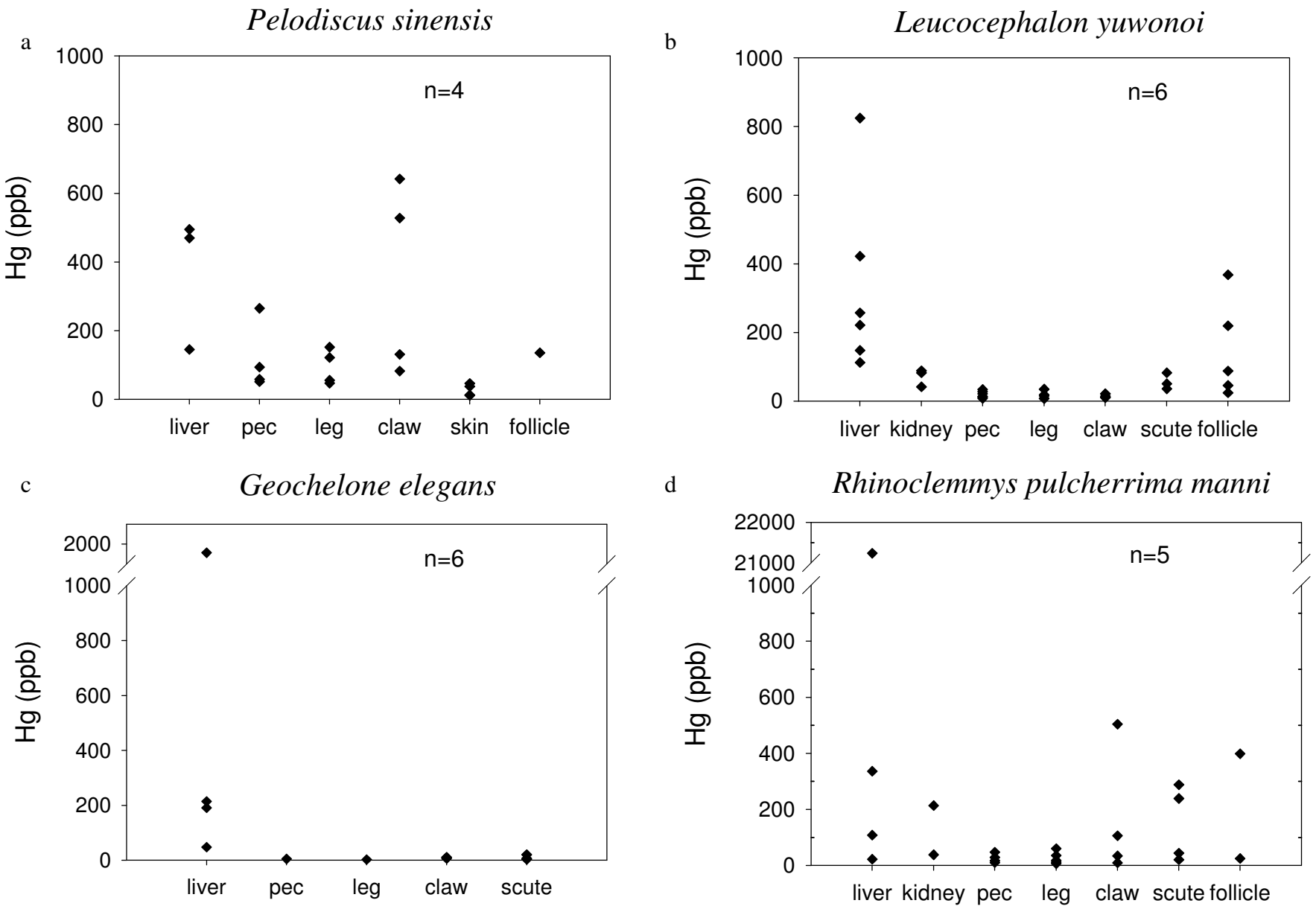
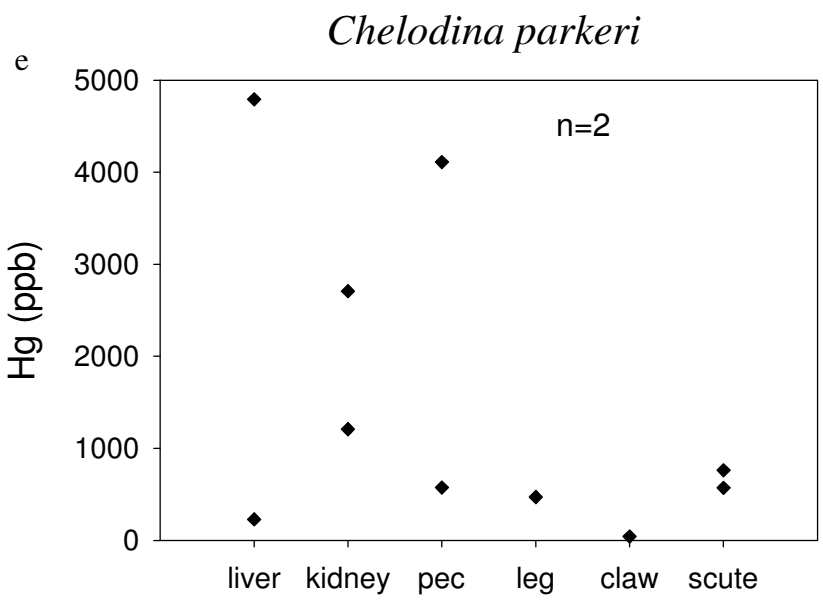
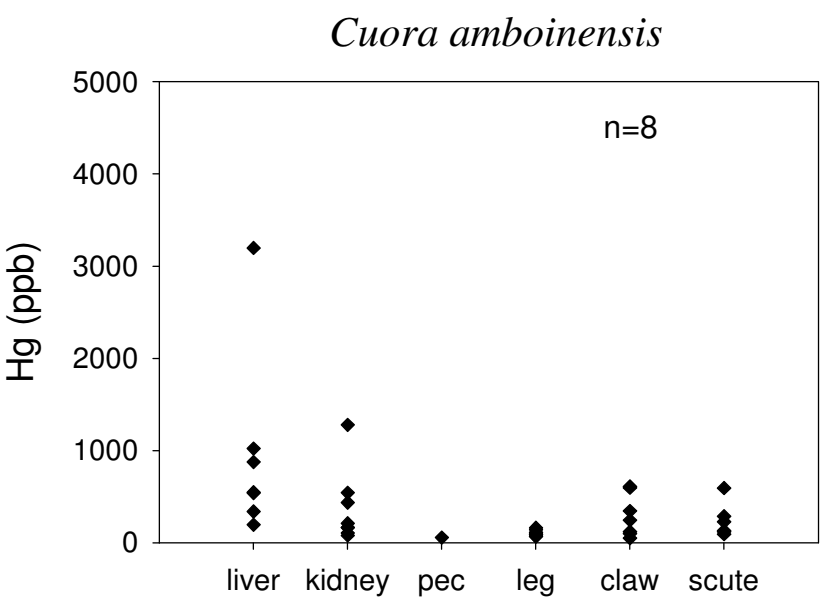


Figure 2: Tissue mercury distribution. Each graph represents one species, and each data point represents one tissue sample. (a): *Pelodiscus sinensis*; (b): *Leucocephalon yuwonoi*; (c): *Geochelone elegans*; (d): *Rhinoclemmys pulcherrima manni*; (e): *Chelodina parkeri*; (f): *Cuora amboinensis*; (g): *Geoemyda spengleri*; (h): *Heosemys spinosa*; (i): *Pelochelys cantorii*; (j): *Chinemys reevesi*; (k): *Kinosternon flavescens*; (l): *Malaclemys terrapin*; (m): *Pyxidea mohoutii*; (n): *Siebenrockiella crassicollis*.

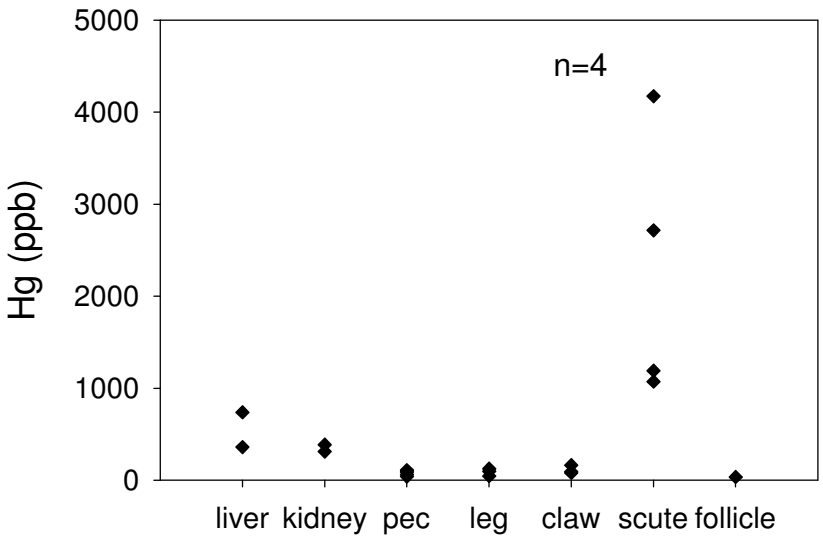
e


Chelodina parkeri
n=2
Hg (ppb)
5000
4000
3000
2000
1000
0
liver
kidney
pec
leg
claw
scute

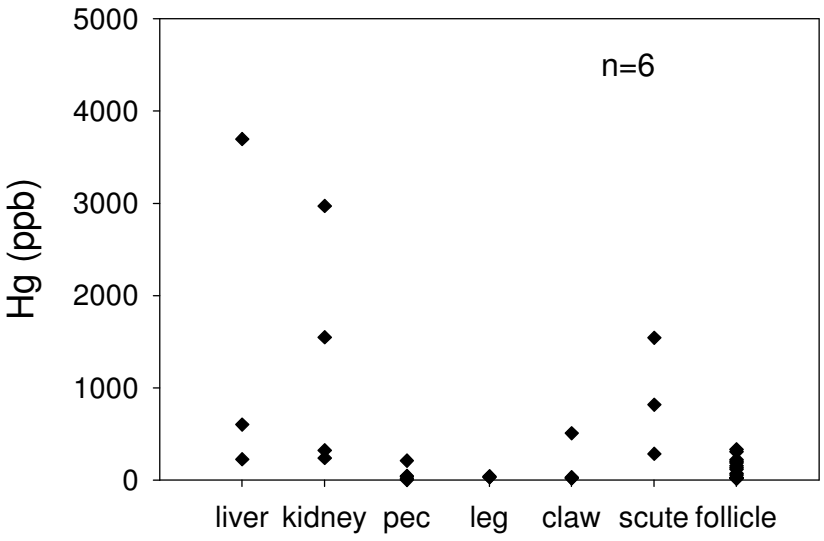
f


Cuora amboinensis
n=8
Hg (ppb)
5000
4000
3000
2000
1000
0
liver
kidney
pec
leg
claw
scute

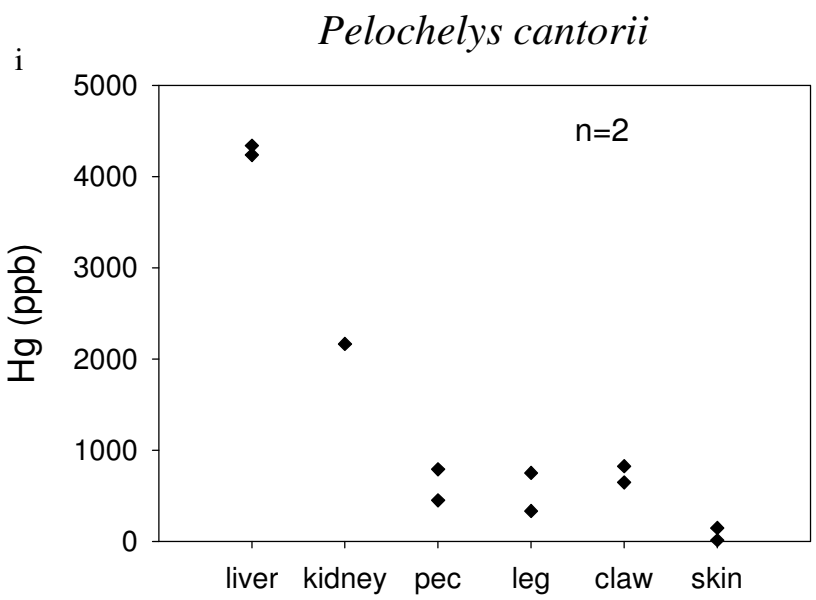
g


Geoemyda spengleri
n=4
Hg (ppb)
5000
4000
3000
2000
1000
0
liver
kidney
pec
leg
claw
scute
follicle

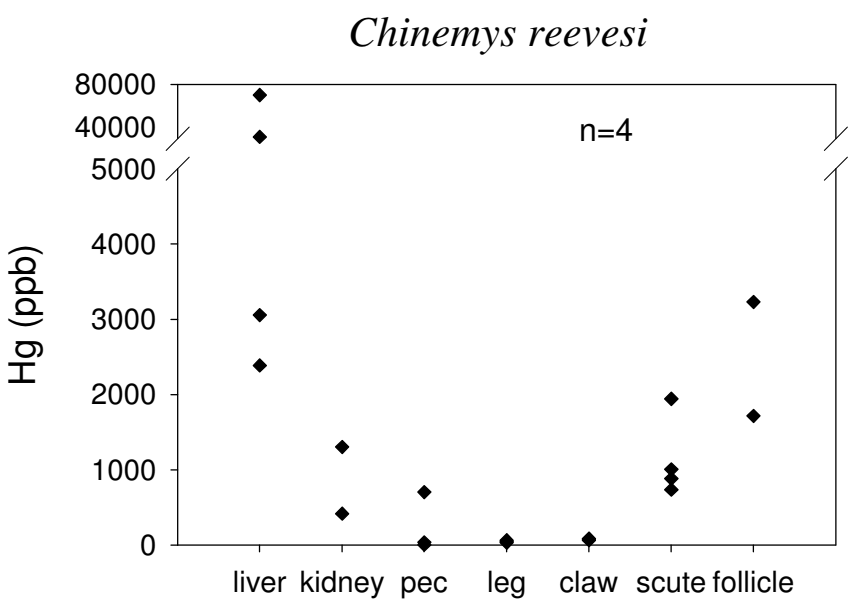
h


Heosemys spinosa
n=6
Hg (ppb)
5000
4000
3000
2000
1000
0
liver
kidney
pec
leg
claw
scute
follicle

i


Pelochelys cantorii
n=2
Hg (ppb)
5000
4000
3000
2000
1000
0
liver
kidney
pec
leg
claw
skin

j


Chinemys reevesi
n=4
Hg (ppb)
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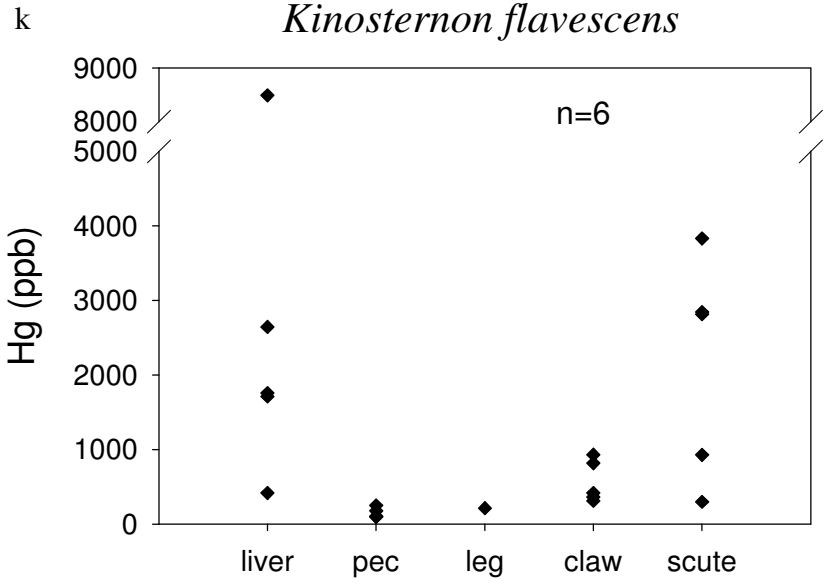
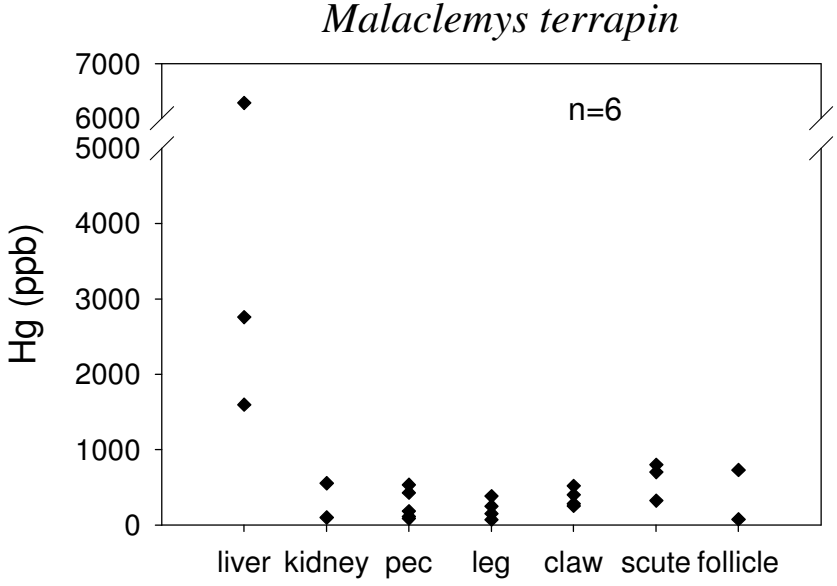
40000
5000
4000
3000
2000
1000
0
liver
kidney
pec
leg
claw
scute
follicle

k


Kinosternon flavescens
n=6
Hg (ppb)
9000
8000
5000
4000
3000
2000
1000
0
liver
pec
leg
claw
scute

l


Malaclemys terrapin
n=6
Hg (ppb)
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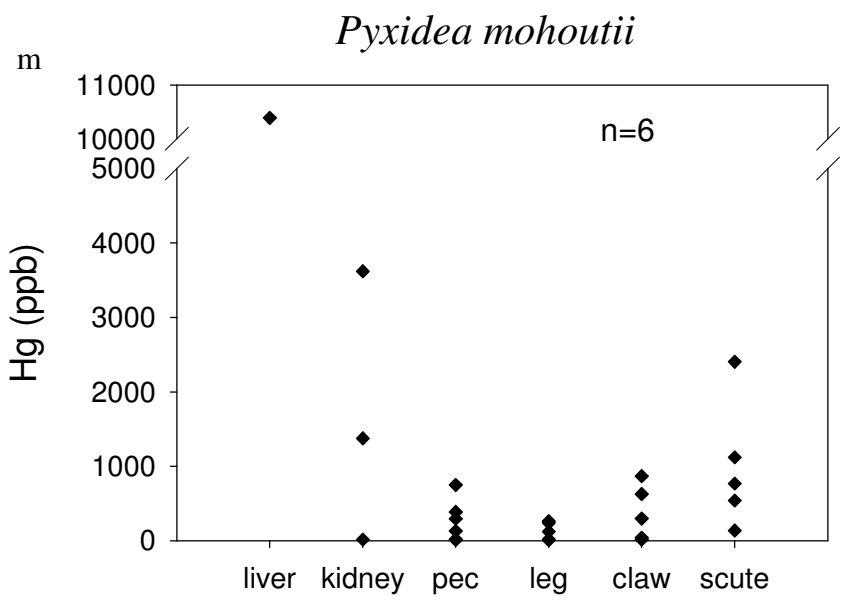
6000
5000
4000
3000
2000
1000
0
liver
kidney
pec
leg
claw
scute
follicle

m


Pyxidea mohoutii
11000
10000
5000
4000
3000
2000
1000
0
Hg (ppb)
n=6
liver
kidney
pec
leg
claw
scute

n

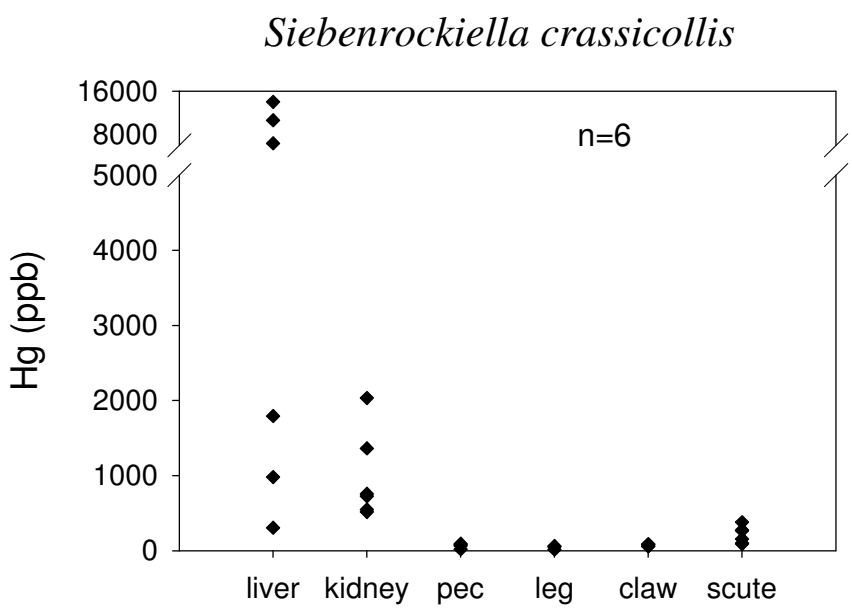

Siebenrockiella crassicollis
16000
8000
5000
4000
3000
2000
1000
0
Hg (ppb)
n=6
liver
kidney
pec
leg
claw
scute

Table 4: Summary of Hg data. (a): *Chinemys reevesi*, (b): *Cuora amboinensis*, (c): *Geochelone elegans*, (d): *Geoemyda spengleri*, (e): *Heosemys spinosa*, (f): *Leucocephalon yuwonoi*, (g): *Pelochelys cantori*, (h): *Pelodiscus sinensis*, (i): *Pyxidea mohoutii*, (j): *Siebenrockiella crasicollis*, (k): *Chelodina parkeri*, (l): *Kinosternon flavescens*, (m): *Malaclemys terrapin*, (n): *Rhinoclemmys pulcherrima manni*.

a *Chinemys reevesi*

tissue	n	max	min	median Hg (ppb)	average Hg (ppb)	standard deviation
liver	4	70094.88	2387.85	17279.78	26760.57	31918.15
kidney	2	1301.71	418.05	859.88	859.88	624.84
leg	4	514.63	34.74	55.47	165.07	233.38
pec	4	703.19	6.99	43.24	199.17	336.48
scute	4	1941.80	737.12	944.45	1141.96	544.33
claw	4	86.99	57.67	71.96	72.14	13.60
follicle	2	3229.98	1715.84	2472.91	2472.91	1070.66

b *Cuora amboinensis*

tissue	n	max	min	median Hg (ppb)	average Hg (ppb)	standard deviation
liver	7	3196.47	194.85	548.22	959.39	1027.15
kidney	8	1279.67	77.89	186.75	372.01	401.66
leg	8	162.19	65.82	97.87	104.32	33.98
pec	1	--	--	55.49	55.49	--
scute	6	591.96	93.72	178.40	241.35	186.53
claw	7	610.51	49.40	245.13	295.07	232.53

c *Geochelone elegans*

tissue	n	max	min	median Hg (ppb)	average Hg (ppb)	standard deviation
liver	5	1911.97	47.35	190.36	489.67	798.18
kidney	0	--	--	--	--	--
leg	4	13.36	2.44	7.54	7.72	5.19
pec	5	19.12	4.24	7.46	9.55	5.69
scute	6	20.71	3.2	5.60	9.77	8.31
claw	6	10.5	5.75	7.93	8.10	1.95

d *Geoemyda spengleri*

tissue	n	max	min	median Hg (ppb)	average Hg (ppb)	standard deviation
liver	2	747.91	361.27	554.59	554.59	273.40
kidney	3	387.93	277.02	312.18	325.71	56.68
leg	4	125.74	46.14	93.94	89.94	32.94
pec	4	110.33	41.29	75.97	75.89	30.57
scute	4	4173.73	1073.1	1953.64	2288.54	1462.62
claw	4	163.92	82.57	97.46	110.35	36.60

e *Heosemys spinosa*

tissue	n	max	min	median Hg (ppb)	average Hg (ppb)	standard deviation
liver	5	20179.92	223.15	3695.46	5837.89	8231.73
kidney	5	2970.38	239.57	1504.18	1316.83	1114.79
leg	6	90.22	14.95	34.55	40.90	25.65
pec	6	210.42	1.78	39.82	66.67	77.05
scute	6	1542.71	68.07	797.72	743.41	522.55
claw	6	507.08	19.84	40.85	115.06	192.73
follicle	16	332.66	15.99	95.35	119.42	105.71

f *Leucocephalon yuwonoi*

tissue	n	max	min	median Hg (ppb)	average Hg (ppb)	standard deviation
liver	6	824.38	112.13	239.07	330.76	264.88
kidney	3	88.50	41.36	82.65	70.84	25.69
leg	6	34.62	7.25	16.74	19.71	12.22
pec	6	33.79	7.57	17.64	19.27	9.99
scute	3	82.37	35.53	50.48	56.13	23.93
claw	6	21.44	10.10	12.84	14.77	5.18
follicle	5	367.64	24.21	87.99	148.95	143.84
egg	2	10.21	8.93	9.57	9.57	0.91

g *Pelochelys cantori*

tissue	n	max	min	median Hg (ppb)	average Hg (ppb)	standard deviation
liver	2	4338.00	2219.82	3278.91	3278.91	1497.78
kidney	0	--	--	--	--	--
leg	2	751.05	333.41	542.23	542.23	295.32
pec	2	789.48	451.21	620.35	620.35	239.19
skin	2	147.15	13.89	80.52	80.52	94.23
claw	2	823.31	648.53	735.92	735.92	123.59

h *Pelodiscus sinensis*

tissue	n	max	min	median Hg (ppb)	average Hg (ppb)	standard deviation
liver	3	494.62	144.60	469.51	369.58	195.24
kidney	0	--	--	--	--	--
leg	4	151.93	46.74	88.30	93.82	51.13
pec	4	264.90	51.20	75.68	116.87	100.41
skin	4	46.00	11.19	25.00	26.80	17.67
claw	4	641.28	82.15	329.22	345.47	280.57
follicle	1	--	--	135.02	135.02	--

i *Pyxidea mohoutii*

tissue	n	max	min	median Hg (ppb)	average Hg (ppb)	standard deviation
liver	4	10384.58	33.31	188.96	2698.95	5124.40
kidney	5	4717.20	15.85	1375.58	1965.00	2116.73
leg	6	729.95	9.94	180.89	230.84	266.83
pec	6	748.37	12.29	213.29	265.19	279.65
scute	6	2403.01	138.30	654.39	856.50	843.28
claw	6	870.47	17.29	351.23	376.26	333.61
follicle	2	3371.11	170.15	1770.63	1770.63	2263.42

j *Siebenrockiella crassicollis*

tissue	n	max	min	median Hg (ppb)	average Hg (ppb)	standard deviation
liver	6	13976.90	306.85	4059.18	5658.93	5643.86
kidney	6	2033.49	516.29	742.20	990.67	595.03
leg	3	59.83	16.47	49.84	42.05	22.71
pec	4	91.85	18.86	73.64	64.49	32.67
scute	6	378.79	88.03	211.66	210.46	115.34
claw	5	84.49	59.39	80.32	73.77	12.83

k *Chelodina parkeri*

tissue	n	max	min	median Hg (ppb)	average Hg (ppb)	standard deviation
liver	2	4793.05	226.48	2509.77	2509.77	3229.05
kidney	2	2708.22	1206.56	1957.39	1957.39	1061.83
leg	1	--	--	468.58	468.58	0.00
pec	2	4108.86	572.63	2340.75	2340.75	2500.49
scute	2	762.54	570.97	666.76	666.76	135.46
claw	2	856.84	41.61	449.23	449.23	576.45

l *Kinosternon flavescens*

tissue	n	max	min	median Hg (ppb)	average Hg (ppb)	standard deviation
liver	6	8489.48	352.26	1734.12	2562.39	3032.80
kidney	0	--	--	--	--	--
leg	6	213.91	92.27	125.44	138.17	46.48
pec	6	250.95	94.63	145.06	155.93	62.01
scute	6	3829.36	300.01	1872.05	1892.91	1452.06
claw	6	930.14	312.47	451.63	554.83	255.95

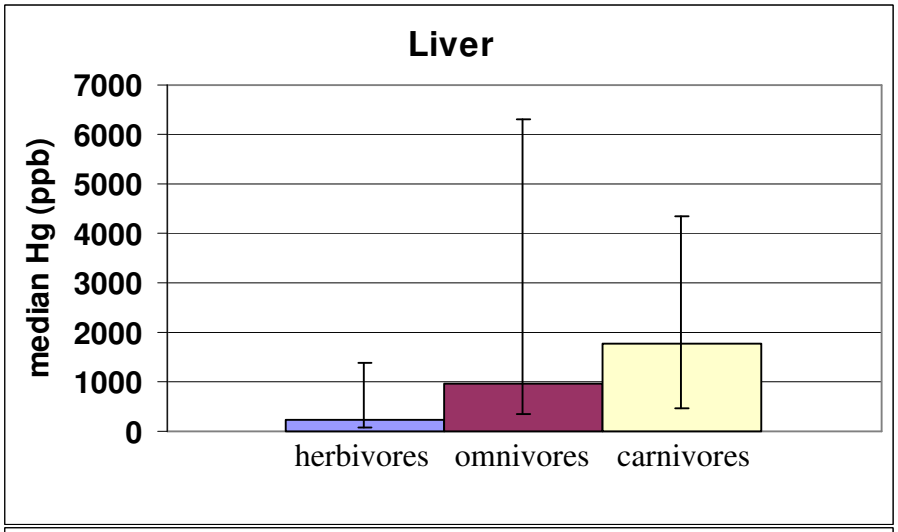
m *Malaclemys terrapin*

tissue	n	max	min	median Hg (ppb)	average Hg (ppb)	standard deviation
liver	4	14645.53	1595.88	4518.18	6319.44	5896.81
kidney	4	919.82	98.42	463.41	486.26	344.30
leg	5	435.23	70.01	251.04	257.72	152.97
pec	5	533.55	84.02	182.76	268.36	200.87
scute	5	1000.65	323.03	699.96	647.46	278.57
claw	5	744.63	255.03	401.97	440.56	199.48
follicle	3	831.09	76.44	728.06	545.20	409.21

n *Rhinoclemmys pulcherrima manni*

tissue	n	max	min	median Hg (ppb)	average Hg (ppb)	standard deviation
liver	4	21233.98	21.76	1047.50	5837.68	10291.98
kidney	3	1481.59	213.37	847.48	847.48	896.77
leg	5	59.94	6.53	18.17	26.42	21.70
pec	4	47.43	10.11	22.77	25.77	16.22
scute	4	288.10	20.46	141.19	147.74	135.53
claw	5	503.56	9.64	34.03	135.11	209.35
follicle	2	20.04	19.12	19.58	19.58	0.65

a



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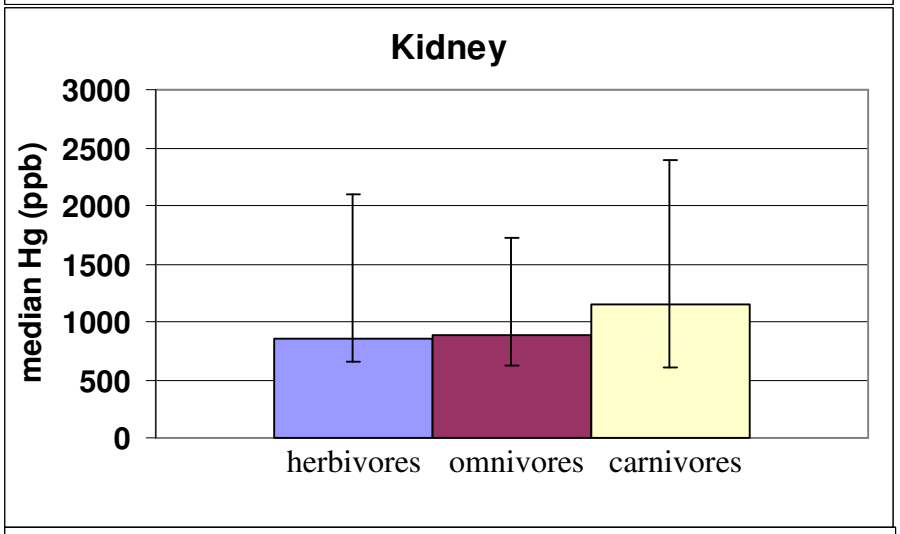

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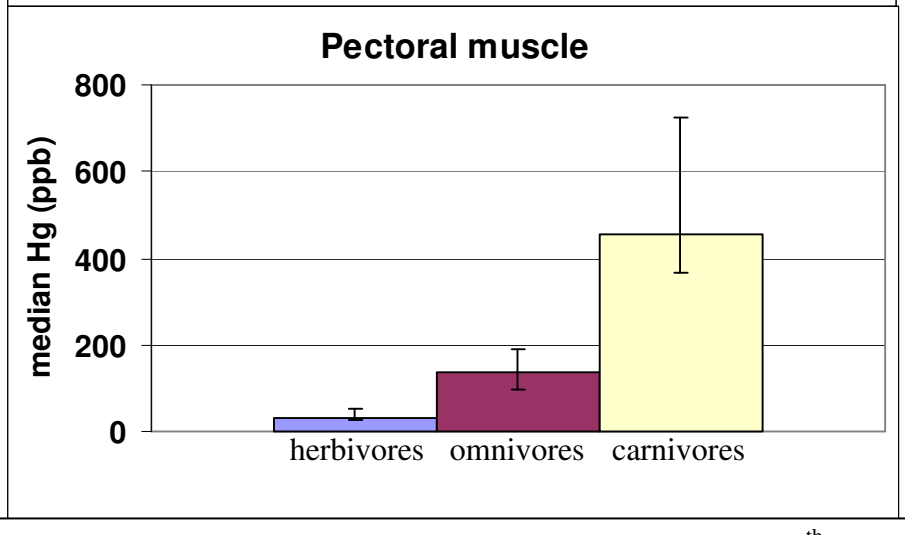
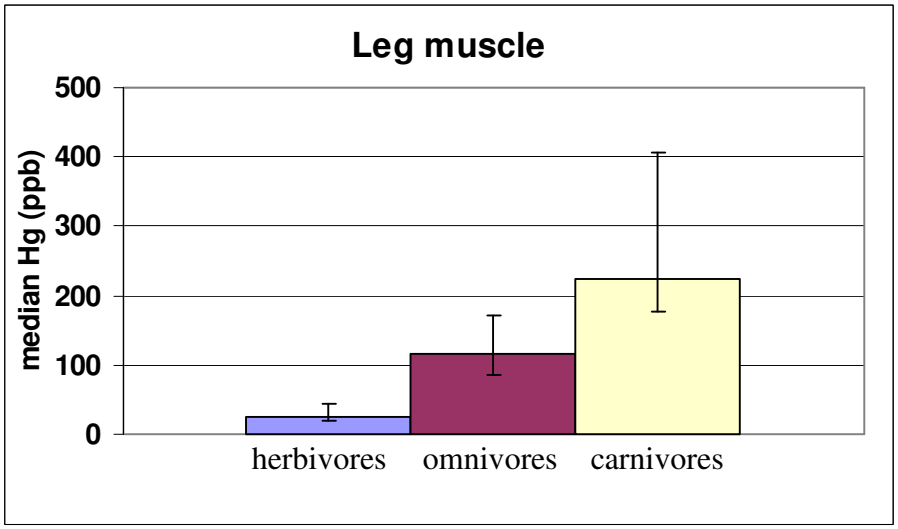
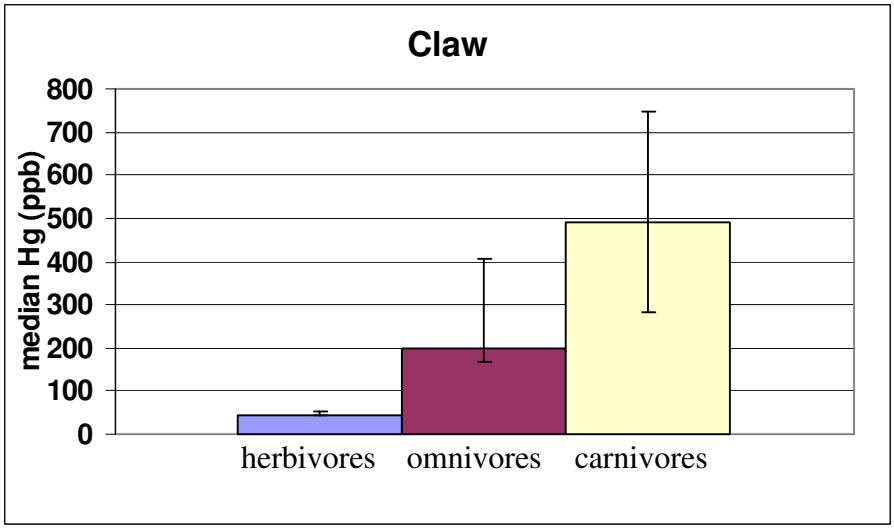


Figure 3: Median Hg concentrations by diet category. Error bars represent the 25th and 75th percentiles. (a): Liver, (b): kidney, (c): pectoral muscle, (d): leg muscle, (e): claw, (f): scute. Herbivores= *Geochelone elegans*, *Heosemys spinosa*, and *Leucocephalon yuwonoi*. Omnivores= *Chinemys reevesi*, *Cuora amboinensis*, *Geoemyda spengleri*, *Pyxidea mohoutii*, *Rhinoclemmys pulcherrima manni*, and *Siebenrockiella crassicollis*. Carnivores= *Chelodina parkeri*, *Kinosternon flavescens*, *Malaclemys terrapin*, *Pelochelys*

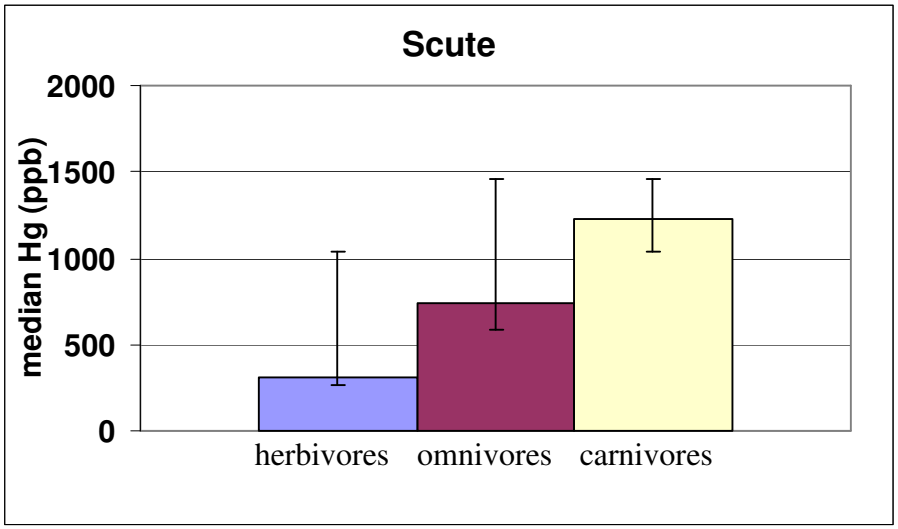
d


Leg muscle
median Hg (ppb)
500
400
300
200
100
0
herbivores
omnivores
carnivores

e


Claw
median Hg (ppb)
800
700
600
500
400
300
200
100
0
herbivores
omnivores
carnivores

f


Scute
median Hg (ppb)
2000
1500
1000
500
0
herbivores
omnivores
carnivores

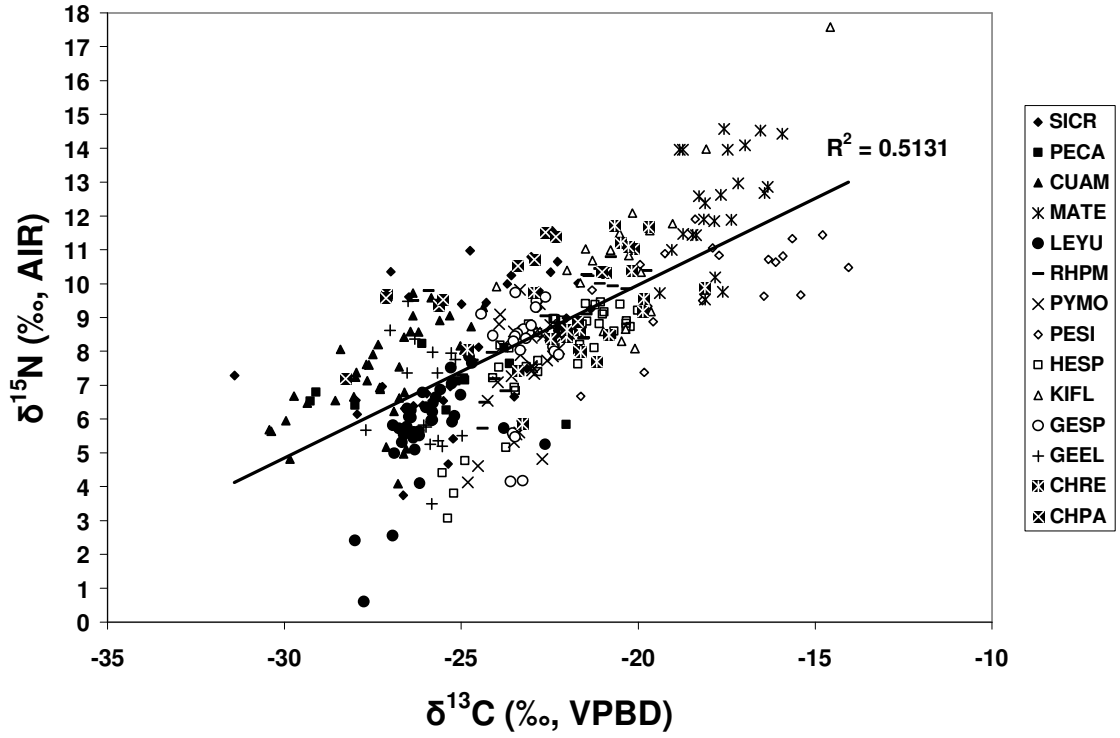


Figure 4: Stable nitrogen isotope values versus stable carbon isotope values by tissue. Each data point represents one tissue sample.

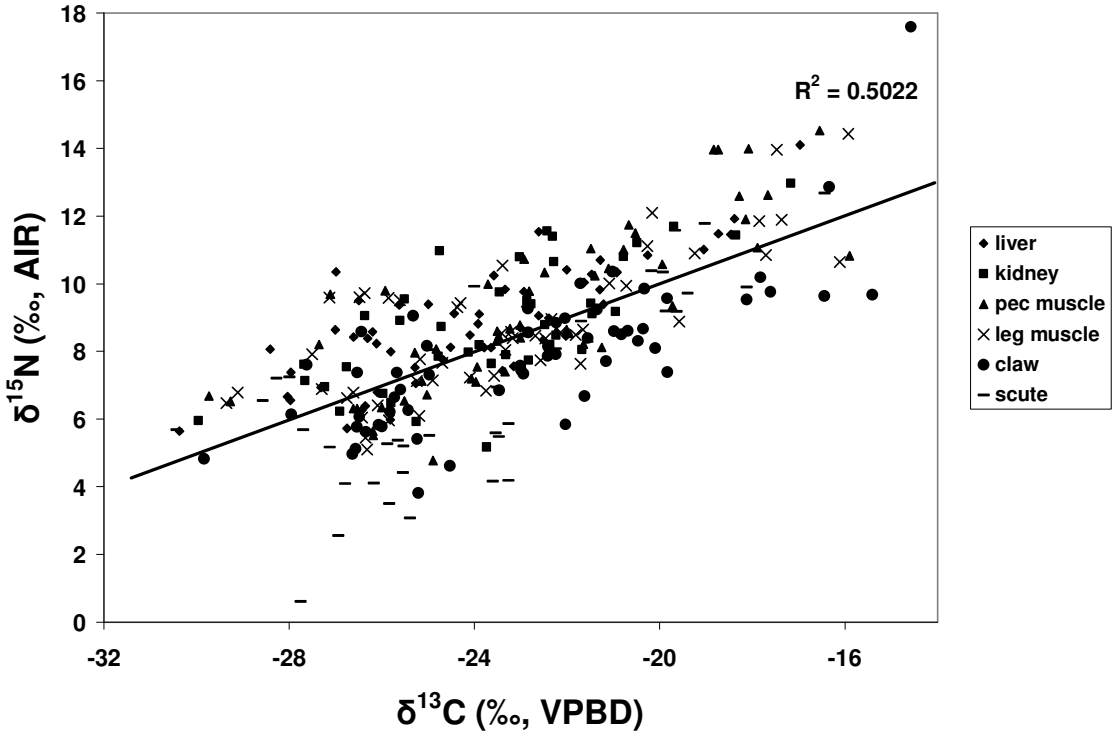


Figure 5: Stable nitrogen isotope values versus stable carbon isotope values by species. Each data point represents one tissue sample. Refer to table 2 for species codes.

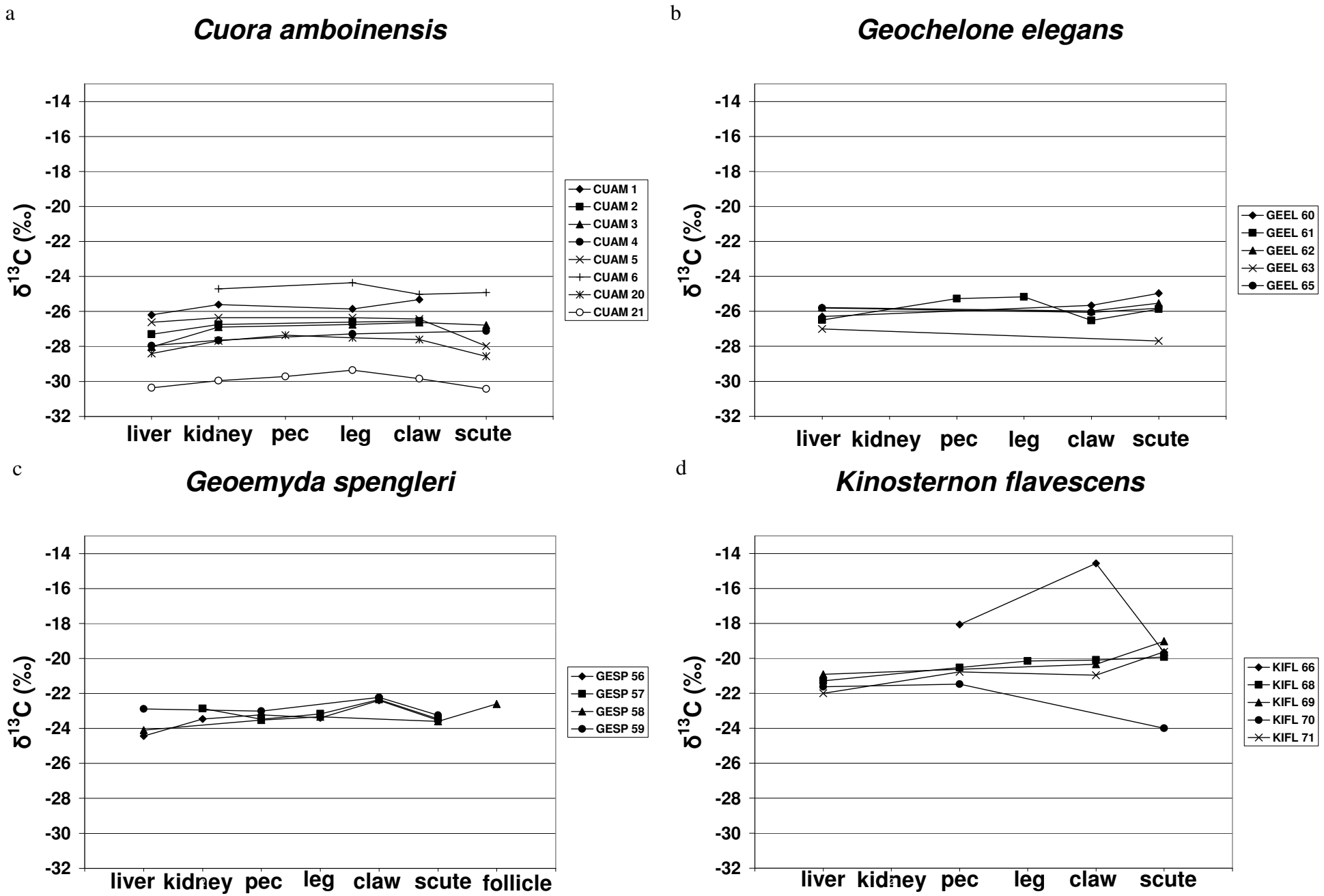
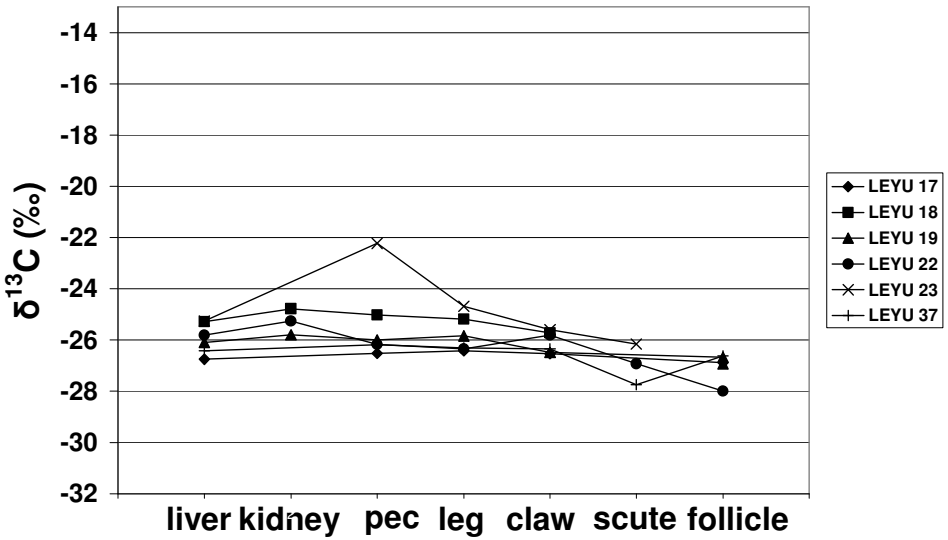
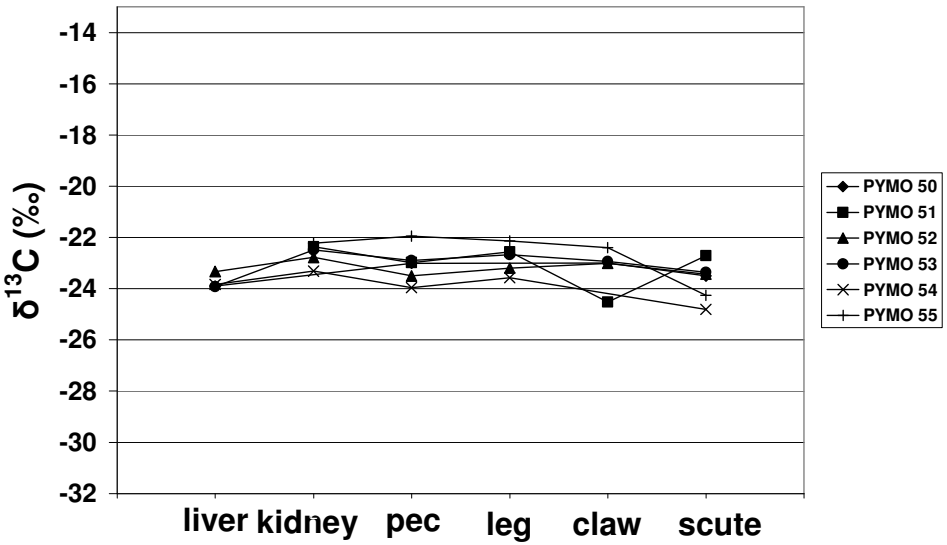


Figure 6: Stable carbon isotope values. Each graph represents one species, and each symbol represents one individual of a species. Lines connecting the points are drawn to demonstrate intra-individual and intra-species variation in stable carbon isotope values, and do not signify a linear relationship. (a): *Cuora amboinensis*; (b): *Geochelone elegans*; (c): *Geoemyda spengleri*; (d): *Kinosternon flavescens*; (e): *Leucocephalon yuwonoi*; (f): *Pyxidea mohoutii*; (g): *Chinemys reevesi*; (h): *Heosemys spinosa*; (i): *Malaclemys terrapin*; (j): *Rhinoclemmys pulcherrima manni*; (k): *Siebenrockiella crassicollis*; (l): *Chelodina parkeri*; (m): *Pelochelys cantorii*; (n): *Pelodiscus sinensis*.

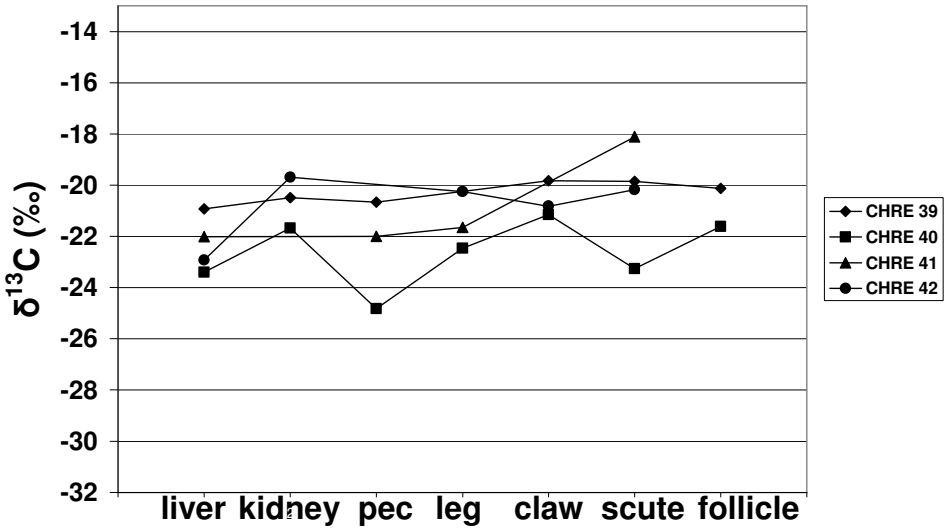
e


Leucocephalon yuwonoi
δ13C (‰)
-14
-16
-18
-20
-22
-24
-26
-28
-30
-32
liver kidney pec leg claw scute follicle
LEYU 17
LEYU 18
LEYU 19
LEYU 22
LEYU 23
LEYU 37

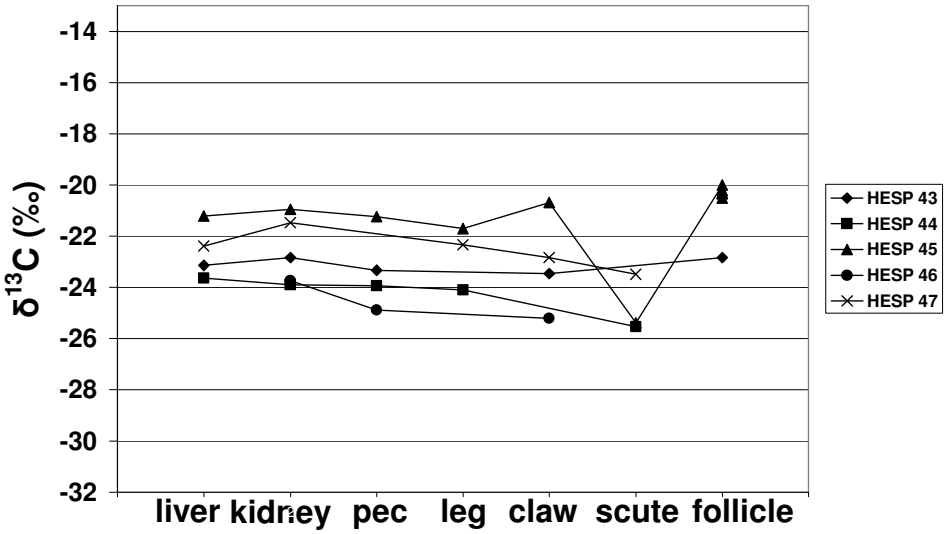
f


Pyxidea mohoutii
δ13C (‰)
-14
-16
-18
-20
-22
-24
-26
-28
-30
-32
liver kidney pec leg claw scute
PYMO 50
PYMO 51
PYMO 52
PYMO 53
PYMO 54
PYMO 55

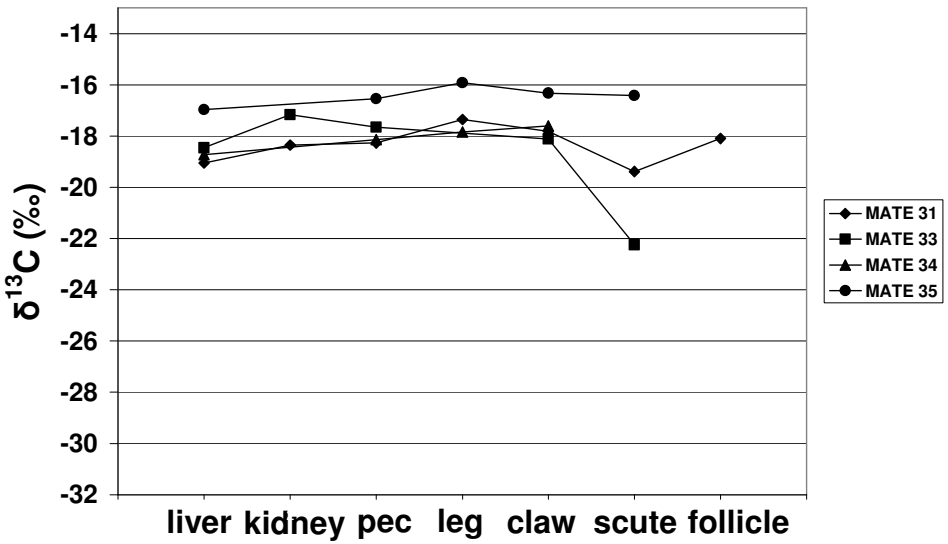
g


Chinemys reevesi
δ13C (‰)
-14
-16
-18
-20
-22
-24
-26
-28
-30
-32
liver kidney pec leg claw scute follicle
CHRE 39
CHRE 40
CHRE 41
CHRE 42

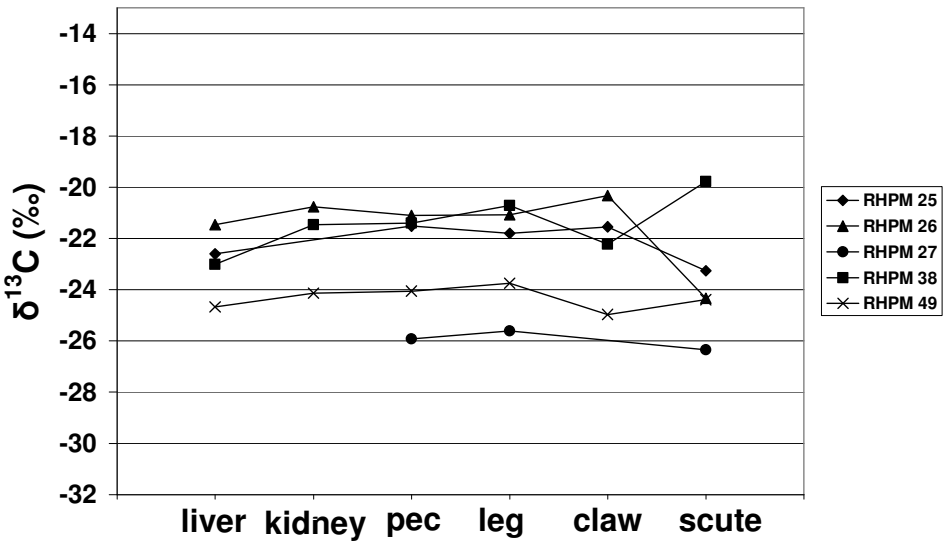
h


Heosemys spinosa
δ13C (‰)
-14
-16
-18
-20
-22
-24
-26
-28
-30
-32
liver kidney pec leg claw scute follicle
HESP 43
HESP 44
HESP 45
HESP 46
HESP 47

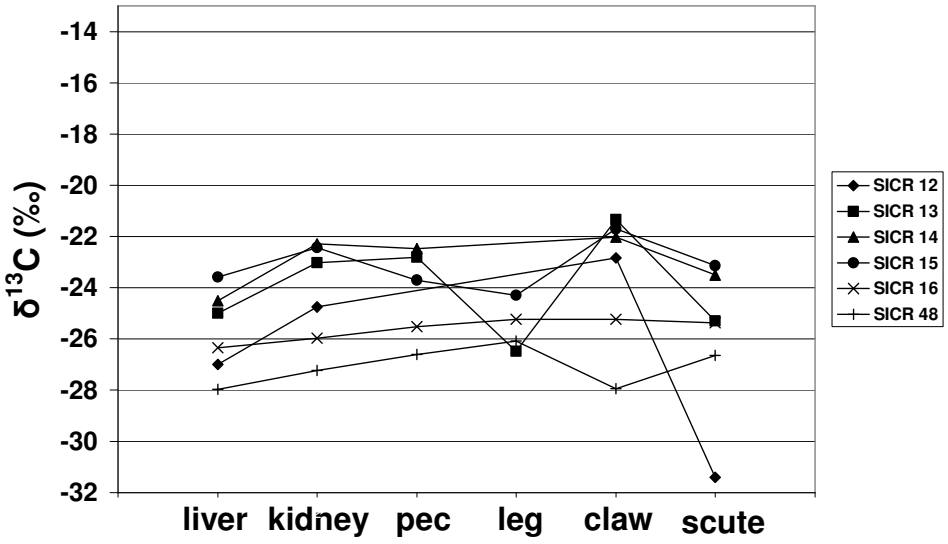
i


Malaclemys terrapin
δ13C (‰)
-14
-16
-18
-20
-22
-24
-26
-28
-30
-32
liver kidney pec leg claw scute follicle
MATE 31
MATE 33
MATE 34
MATE 35

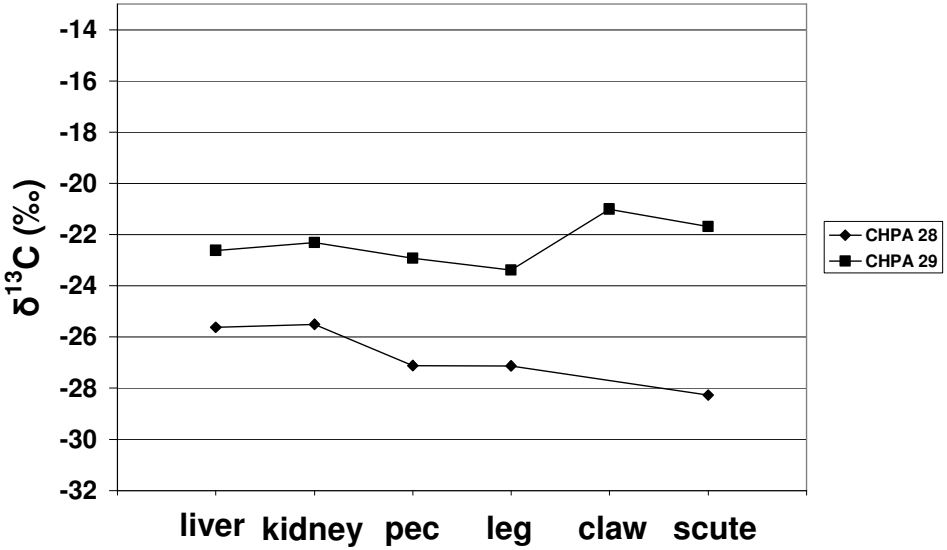
j


Rhinoclemmys pulcherrima manni
δ13C (‰)
-14
-16
-18
-20
-22
-24
-26
-28
-30
-32
liver kidney pec leg claw scute
RHPM 25
RHPM 26
RHPM 27
RHPM 38
RHPM 49

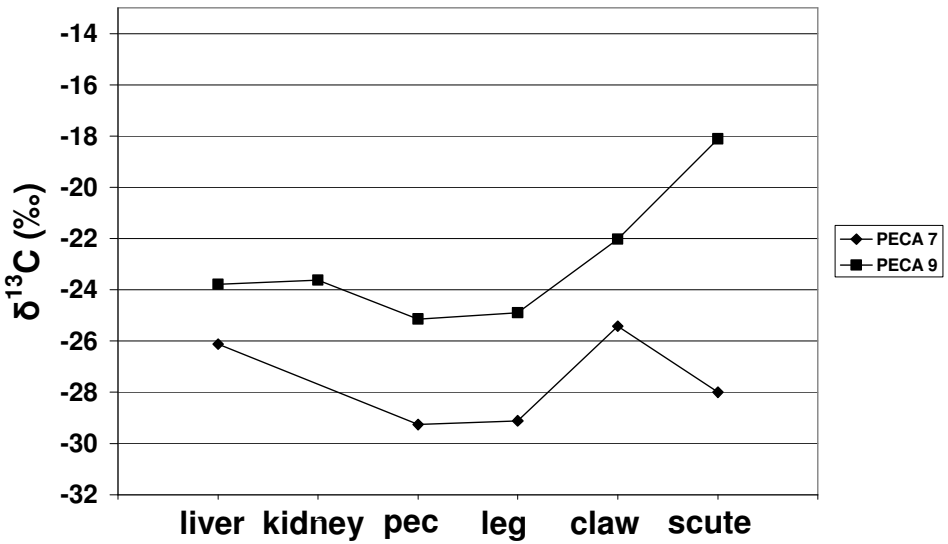
k


Siebenrockiella crassicollis
δ13C (‰)
-14
-16
-18
-20
-22
-24
-26
-28
-30
-32
liver kidney pec leg claw scute
SICR 12
SICR 13
SICR 14
SICR 15
SICR 16
SICR 48

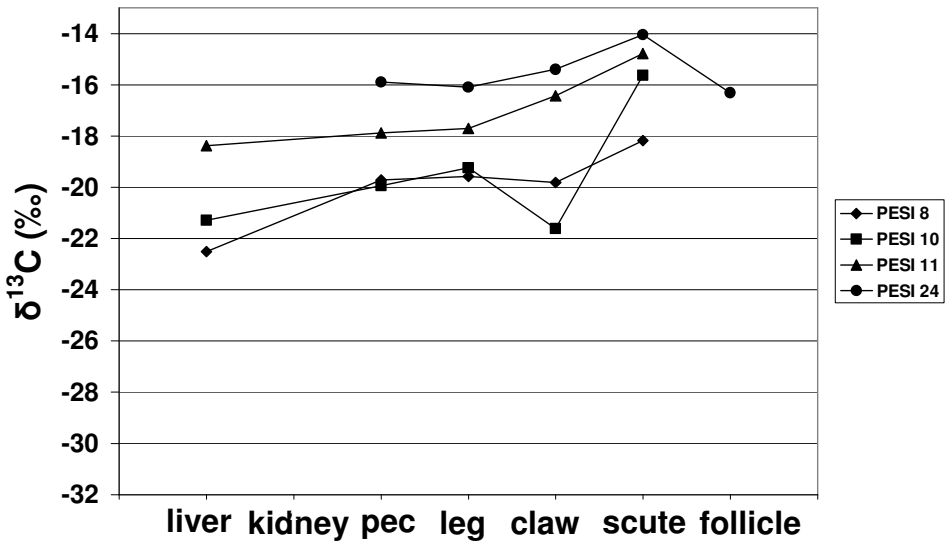
l


Chelodina parkeri
δ13C (‰)
-14
-16
-18
-20
-22
-24
-26
-28
-30
-32
liver kidney pec leg claw scute
CHPA 28
CHPA 29

m


Pelochelys cantorii
-14
-16
-18
-20
-22
-24
-26
-28
-30
-32
δ13C (‰)
liver
kidney
pec
leg
claw
scute
PECA 7
PECA 9

n


Pelodiscus sinensis
-14
-16
-18
-20
-22
-24
-26
-28
-30
-32
δ13C (‰)
liver
kidney
pec
leg
claw
scute
follicle
PESI 8
PESI 10
PESI 11
PESI 24

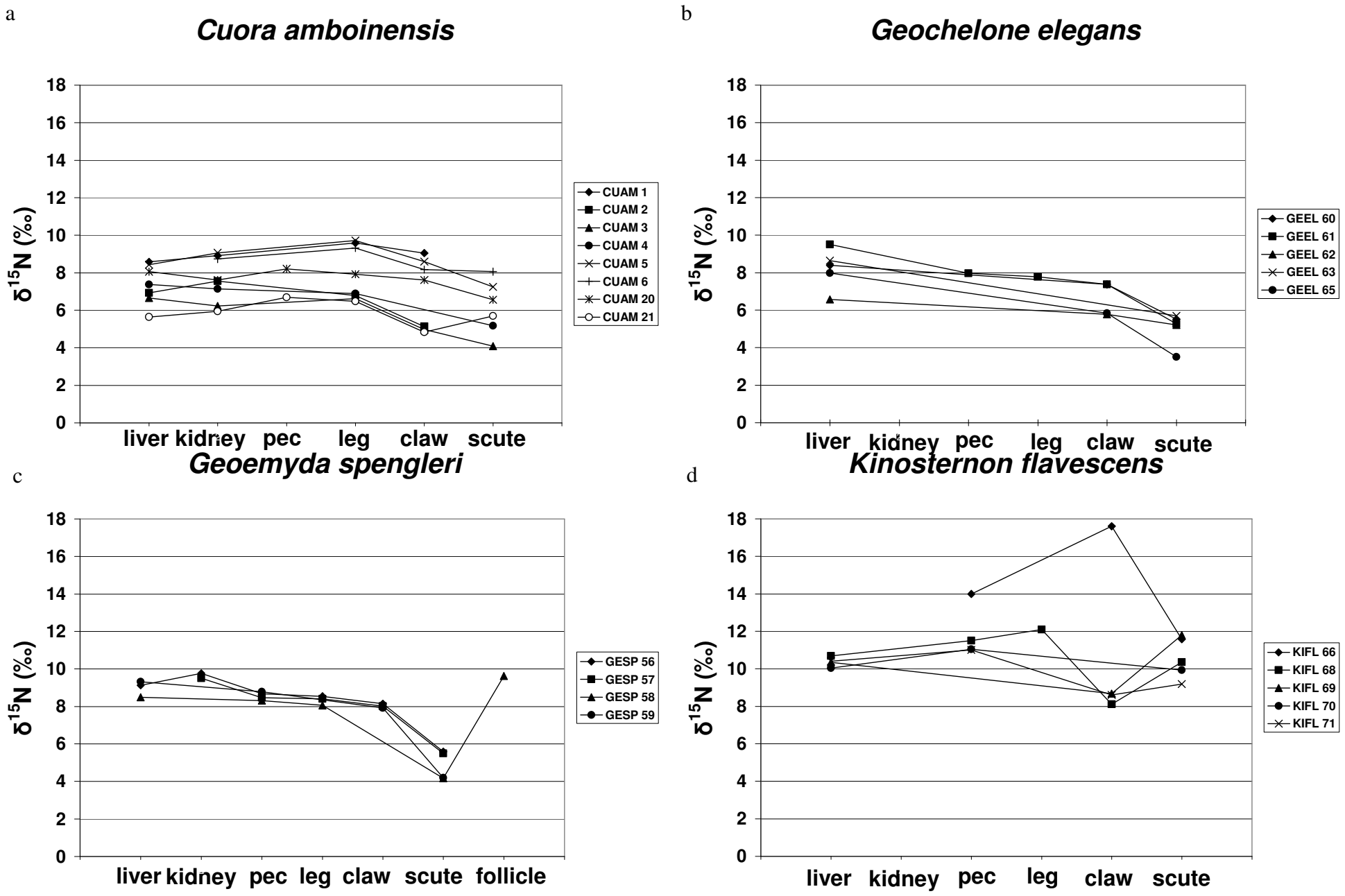
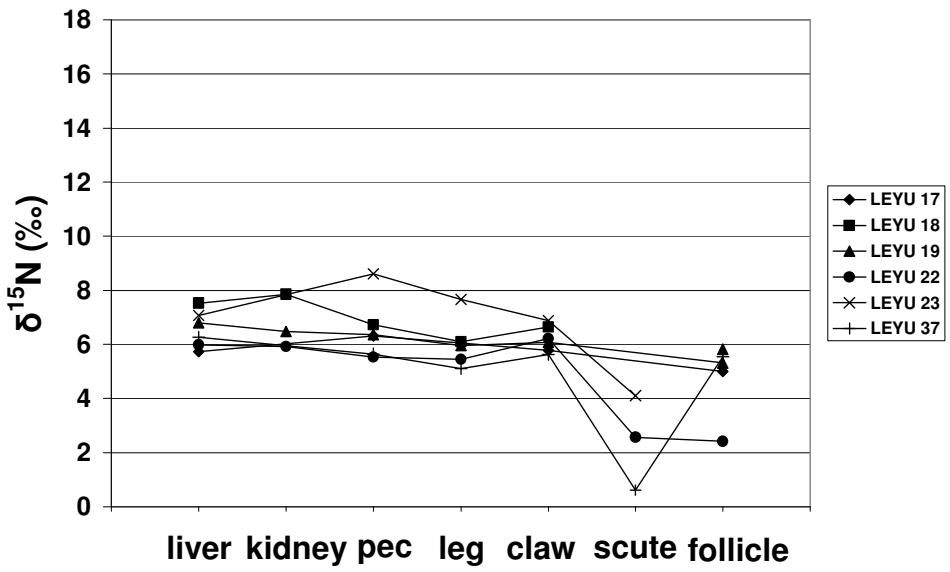
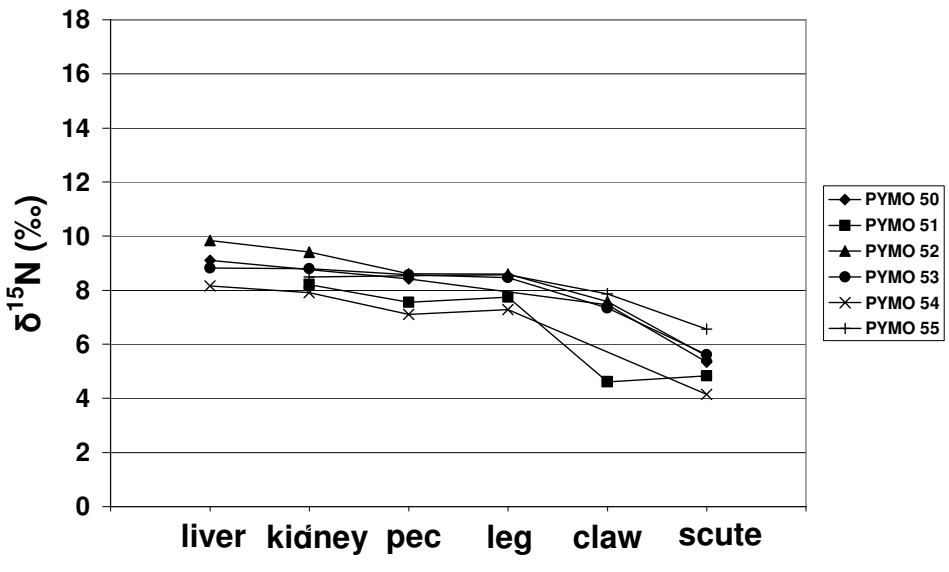


Figure 2.3: Stable nitrogen isotope values. Each graph represents one species, and each symbol represents one individual of a species. Lines connecting the points are drawn to demonstrate intra-individual and intra-species variation in stable nitrogen isotope values, and do not signify a linear relationship. (a): *Cuora amboinensis*; (b): *Geochelone elegans*; (c): *Geoemyda spengleri*; (d): *Kinosternon flavescens*; (e): *Leucocephalon yuwonoi*; (f): *Pyxidea mohoutii*; (g): *Chinemys reevesi*; (h): *Heosemys spinosa*; (i): *Malaclemys terrapin*; (j): *Rhinoclemmys pulcherrima manni*; (k): *Siebenrockiella crassicollis*; (l): *Chelodina parkeri*; (m): *Pelochelys cantorii*; (n): *Pelodiscus sinensis*.

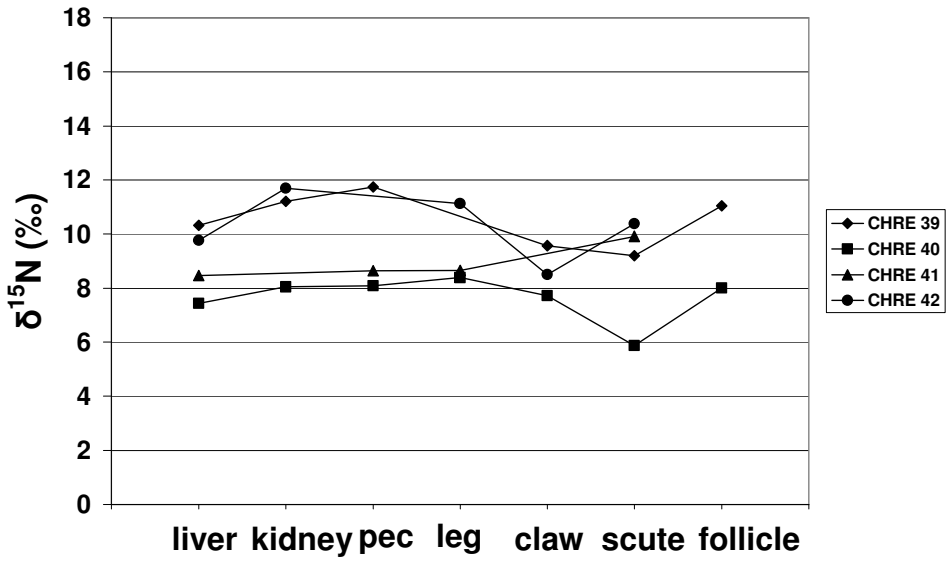
e


Leucocephalon yuwonoi
δ15N (‰)
0
2
4
6
8
10
12
14
16
18
liver
kidney
pec
leg
claw
scute
follicle
LEYU 17
LEYU 18
LEYU 19
LEYU 22
LEYU 23
LEYU 37

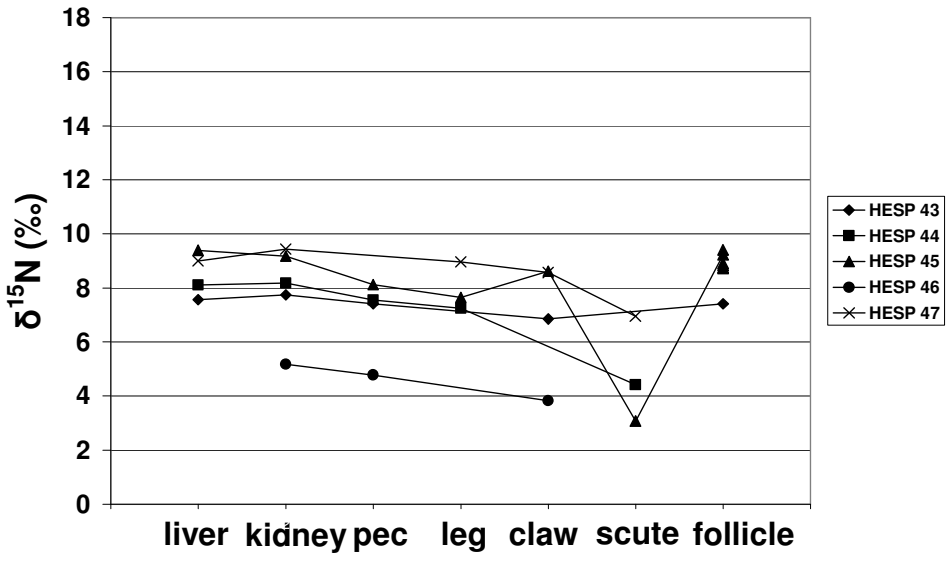
f


Pyxidea mohoutii
δ15N (‰)
0
2
4
6
8
10
12
14
16
18
liver
kidney
pec
leg
claw
scute
PYMO 50
PYMO 51
PYMO 52
PYMO 53
PYMO 54
PYMO 55

g

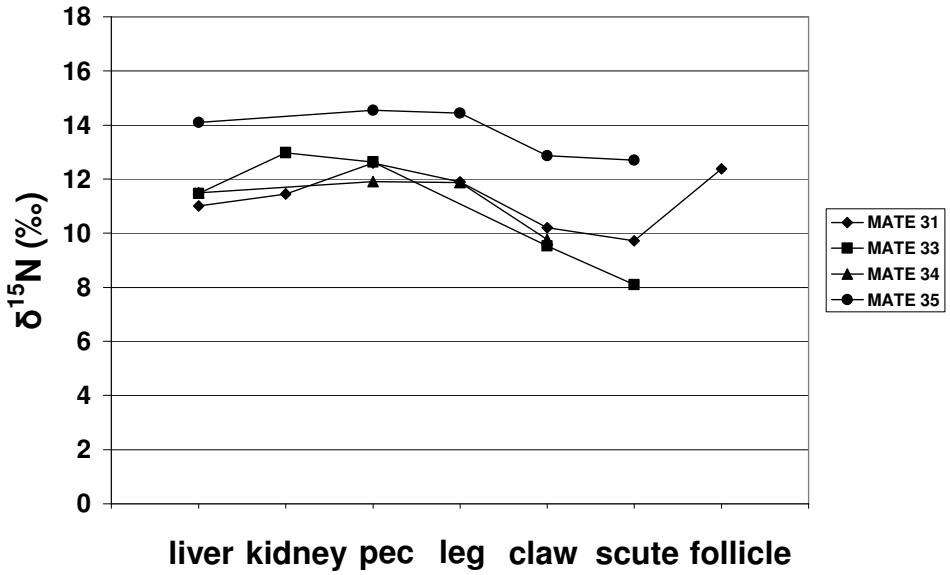

Chinemys reevesi
δ15N (‰)
0
2
4
6
8
10
12
14
16
18
liver
kidney
pec
leg
claw
scute
follicle
CHRE 39
CHRE 40
CHRE 41
CHRE 42

h


Heosemys spinosa
δ15N (‰)
0
2
4
6
8
10
12
14
16
18
liver
kidney
pec
leg
claw
scute
follicle
HESP 43
HESP 44
HESP 45
HESP 46
HESP 47

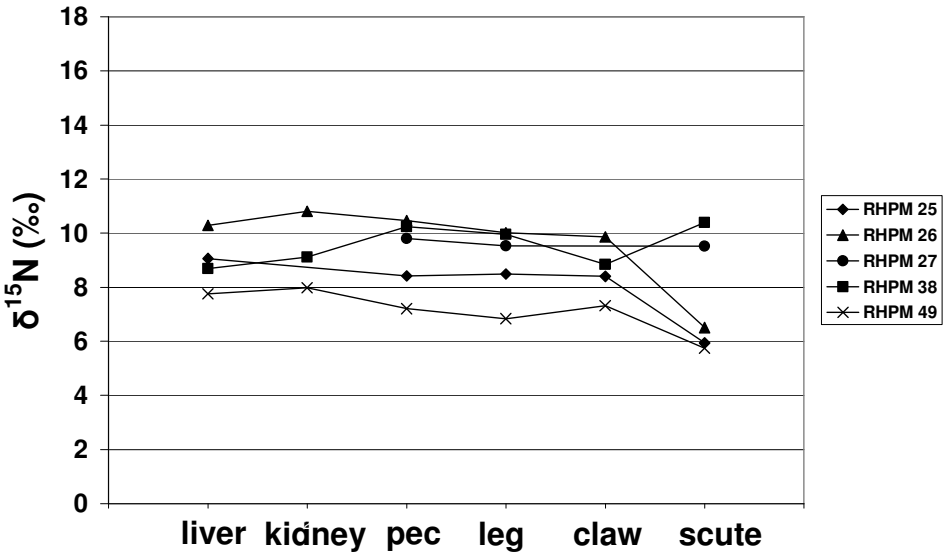
i

Malaclemys terrapin



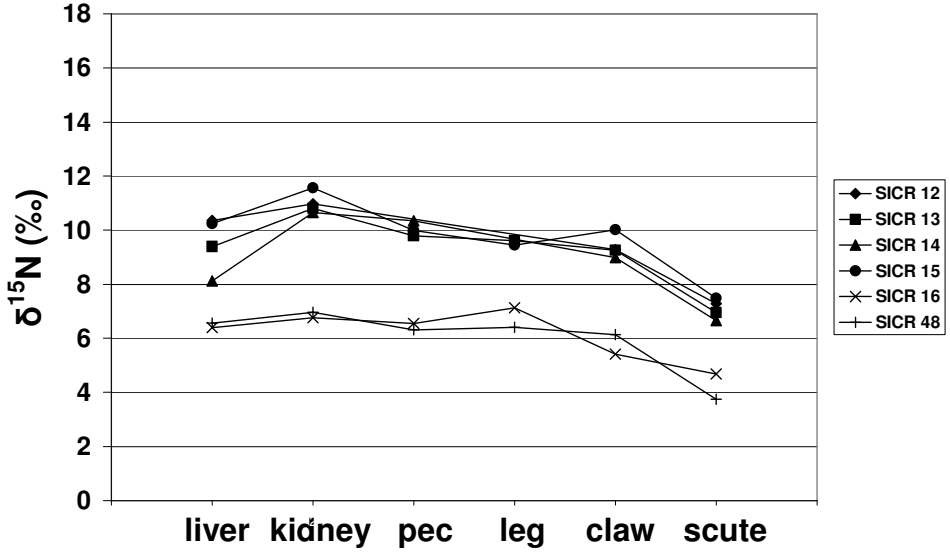
j

Rhinoclemmys pulcherrima manni



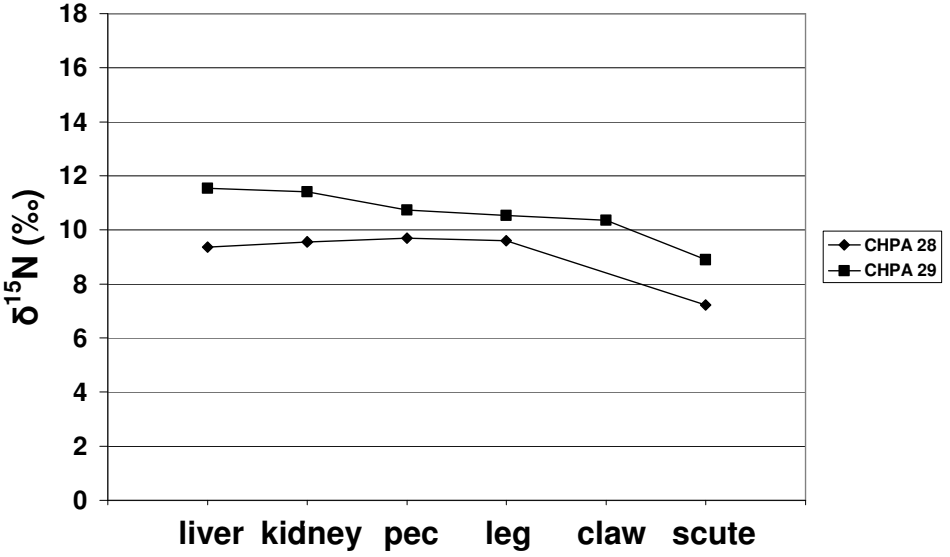
k

Siebenrockiella crassicollis



l

Chelodina parkeri



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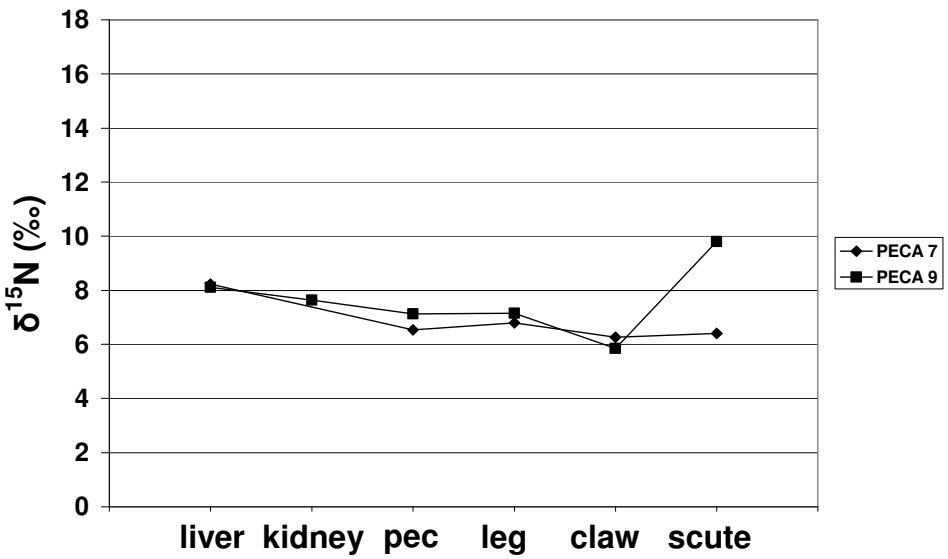
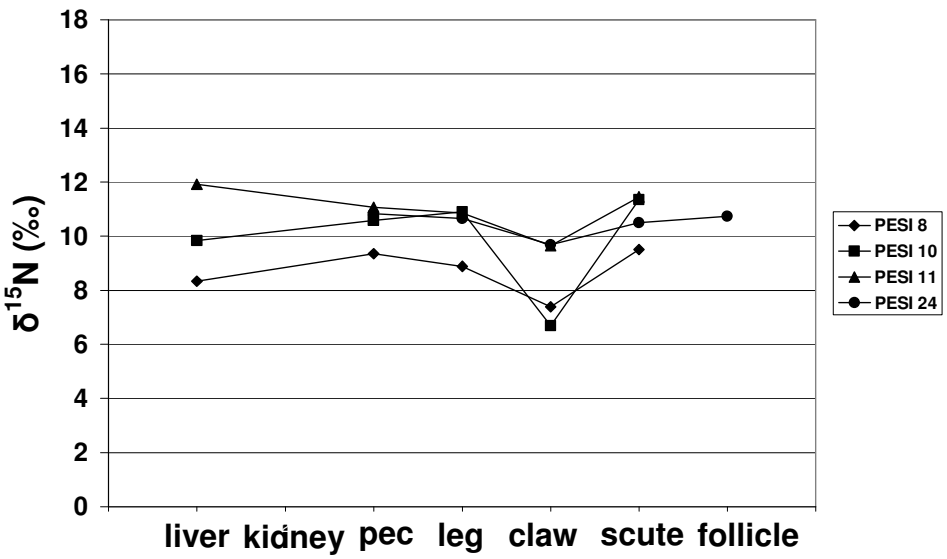

Pelochelys cantorii
δ15N (‰)
18
16
14
12
10
8
6
4
2
0
liver
kidney
pec
leg
claw
scute
PECA 7
PECA 9

n


Pelodiscus sinensis
δ15N (‰)
18
16
14
12
10
8
6
4
2
0
liver
kidney
pec
leg
claw
scute
follicle
PESI 8
PESI 10
PESI 11
PESI 24

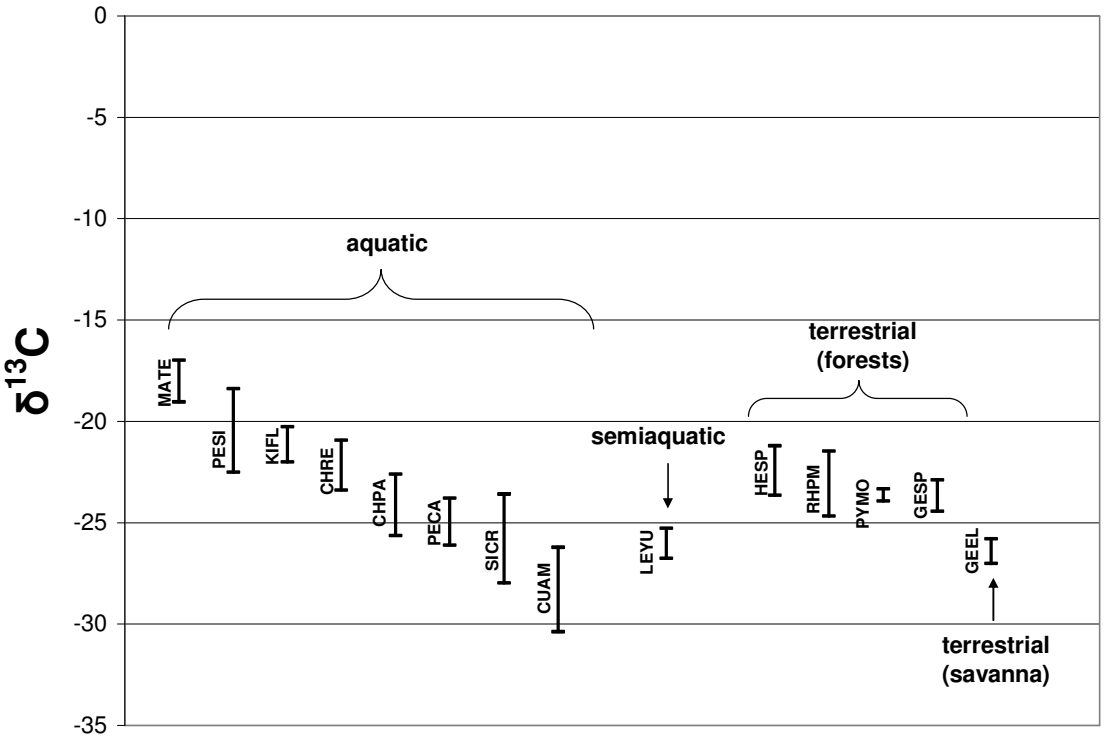


Figure 8: Ranges of liver stable carbon isotope values. Species are grouped into categories based on habitat preferences. Refer to table 2 for species codes.

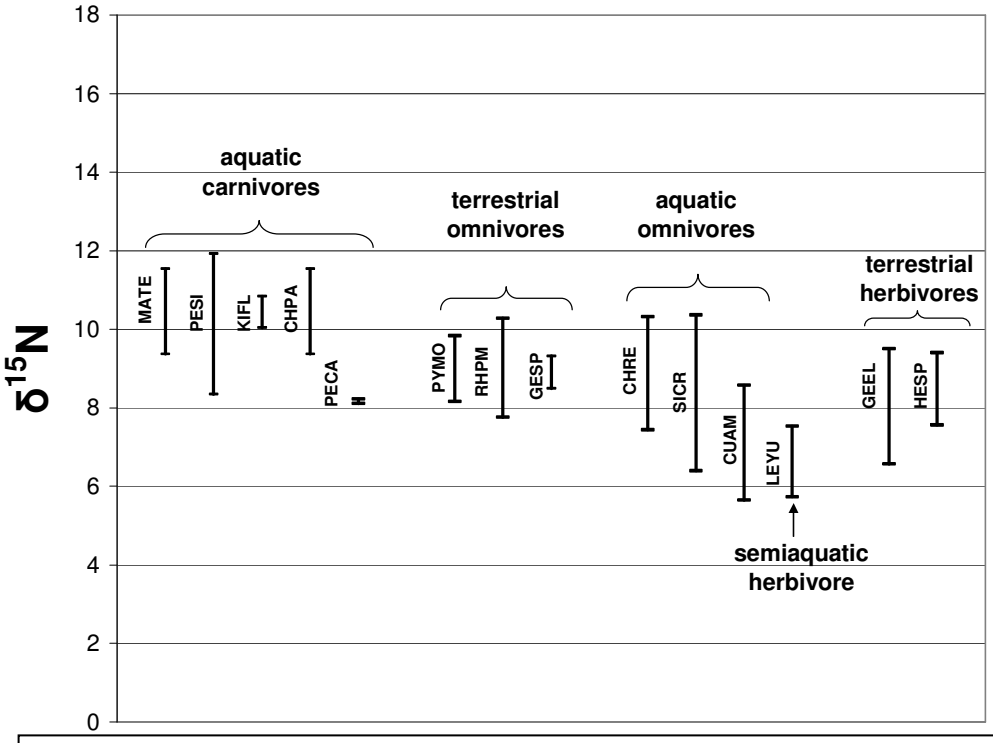
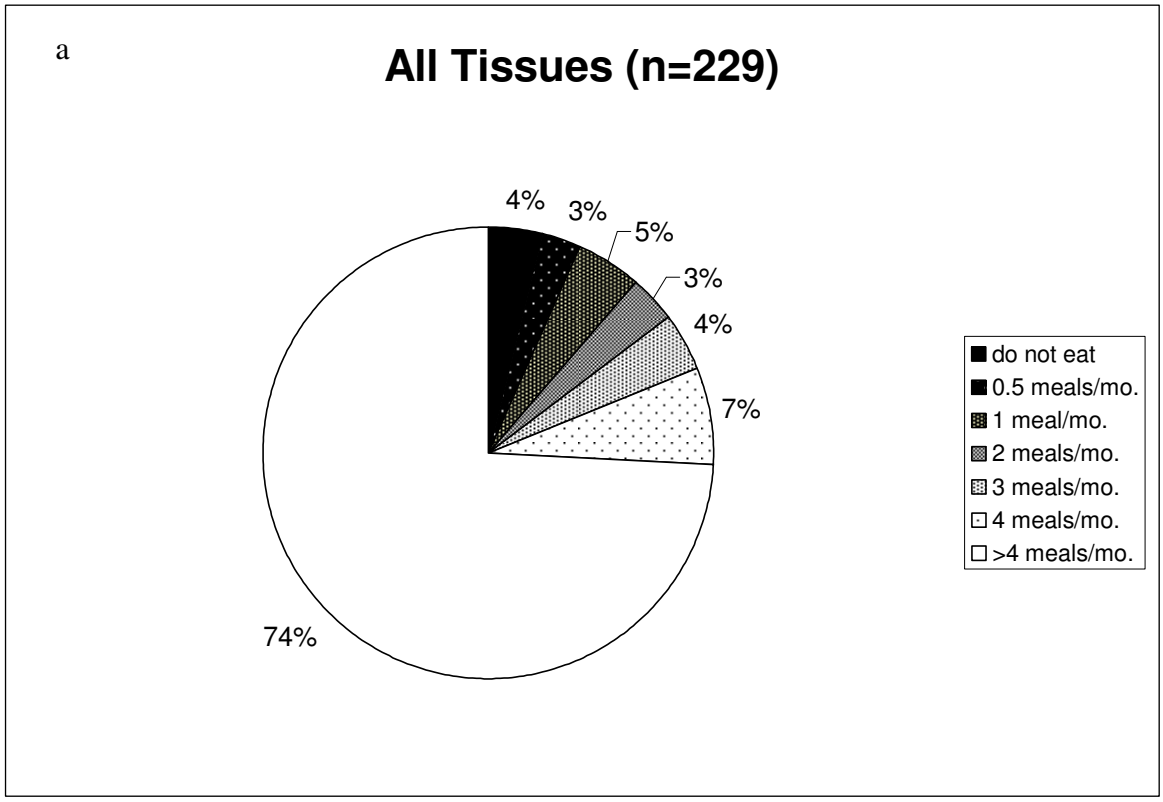


Figure 9: Ranges of liver stable nitrogen isotope values. Species are grouped into categories based on habitat and diet preferences. Refer to table 2 for species codes.

a



b

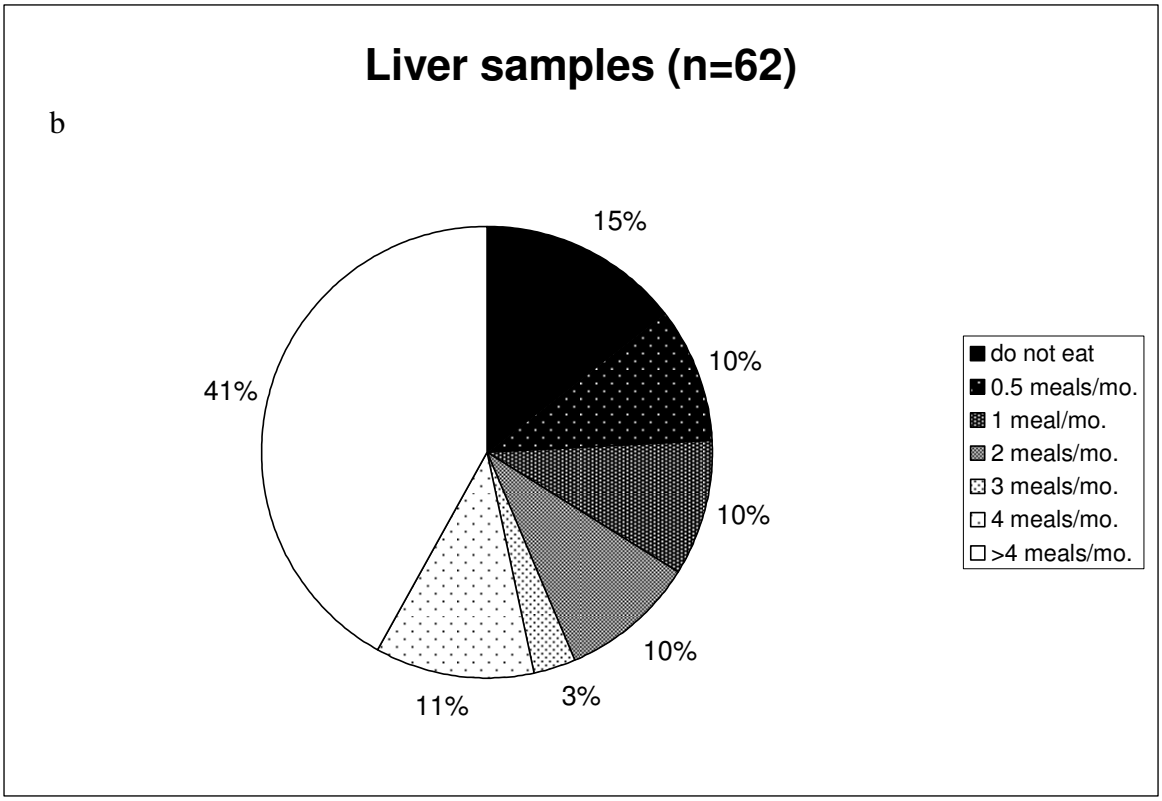
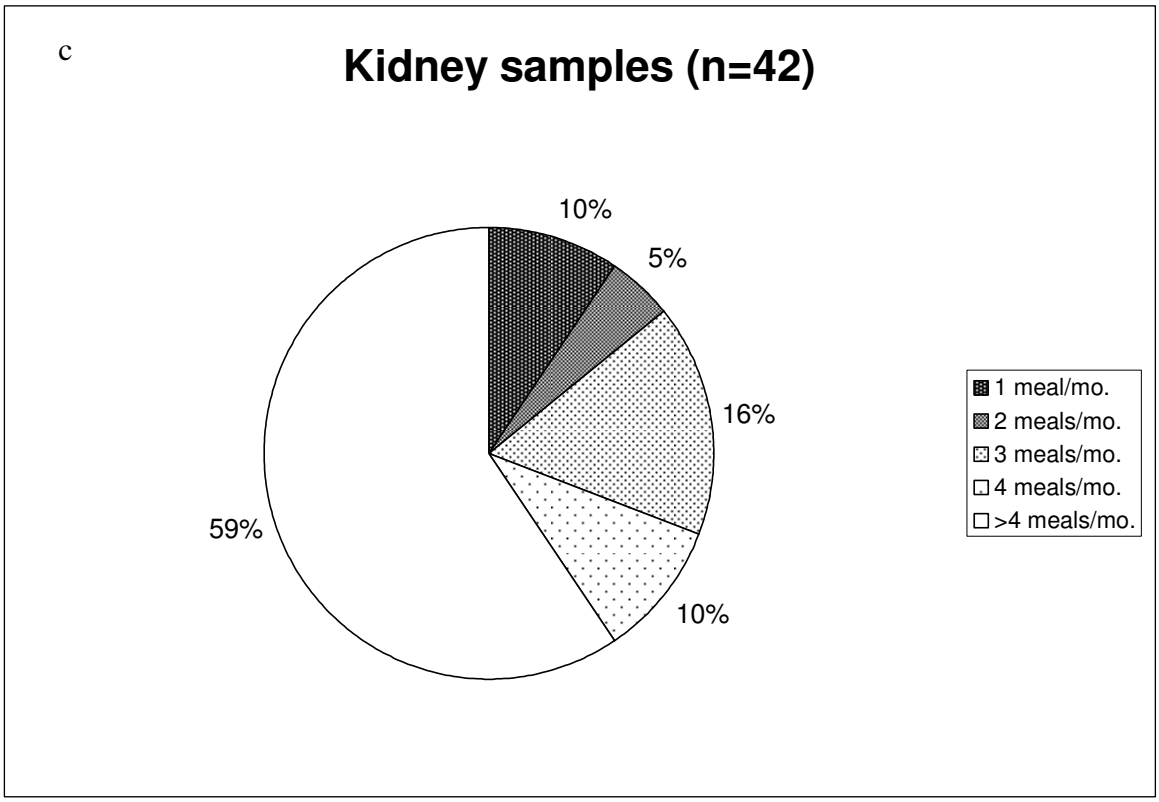
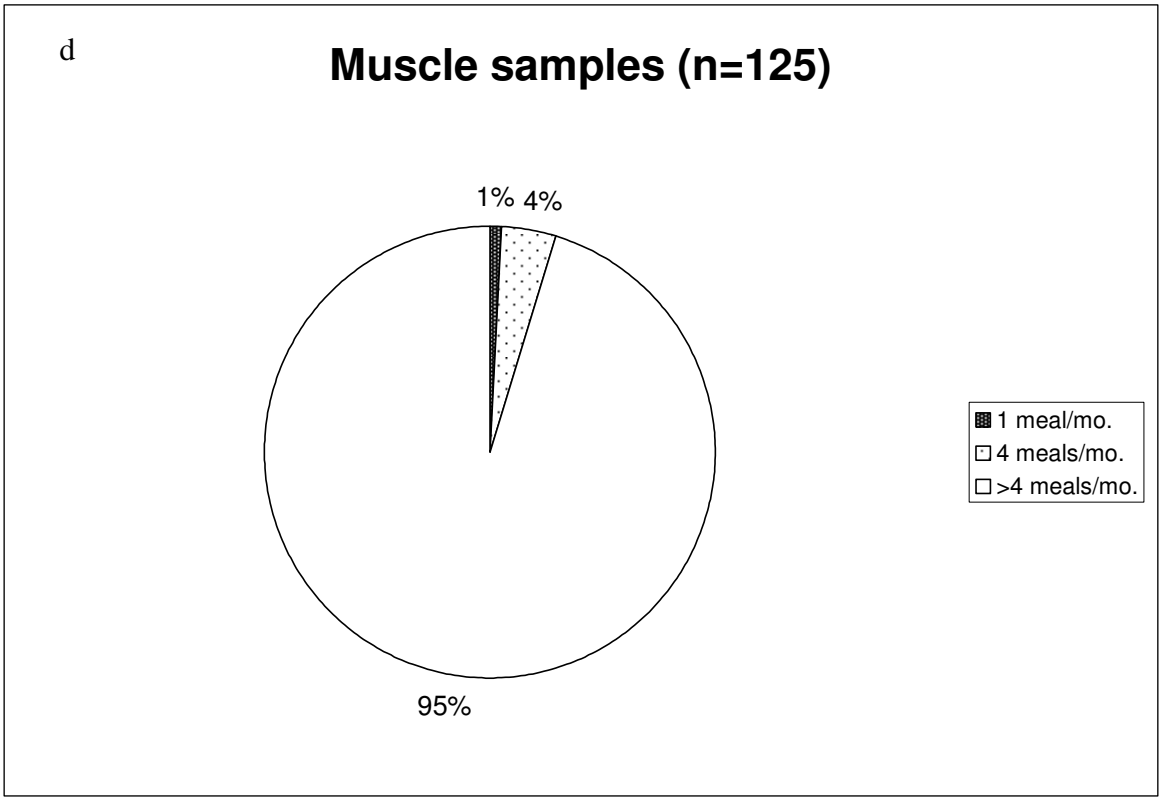


Figure 10: Percentage of samples in each consumption limit category, all species pooled. A: All tissues, b: liver samples, c: kidney samples, d: muscle samples.

c


Kidney samples (n=42)
10%
5%
16%
59%
10%
1 meal/mo.
2 meals/mo.
3 meals/mo.
4 meals/mo.
>4 meals/mo.

d


Muscle samples (n=125)
1%
4%
95%
1 meal/mo.
4 meals/mo.
>4 meals/mo.

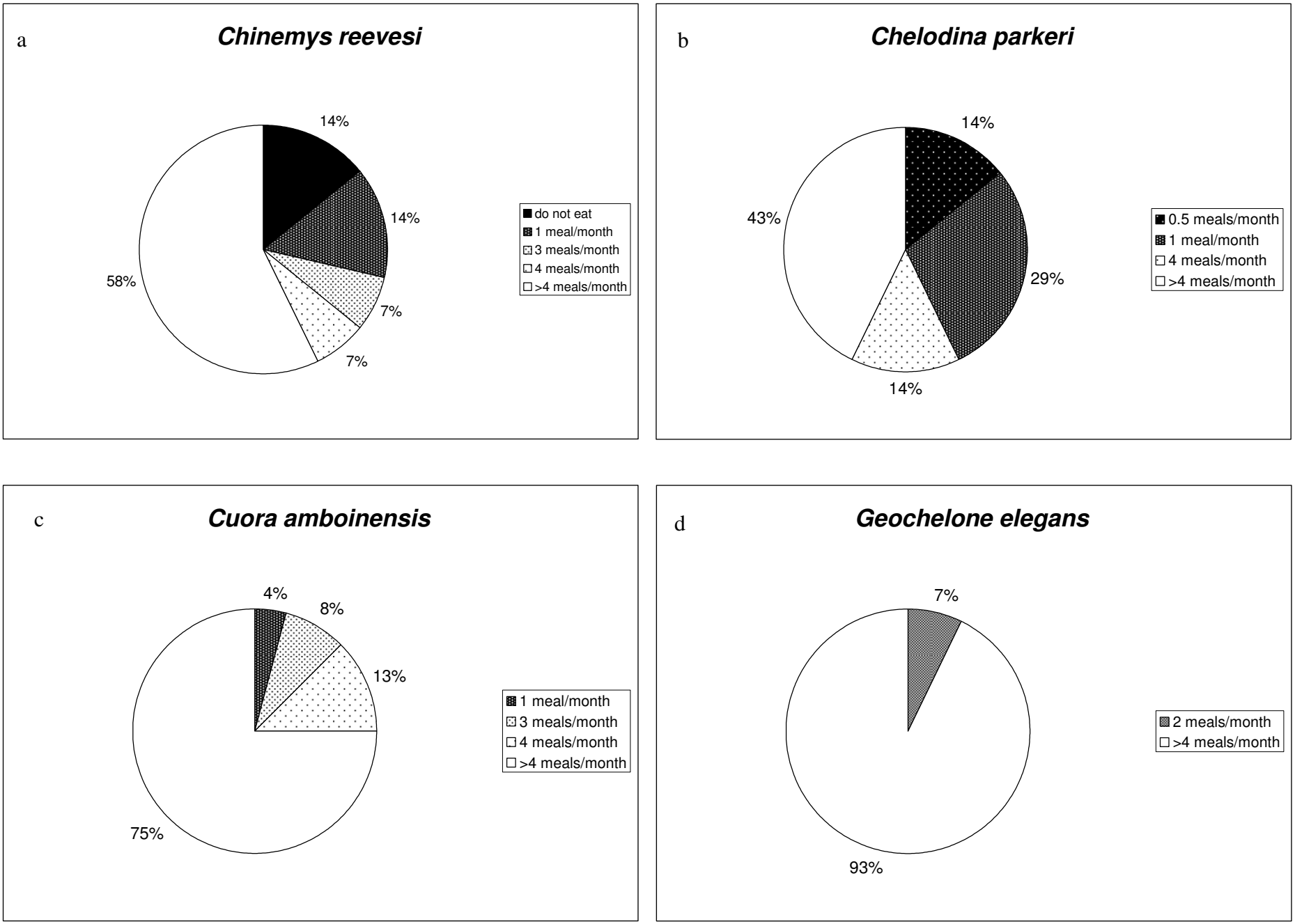
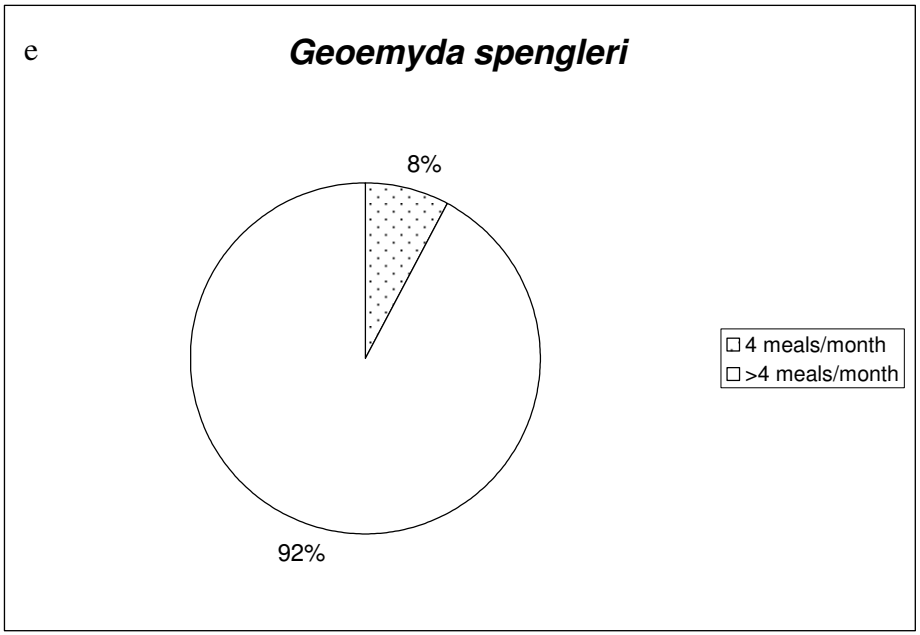
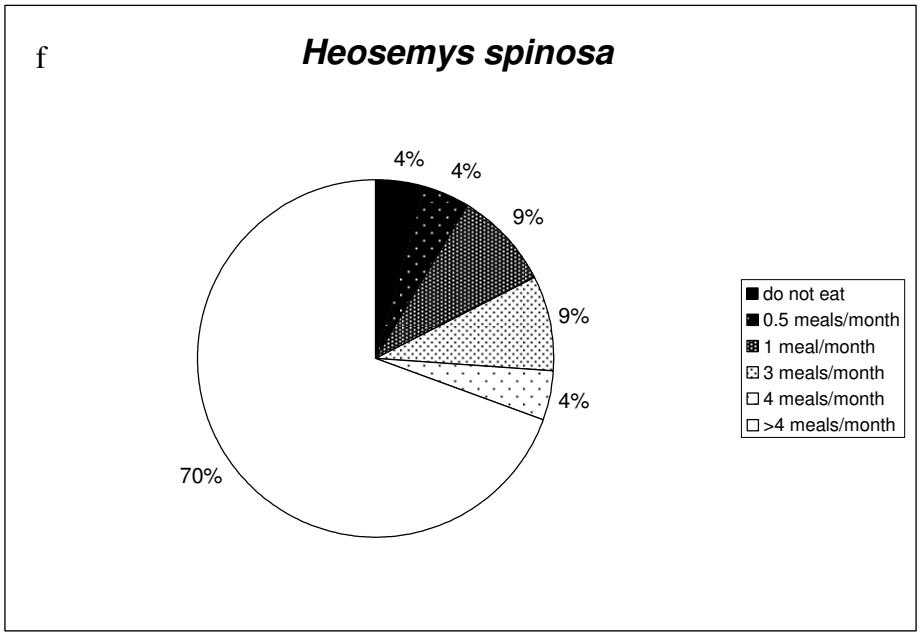


Figure 11: Percentages of samples in each consumption limit category, all tissues pooled. (a): *Chinemys reevesi*; (b): *Chelodina parkeri*; (c): *Cuora amboinensis*; (d): *Geochelone elegans*; (e): *Geoemyda spengleri*; (f): *Heosemys spinosa*; (g): *Kinosternon flavescens*; (h): *Leucocephalon yuwonoi*; (i): *Malaclemys terrapin*; (j): *Pelochelys cantorii*; (k): *Pelodiscus sinensis*; (l): *Pyxidea mohoutii*; (m): *Rhinoclemmys pulcherrima manni*; (n): *Siebenrockiella crassicollis*.

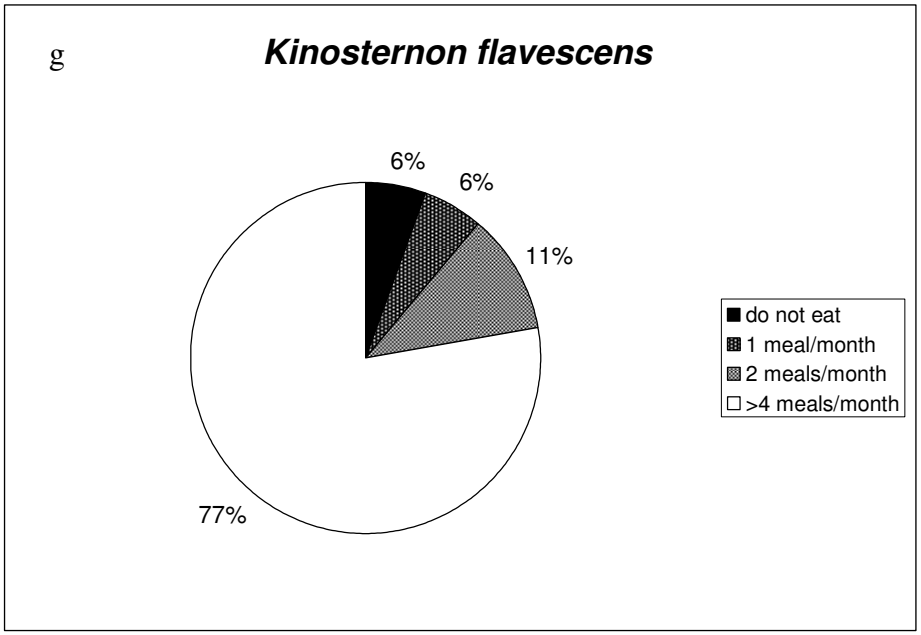
e


Geoemyda spengleri
8%
92%
4 meals/month
>4 meals/month

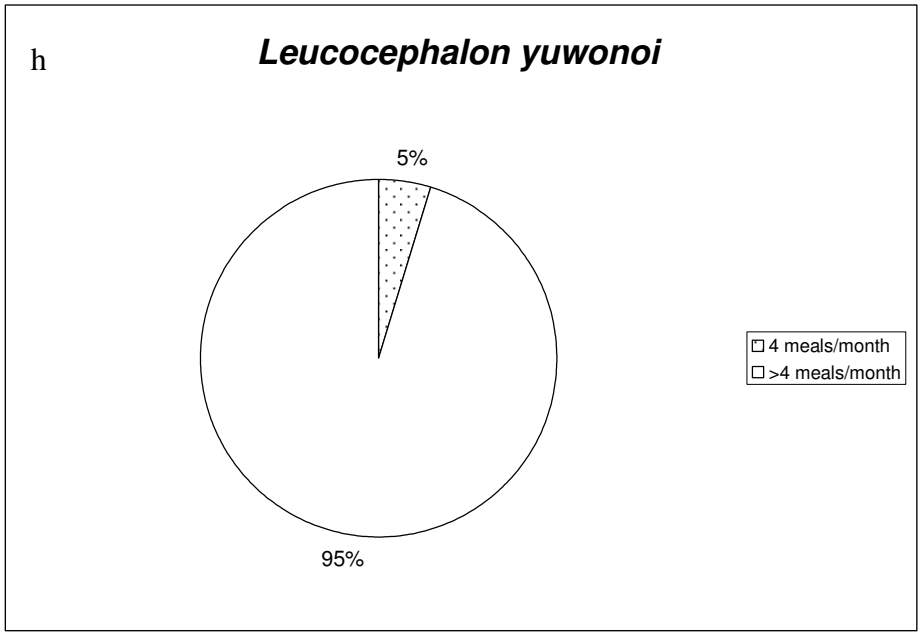
f


Heosemys spinosa
4%
4%
9%
9%
4%
70%
do not eat
0.5 meals/month
1 meal/month
3 meals/month
4 meals/month
>4 meals/month

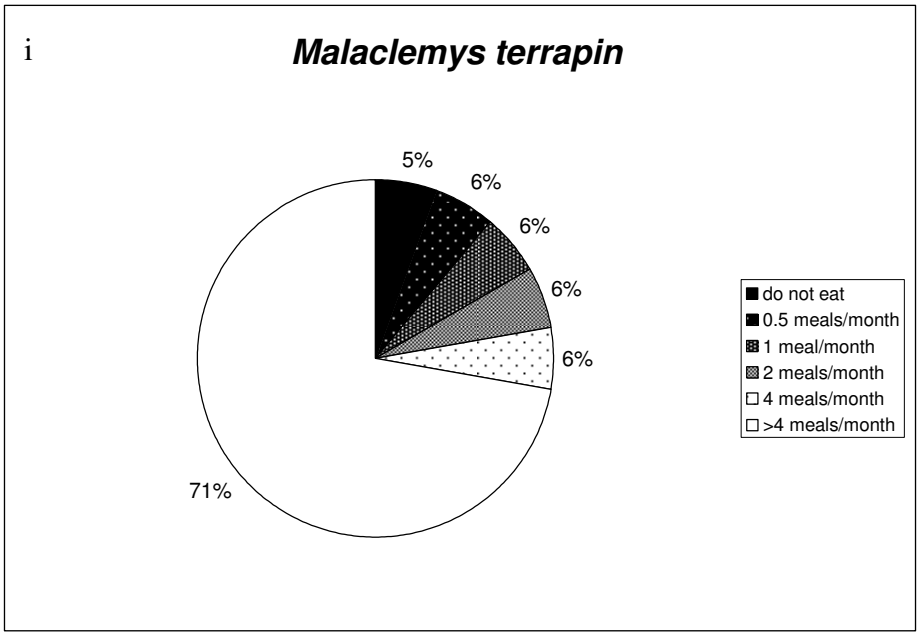
g


Kinosternon flavescens
6%
6%
11%
77%
do not eat
1 meal/month
2 meals/month
>4 meals/month

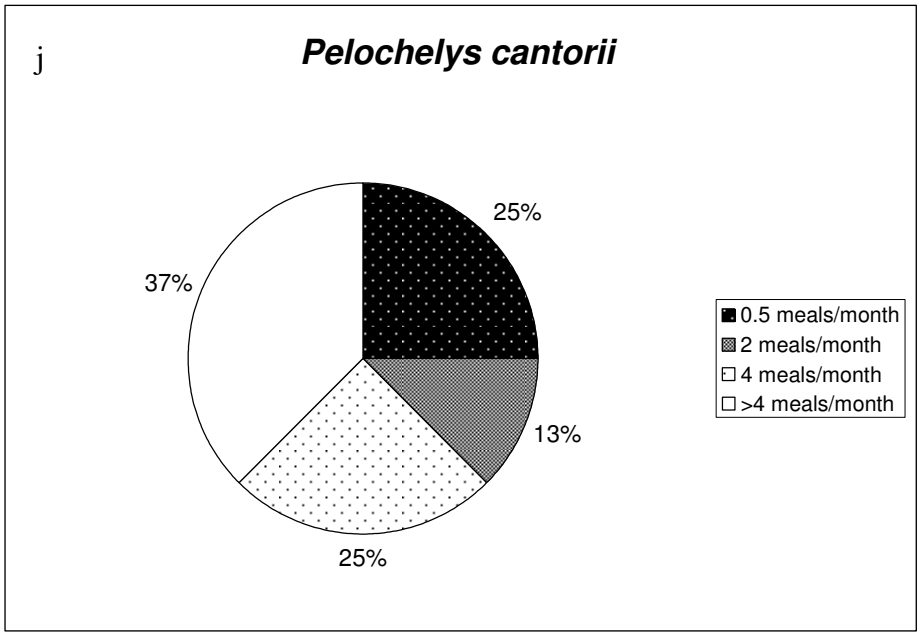
h


Leucocephalon yuwonoi
5%
95%
4 meals/month
>4 meals/month

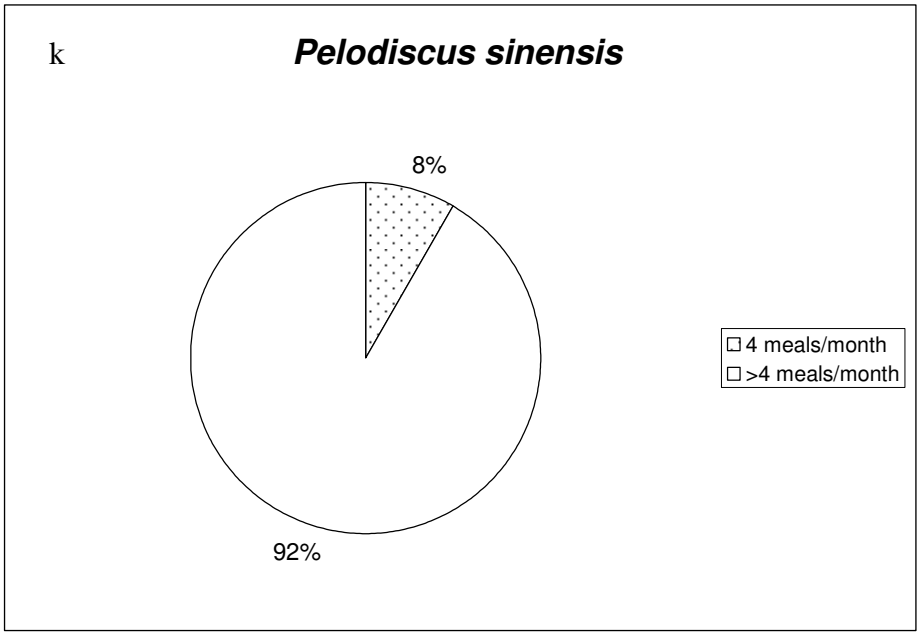
i


Malaclemys terrapin
5%
6%
6%
6%
6%
71%
do not eat
0.5 meals/month
1 meal/month
2 meals/month
4 meals/month
>4 meals/month

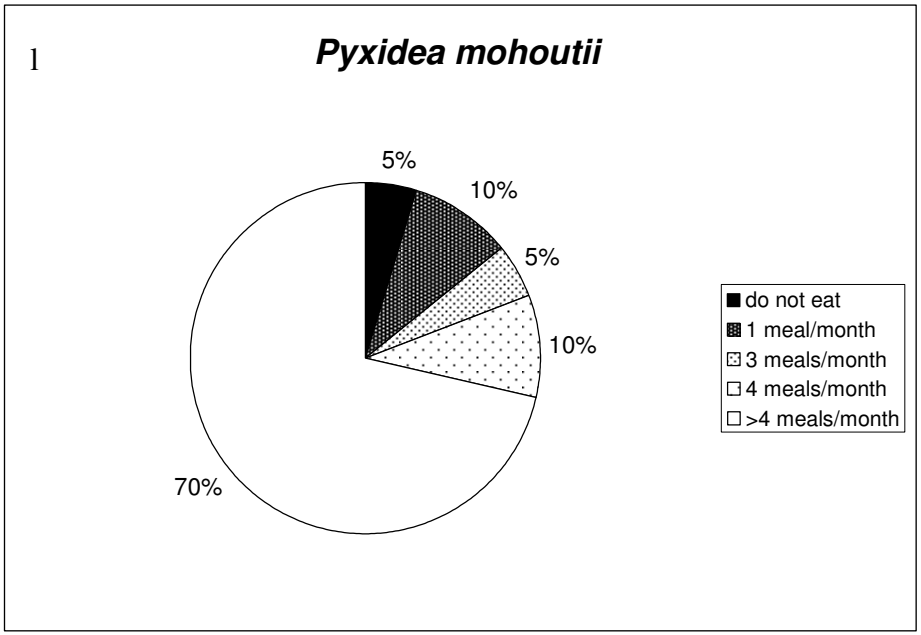
j


Pelochelys cantorii
25%
37%
13%
25%
0.5 meals/month
2 meals/month
4 meals/month
>4 meals/month

k

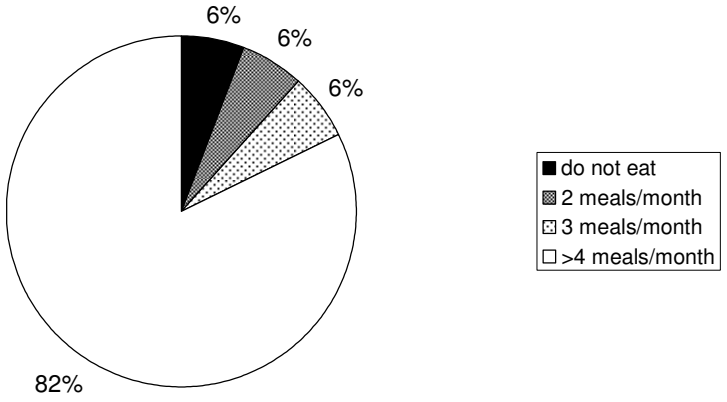

Pelodiscus sinensis
8%
92%
4 meals/month
>4 meals/month

l


Pyxidea mohoutii
5%
10%
5%
10%
70%
do not eat
1 meal/month
3 meals/month
4 meals/month
>4 meals/month

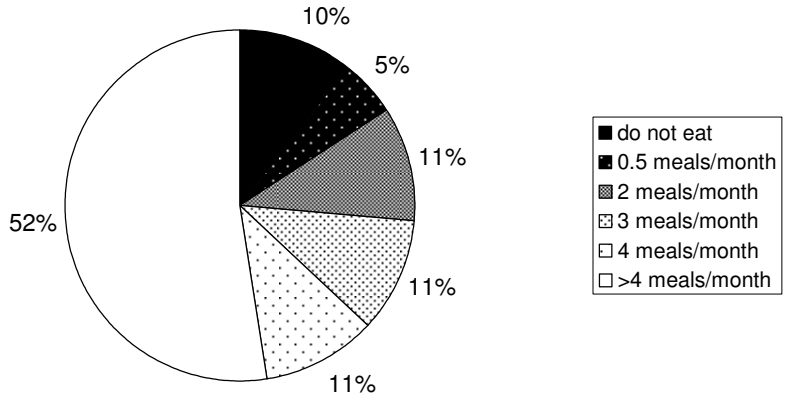
m

Rhinoclemmys pulcherrima manni



n

Siebenrockiella crassicollis



DISCUSSION

Mercury

Tissue distribution:

The general pattern of tissue Hg distribution (liver>kidney≥scutes>muscle) displayed by the majority of specimens is consistent with past studies of sea turtles (Sakai *et al*., 1995; Sakai *et al*., 2000a; Anan *et al*., 2001; Day *et al*., 2005), snapping turtles (Albers *et al*., 1986; Meyers-Schone *et al*., 1993), terrapins (Burger, 2001), and sliders (Meyers-Schone *et al*., 1993). Specimens with extremely high liver Hg had Hg levels in all other tissues that were comparable to those of specimens with relatively low liver Hg. This suggests that, above a certain threshold of Hg exposure, "excess" Hg accumulates in the liver, while Hg in other tissues remains relatively low. However, for *G. spengleri*, it seems as if this excess Hg is allocated to the scutes. As shown in Figure 2e, scute Hg for this species greatly exceeded Hg in all other tissues, including liver. This is displayed to a lesser extent in *K. flavescens* specimens (Figure 2g), whose liver Hg nearly equaled scute Hg in most specimens. It is interesting that these were also the two species which had the highest scute Hg concentrations in the entire study. Scute Hg concentrations that exceed liver Hg concentrations have also been reported in *C. caretta* (Day *et al*., 2005). Scutes are comprised of non-living keratin. Unlike in other tissues, nutrients and other dietary components allocated to scutes do not return to the total body circulation and are metabolically unavailable. It is possible that the allocation of excess Hg to the scutes rather than the liver allows these species to fare better than others in contaminated environments.

Tissues with high metabolic rates, such as liver and kidneys, have shorter nutrient turnover periods than less metabolically-active tissues, such as muscle, cartilage, and bone. This

means that liver cells are replaced more often and reflect more recent dietary components than do muscle cells.

Differences in Hg among diet groups:

As expected, carnivorous species had significantly greater Hg in most tissues than omnivorous species, and omnivorous species had significantly greater Hg in most tissues than herbivorous species. Although the same pattern was detected in liver and kidney samples (Figure 3) differences in Hg among diet groups were not significantly different for these two tissue types. Since Hg tends to accumulate in liver and kidneys, they are most sensitive to Hg contamination. This tendency makes it difficult to detect differences in Hg between diet groups when species are exposed to a wide spectrum of environmental Hg levels. Tissue Hg concentrations are influenced by several factors, including trophic position as well as environmental Hg levels. However, any differences in tissue Hg associated with diet type may be confounded if some species are obtained from locations with greater levels of contaminants that others. For example, an omnivore in a highly polluted environment may have higher liver Hg than a carnivore in a less contaminated habitat. Differences in tissue Hg between dietary groups are more likely to be detected in muscle, claws, and follicles, which are generally low in Hg compared to other tissues.

Stable Isotopes

Macronutrients from the diet, including their associated isotopes, can be preferentially directed into particular tissues before they enter the total body pool of carbon or nitrogen in a process known as isotopic routing (Martinez del Rio and Wolf, 2005). This results in isotopic compositions that differ among tissues and between tissues and the diet. Carbon and nitrogen from the diet is routed through the liver before incorporation into other tissues. As a result, liver has high carbon and nitrogen turnover rates and its isotopic composition resembles that of the

recent diet more closely than less metabolically-active tissues such as muscle and claws (Tieszen *et al*., 1983). It was for this reason that only liver $\delta^{13}C$ and $\delta^{15}N$ values were used to assess groups of species based on life history traits as shown in Figures 8 and 9. Because tissues differ in their rates of carbon and nitrogen turnover, analysis of several types of tissues can provide information on the isotopic composition of the diet at different intervals in the past.

Stable carbon and nitrogen isotope values were analyzed to gain information on carbon and nitrogen sources. Since we had limited information on the geographic origin of the study specimens, we also used $\delta^{13}C$ and $\delta^{15}N$ values along with life history information to develop hypotheses about the types of environments they occupied. Species were divided into three groups based on the pattern presented in plots of $\delta^{13}C$ values by tissue. Group 1 was characterized by relatively invariant $\delta^{13}C$ values between all tissues. This relationship signifies that the tissues are in isotopic equilibrium with each other and the diet. This is indicative of an individual that has remained in a single ecosystem and maintained the same diet for a relatively long period of time (i.e., several months). Species in group 1 included *C. amboinensis*, *L. yuwonoi*, *P. mohoutii*, *G. elegans*, *G. spengleri*, and *K. flavescens*. All of the species in this group, except *C. amboinensis*, had very narrow ranges in $\delta^{15}N$ and $\delta^{13}C$ values for tissues, with a maximum average difference of 2.6‰ between tissues. The low variance of both $\delta^{15}N$ and $\delta^{13}C$ between tissues suggests that individuals within a species had similar prey compositions and are likely from the same population or area.

Group 2 consisted of *S. crassicollis*, *C. reevesi*, *M. terrapin*, *R. pulcherrima manni*, and *H. spinosa*, and was characterized by relatively invariant $\delta^{13}C$ values for the internal tissues (liver, kidney, muscle, and follicles), but a significantly different $\delta^{13}C$ value (up to 3.1‰) and $\delta^{15}N$ value (up to 4‰) for the scutes. As stated previously, nutrients in scutes represent dietary components consumed at the time of scute formation, while nutrients in internal tissues represent

more recent dietary items. This suggests that the individuals in group 2 had undergone a recent dietary change that had not yet been reflected in the scutes at the time of death.

Interestingly, four of the species in group 2 (*M. terrapin*, *C. reevesi*, *S. crassicollis*, and *H. spinosa*) also had a bimodal pattern $\delta^{15}N$ values, where individuals within the species split into two or more distinct, nonoverlapping groups for all tissues. These bimodal groups did not differ in tissue Hg. In the case of both *M. terrapin* and *H. spinosa*, one individual was an outlier in both the carbon and nitrogen isotope compositions, suggesting that these two turtles were likely from different environments than their conspecifics.

Group 3 included *P. sinensis*, *P. cantorii*, and *C. parkeri*. For these species, there was a larger difference in $\delta^{13}C$ values between tissues within an individual than there was between species for the other two groups. This suggests that the carbon isotope composition of the tissues were not in equilibrium with each other or the diet, which may reflect recent dietary changes. The $\delta^{15}N$ values for these species spanned a much narrower range, which may be indicative of conspecifics that were collected from a single location, but occupying slightly different niches. In the case of *P. cantorii*, there is an average difference in $\delta^{15}N$ values of 0.4‰ between individuals, and an average difference of 3.6‰ in $\delta^{13}C$ values. This could represent two individuals from the same river system, with one feeding in a more freshwater environment, and the other feeding closer to the sea in a more estuarine environment.

Evaluation of Risk to Human Consumers

Comparisons to high Hg fish species:

The EPA advises consumers to avoid eating king mackerel, Gulf of Mexico tilefish, swordfish, and shark, as they have the highest levels of Hg of all commercial US fish species. The EPA's "Mercury Levels in Commercial Fish and Shellfish" (EPA, 2001) lists the mean Hg concentration of these four species collected from four prior studies as follows: king mackerel,

730 ppb; shark, 988 ppb; swordfish, 976 ppb; tilefish, 1,450 ppb. Of all the edible turtle tissue samples analyzed, 19 of 229 (8.7%) are within or above this range of values.

Comparisons among tissues:

Of the edible tissue types sampled, liver is the riskiest to consume, as it had the highest proportion of samples in each of the four highest consumption limit categories (do not eat, 0.5 meals/month, 1 meal/month, and 2 meals/month). Liver samples from several popular food trade species exceeded the 1900 ppb consumption threshold. A single 8 oz. meal of any of these samples will exceed the reference dose. Frequent consumption of turtles with liver Hg concentrations that are this high is likely to put consumers at risk for harmful health effects.

Kidneys are less risky to consume than liver, but nearly half (41%) of kidney samples contained levels of Hg requiring consumption limitations. Muscle samples from all species were substantially lower in Hg than liver and kidney samples. It is reasonable to assume that consumers who eat only muscle will be at relatively low risk of harmful health effects due to Hg exposure. However, turtles are frequently cooked and eaten whole in soups and stews (K. Buhlmann and C. Hagen, pers. comm.), so it is likely that most consumers will ingest several types of tissue in one meal.

Comparisons among species:

For ten species (*C. amboinensis*, *G. elegans*, *G. spengleri*, *H. spinosa*, *K. flavescens, L. yuwonoi*, *M. terrapin*, *P. sinensis*, *P. mohoutii*, and *R. pulcherrima manni*), 70% of the edible samples (liver, kidney, and muscle) can be safely consumed more than four times per month. The remaining four species include *C. reevesi* (58%), *S. crassicollis* (52%), *C. parkeri* (43%) and *P. cantorii* (37%).

Based on the range of Hg values measured, the proportion of edible samples in each monthly consumption limit category, and the popularity of the turtles in the food trade, it may be

concluded that the turtles with the greatest potential to cause health risks to consumers to include *S. crassicollis*, *C. reevesi*, *P. cantorii*, *C. parkeri*, and *M. terrapin*.

Conclusions

Turtles in this study showed a similar pattern of tissue Hg distribution observed in many other species of turtles (liver>kidney≥scutes>muscle). Mercury accumulates in the liver and kidneys, and remains relatively low in muscle tissue. The two species that did not follow this pattern, *G. spengleri* and *K. flavescens*, also displayed the highest scute Hg concentratons in the entire study. These two species also occupy habitats with a high potential for mercury contamination. *Geoemyda spengleri* lives in high-elevation montane forests in southern China, and is likely exposed to Hg from atmospheric deposition originating from nearby industrial facilities. This species may also retain its scutes throughout its lifetime instead of shedding them, which may explain why it had such large scute Hg concentrations. *Kinosternon flavescens* aestivates in terrestrial soils, so it may be exposed to Hg not only in its diet, but also from direct contact with Hg-contaminated sediments. *Geoemyda spengleri* and *K. flavescens* may metabolize Hg differently than the rest of the species in this study, allocating most Hg to their scutes rather than accumulating it in their livers. This may make these species better able to handle conditions in a contaminated environment.

As expected, species with more carnivorous diets had higher Hg in all tissues than more herbivorous species. One species in particular, *P. sinensis*, had relatively low tissue Hg despite being piscivorous. Many Chinese softshell turtles are now raised on farms to supply the food trade, where they are likely fed a diet containing little to no fish. It is possible that the *P. sinensis* individuals in this study originated from farms.

Many samples in this study had enough Hg to require consumption limitations according to EPA standards. A small proportion of all samples contained levels of Hg that were several

times higher than the 1900 ppb consumption threshold recommended for most adults. A single 8 oz. meal of a turtle with Hg concentrations such as those measured in the livers of *S. crassicollis* or *C. reevesi*, for example, would exceed the reference dose of 0.1 ppb/day. The short- and long-term effects of consuming these high levels of Hg are uncertain, but it is clear that many of the individuals in this study should be consumed very infrequently or not at all. Although only a small proportion (~6%) of edible tissues sampled were above the recommended consumption threshold, this study's sample size of 71 individuals represents an extremely small percentage of the several million turtles sold for human consumption worldwide. Assuming that the turtles analyzed here are a representative sample of the total population of food trade turtles, several hundred thousand turtles on the market may have concentrations of mercury making them unfit for human consumption. This could signify a troubling public health situation, although consumers of turtles in Asia are likely to be exposed to Hg from fish and the environment as well.

This was a reconnaissance study aimed at identifying ranges and patterns of Hg contamination in food trade turtle species. Conclusions from this study raise several questions that may be addressed in future research projects. The study specimens consisted of few individuals from 14 species. There are approximately 310 species of turtles worldwide. To further investigate the relationship between tissue Hg and certain life history characteristics, such as body size, sex, and habitat type, a future study would include a higher number of individuals from one or a few species. Limited location data on the study specimens prevented any examination of the relationship between environmental Hg levels and tissue Hg levels. To address this question, a future research project would compare tissue Hg concentrations to Hg levels in water, soil, and known dietary components from the turtles' habitats.

The majority of the species in this study originated from Asia, with one and two species respectively from Central America and North America. This impeded a complete analysis of possible differences in tissue Hg among turtles from different regions. To accurately analyze any possible regional differences, a future study would contain an equal number of individuals and species from each region, while controlling for possible confounding factors such as diet. Revelation of the extremely high tissue Hg concentrations present in some turtles raises the question of the biological effects of Hg on individual specimens. This question could be addressed with a study comparing tissue Hg levels with indicators of immune function, such as plasma lysozyme activity. Several contaminants would have to be analyzed and compared to be able to accurately attribute any difference in immune function to Hg alone.

Kjellstrom *et al*. (1986) and Grandjean *et al*. (1997) were two important public health studies that linked frequent maternal fish consumption during pregnancy to subtle neurotoxic effects in children. Replicating these studies with mothers who consumed turtles during pregnancy would shed light on the health effects of consuming turtles.

Although there is still much to be learned about Hg levels in food trade turtles, greater public awareness and education about the Hg content of turtles and the possible health consequences of dietary Hg exposure may help to both protect human consumers and decrease consumption demand for some turtle species. This may stimulate greater public awareness of the problem of environmental Hg contamination and encourage citizens to demand greater restrictions on Hg emissions. This may also help to relax pressure on turtle populations suffering from extensive harvesting for the food trade.

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APPENDIX A: MERCURY VALUES

specimen ID#	species code	tissue	Hg (ppb dry wt.)	Hg (ppb wet wt.)
1	CUAM	claw	49.40	33.38
1	CUAM	kidney	163.43	30.14
1	CUAM	leg muscle	65.82	14.54
1	CUAM	liver	548.22	129.53
2	CUAM	claw	245.13	165.66
2	CUAM	kidney	542.16	99.98
2	CUAM	leg muscle	162.19	35.83
2	CUAM	liver	1021.47	241.34
3	CUAM	claw	596.14	402.87
3	CUAM	kidney	77.89	14.36
3	CUAM	leg muscle	86.24	19.05
3	CUAM	liver	194.85	46.04
3	CUAM	scute	131.07	99.46
4	CUAM	kidney	436.85	80.56
4	CUAM	leg muscle	91.36	20.18
4	CUAM	liver	538.02	127.12
4	CUAM	scute	93.72	71.11
5	CUAM	claw	344.90	233.08
5	CUAM	kidney	1279.67	236.00
5	CUAM	leg muscle	142.32	31.44
5	CUAM	liver	3196.47	755.23
5	CUAM	scute	225.73	171.29
6	CUAM	claw	120.07	81.14
6	CUAM	kidney	102.95	18.99
6	CUAM	leg muscle	104.38	23.06
6	CUAM	scute	119.56	90.73
7	PECA	claw	823.31	556.38
7	PECA	leg muscle	751.05	165.90
7	PECA	liver	203.97	48.19
7	PECA	liver	4235.66	1000.76
7	PECA	pectoral muscle	789.48	148.80
7	PECA	skin	147.15	111.66
8	PESI	claw	130.73	88.35
8	PESI	leg muscle	44.33	9.79
8	PESI	leg muscle	49.15	10.86
8	PESI	liver	144.60	34.16
8	PESI	pectoral muscle	51.20	9.65
8	PESI	skin	11.19	8.49
9	PECA	claw	648.53	438.27

9	PECA	kidney	2163.63	399.02
9	PECA	leg muscle	333.41	73.65
9	PECA	liver	4338.00	1024.94
9	PECA	pectoral muscle	451.21	85.04
9	PECA	skin	13.89	10.54
10	PESI	claw	527.71	356.62
10	PESI	leg muscle	121.38	26.81
10	PESI	liver	685.03	161.85
10	PESI	pectoral muscle	93.38	17.60
10	PESI	skin	37.61	28.54
11	PESI	claw	641.28	433.37
11	PESI	leg muscle	151.93	33.56
11	PESI	liver	469.51	110.93
11	PESI	pectoral muscle	264.90	49.93
11	PESI	skin	46.00	34.90
12	SICR	claw	60.30	40.75
12	SICR	kidney	758.54	139.89
12	SICR	liver	6326.46	1494.75
12	SICR	scute	269.03	204.15
13	SICR	claw	80.32	54.28
13	SICR	kidney	725.86	133.86
13	SICR	leg muscle	49.84	11.01
13	SICR	liver	10571.88	2497.81
13	SICR	pectoral muscle	83.72	15.78
13	SICR	scute	274.18	208.06
14	SICR	claw	59.39	40.14
14	SICR	kidney	547.96	101.05
14	SICR	liver	1791.91	423.37
14	SICR	scute	378.79	287.44
15	SICR	kidney	2033.49	375.02
15	SICR	liver	13976.90	3302.31
15	SICR	pectoral muscle	91.85	17.31
15	SICR	scute	88.03	66.80
16	SICR	claw	84.49	57.10
16	SICR	kidney	516.29	95.21
16	SICR	leg muscle	59.83	13.22
16	SICR	liver	979.61	231.45
16	SICR	pectoral muscle	63.55	11.98
16	SICR	scute	154.29	117.08
17	LEYU	claw	20.76	14.03
17	LEYU	follicle	219.39	71.17
17	LEYU	leg muscle	18.35	4.05
17	LEYU	liver	824.38	194.78
17	LEYU	pectoral muscle	27.02	5.09
18	LEYU	claw	10.90	7.37
18	LEYU	kidney	82.65	15.24

18	LEYU	leg muscle	34.50	7.62
18	LEYU	liver	147.67	34.89
18	LEYU	pectoral muscle	11.95	2.25
19	LEYU	claw	10.10	6.83
19	LEYU	egg #1	10.21	3.31
19	LEYU	egg #2	8.93	2.90
19	LEYU	follicle #1	45.53	14.77
19	LEYU	follicle #2	24.21	7.85
19	LEYU	kidney	41.36	7.63
19	LEYU	leg muscle	34.62	7.65
19	LEYU	liver	422.27	99.77
19	LEYU	pectoral muscle	13.63	2.57
20	CUAM	claw	610.51	412.58
20	CUAM	kidney	163.02	30.06
20	CUAM	leg muscle	113.07	24.98
20	CUAM	liver	339.82	80.29
20	CUAM	scute	591.96	449.20
21	CUAM	claw	99.34	67.13
21	CUAM	kidney	210.07	38.74
21	CUAM	leg muscle	69.18	15.28
21	CUAM	liver	876.90	207.18
21	CUAM	pectoral muscle	55.49	10.46
21	CUAM	scute	286.09	217.09
22	LEYU	claw	21.44	14.49
22	LEYU	follicle	367.64	119.26
22	LEYU	kidney	88.50	16.32
22	LEYU	leg muscle	15.12	3.34
22	LEYU	liver	221.23	52.27
22	LEYU	pectoral muscle	21.65	4.08
22	LEYU	scute	82.37	62.50
23	LEYU	claw	10.63	7.18
23	LEYU	leg muscle	8.44	1.86
23	LEYU	liver	256.91	60.70
23	LEYU	pectoral muscle	33.79	6.37
23	LEYU	scute	50.48	38.31
24	PESI	claw	82.15	55.52
24	PESI	follicle	135.02	43.80
24	PESI	leg muscle	55.21	12.20
24	PESI	pectoral muscle	57.98	10.93
24	PESI	skin	12.39	9.40
25	RHPM	claw	106.21	71.78
25	RHPM	leg muscle	35.62	7.87
25	RHPM	liver	21233.98	5016.94
25	RHPM	pectoral muscle	28.10	5.30
25	RHPM	scute	238.97	181.34
26	RHPM	claw	503.56	340.30

26	RHPM	follicle	398.57	129.30
26	RHPM	leg muscle	18.17	4.01
26	RHPM	liver	336.14	79.42
27	RHPM	claw	22.12	14.95
27	RHPM	follicle	24.48	7.94
27	RHPM	kidney	37.83	6.98
27	RHPM	leg muscle	6.53	1.44
27	RHPM	liver	107.97	25.51
27	RHPM	pectoral muscle	17.44	3.29
27	RHPM	scute	20.46	15.53
28	CHPA	claw	856.84	579.04
28	CHPA	kidney	1206.56	222.51
28	CHPA	liver	226.48	53.51
28	CHPA	pectoral muscle	4108.86	774.42
28	CHPA	scute	762.54	578.64
29	CHPA	claw	41.61	28.12
29	CHPA	kidney	2708.22	499.45
29	CHPA	leg muscle	468.58	103.50
29	CHPA	liver	4793.05	1132.45
29	CHPA	pectoral muscle	572.63	107.93
29	CHPA	scute	570.97	433.27
30	MATE	claw	744.63	503.21
30	MATE	follicle	831.09	269.61
30	MATE	kidney	919.82	169.63
30	MATE	leg muscle	435.23	96.14
30	MATE	liver	14645.53	3460.29
30	MATE	pectoral muscle	428.46	80.75
30	MATE	scute	414.02	314.17
31	MATE	claw	401.97	271.65
31	MATE	follicle	728.06	236.18
31	MATE	kidney	553.39	102.06
31	MATE	leg muscle	381.63	84.30
31	MATE	pectoral muscle	533.55	100.56
31	MATE	scute	699.96	531.15
32	MATE	follicle	76.44	24.80
32	MATE	leg muscle	251.04	55.45
33	MATE	claw	283.09	191.31
33	MATE	kidney	98.42	18.15
33	MATE	liver	2758.57	651.76
33	MATE	pectoral muscle	113.01	21.30
33	MATE	scute	799.66	606.80
34	MATE	claw	518.06	350.10
34	MATE	leg muscle	70.01	15.46
34	MATE	liver	1595.88	377.06
34	MATE	pectoral muscle	84.02	15.84
34	MATE	scute	1000.65	759.32

35	MATE	claw	255.03	172.35
35	MATE	kidney	373.42	68.87
35	MATE	leg muscle	150.67	33.28
35	MATE	liver	6277.80	1483.25
35	MATE	pectoral muscle	182.76	34.45
35	MATE	scute	323.03	245.12
36	HESP	claw	52.37	35.39
36	HESP	follicle #1	221.63	71.90
36	HESP	follicle #2	332.66	107.92
36	HESP	follicle #3	306.23	99.34
36	HESP	follicle #4	204.50	66.34
36	HESP	follicle #5	153.57	49.82
36	HESP	follicle #6	186.26	60.42
36	HESP	follicle #7	131.56	42.68
36	HESP	follicle #8	116.95	37.94
36	HESP	follicle #9	73.74	23.92
36	HESP	kidney	1504.18	277.40
36	HESP	leg muscle	35.29	7.80
36	HESP	liver	20179.92	4767.90
36	HESP	pectoral muscle	1.78	0.34
36	HESP	scute	779.27	591.33
37	LEYU	claw	14.78	9.99
37	LEYU	follicle	87.99	28.54
37	LEYU	leg muscle	7.25	1.60
37	LEYU	liver	112.13	26.49
37	LEYU	pectoral muscle	7.57	1.43
37	LEYU	scute	35.53	26.96
38	RHPM	claw	34.03	23.00
38	RHPM	kidney	1481.59	273.23
38	RHPM	leg muscle	59.94	13.24
38	RHPM	liver	1758.85	415.56
38	RHPM	pectoral muscle	47.43	8.94
38	RHPM	scute	288.10	218.62
39	CHRE	claw	86.99	58.79
39	CHRE	follicle	1715.84	556.62
39	CHRE	kidney	1301.71	240.06
39	CHRE	leg muscle	514.63	113.68
39	CHRE	liver	70094.88	16561.28
39	CHRE	pectoral muscle	703.19	132.53
39	CHRE	scute	884.30	671.03
40	CHRE	claw	64.07	43.29
40	CHRE	follicle	3229.98	1047.81
40	CHRE	kidney	418.05	77.10
40	CHRE	leg muscle	34.74	7.67
40	CHRE	liver	31503.73	7443.37
40	CHRE	pectoral muscle	6.99	1.32

40	CHRE	scute	1941.80	1473.49
41	CHRE	claw	57.67	38.97
41	CHRE	leg muscle	45.62	10.08
41	CHRE	liver	3055.82	722.00
41	CHRE	pectoral muscle	38.10	7.18
41	CHRE	scute	1004.60	762.32
42	CHRE	claw	79.85	53.96
42	CHRE	leg muscle	65.31	14.43
42	CHRE	liver	2387.85	564.17
42	CHRE	pectoral muscle	48.39	9.12
42	CHRE	scute	737.12	559.35
43	HESP	claw	22.22	15.02
43	HESP	follicle	15.99	5.19
43	HESP	kidney	239.57	44.18
43	HESP	leg muscle	13.85	3.06
43	HESP	leg muscle	16.05	3.55
43	HESP	pectoral muscle	15.14	2.85
43	HESP	scute	68.07	51.65
44	HESP	claw	59.54	40.24
44	HESP	kidney	2970.38	547.80
44	HESP	leg muscle	30.84	6.81
44	HESP	liver	3695.46	873.12
44	HESP	pectoral muscle	43.02	8.11
44	HESP	scute	1542.71	1170.65
45	HESP	claw	29.32	19.81
45	HESP	follicle #1	27.30	8.86
45	HESP	follicle #2	17.85	5.79
45	HESP	follicle #3	25.49	8.27
45	HESP	follicle #4	24.32	7.89
45	HESP	follicle #5	20.28	6.58
45	HESP	follicles 41	52.39	17.00
45	HESP	leg muscle	33.82	7.47
45	HESP	liver	601.69	142.16
45	HESP	pectoral muscle	36.61	6.90
45	HESP	scute	816.16	619.33
46	HESP	claw	507.08	342.68
46	HESP	kidney	1547.23	285.34
46	HESP	leg muscle	90.22	19.93
46	HESP	liver	4489.25	1060.67
46	HESP	pectoral muscle	210.42	39.66
46	HESP	scute	971.18	736.96
47	HESP	claw	19.84	13.41
47	HESP	kidney	322.79	59.53
47	HESP	leg muscle	40.31	8.90
47	HESP	liver	223.15	52.72
47	HESP	pectoral muscle	93.02	17.53

47	HESP	scute	283.09	214.82
48	SICR	claw	84.37	57.02
48	SICR	kidney	1361.86	251.15
48	SICR	leg muscle	16.47	3.64
48	SICR	liver	306.85	72.50
48	SICR	pectoral muscle	18.86	3.55
48	SICR	scute	98.43	74.69
49	RHPM	claw	9.64	6.51
49	RHPM	kidney	213.37	39.35
49	RHPM	leg muscle	11.86	2.62
49	RHPM	liver	21.76	5.14
49	RHPM	pectoral muscle	10.11	1.91
49	RHPM	scute	43.41	32.94
50	PYMO	claw	870.47	588.25
50	PYMO	follicle	3371.11	1093.59
50	PYMO	kidney	4717.20	869.94
50	PYMO	leg muscle	729.95	161.24
50	PYMO	pectoral muscle	748.37	141.05
50	PYMO	scute	2403.01	1823.47
51	PYMO	claw	297.65	201.15
51	PYMO	follicles 4	170.15	55.20
51	PYMO	kidney	1375.58	253.68
51	PYMO	leg muscle	122.55	27.07
51	PYMO	liver	231.82	54.77
51	PYMO	pectoral muscle	132.25	24.93
51	PYMO	scute	541.67	411.03
52	PYMO	claw	39.77	26.88
52	PYMO	kidney	100.60	18.55
52	PYMO	leg muscle	18.44	4.07
52	PYMO	liver	146.09	34.52
52	PYMO	pectoral muscle	18.81	3.55
52	PYMO	scute	169.56	128.67
53	PYMO	claw	627.59	424.12
53	PYMO	leg muscle	264.92	58.52
53	PYMO	pectoral muscle	294.33	55.47
53	PYMO	scute	767.11	582.11
54	PYMO	claw	404.81	273.57
54	PYMO	kidney	3615.75	666.82
54	PYMO	leg muscle	239.22	52.84
54	PYMO	liver	10384.58	2453.56
54	PYMO	pectoral muscle	385.06	72.57
54	PYMO	scute	1119.36	849.40
55	PYMO	claw	17.29	11.68
55	PYMO	kidney	15.85	2.92
55	PYMO	leg muscle	9.94	2.20
55	PYMO	liver	33.31	7.87

55	PYMO	pectoral muscle	12.29	2.32
55	PYMO	scute	138.30	104.95
56	GESP	claw	82.57	55.80
56	GESP	kidney	312.18	57.57
56	GESP	leg muscle	97.39	21.51
56	GESP	pectoral muscle	90.45	17.05
56	GESP	scute	4173.73	3167.15
57	GESP	claw	92.78	62.70
57	GESP	kidney	387.93	71.54
57	GESP	leg muscle	125.74	27.77
57	GESP	pectoral muscle	110.33	20.79
57	GESP	scute	2715.97	2060.96
58	GESP	claw	102.13	69.02
58	GESP	follicle	35.11	11.39
58	GESP	leg muscle	46.14	10.19
58	GESP	liver	361.27	85.36
58	GESP	pectoral muscle	41.29	7.78
58	GESP	scute	1073.14	814.33
59	GESP	claw	163.92	110.78
59	GESP	kidney	277.02	51.09
59	GESP	leg muscle	90.50	19.99
59	GESP	liver	737.17	174.17
59	GESP	pectoral muscle	61.49	11.59
59	GESP	scute	1191.32	904.00
60	GEEL	claw	10.20	6.89
60	GEEL	leg muscle	13.36	2.95
60	GEEL	liver	1911.97	451.74
60	GEEL	pectoral muscle	19.12	3.60
60	GEEL	scute	20.04	15.21
61	GEEL	claw	5.75	3.89
61	GEEL	leg muscle	2.44	0.54
61	GEEL	liver	47.35	11.19
61	GEEL	pectoral muscle	4.24	0.80
61	GEEL	scute	3.48	2.64
62	GEEL	claw	10.50	7.10
62	GEEL	leg muscle	4.29	0.95
62	GEEL	liver	214.67	50.72
62	GEEL	pectoral muscle	7.24	1.36
62	GEEL	scute	6.73	5.11
63	GEEL	claw	8.07	5.45
63	GEEL	scute	4.46	3.38
64	GEEL	claw	6.32	4.27
64	GEEL	liver	83.98	19.84
64	GEEL	pectoral muscle	7.46	1.41
64	GEEL	scute	3.20	2.43
65	GEEL	claw	7.78	5.26

65	GEEL	leg muscle	10.79	2.38
65	GEEL	liver	190.36	44.98
65	GEEL	pectoral muscle	9.67	1.82
65	GEEL	scute	20.71	15.72
66	KIFL	claw	416.11	281.20
66	KIFL	leg muscle	167.79	37.06
66	KIFL	liver	352.26	83.23
66	KIFL	pectoral muscle	177.95	33.54
66	KIFL	scute	300.01	227.66
67	KIFL	claw	366.35	247.58
67	KIFL	leg muscle	104.19	23.01
67	KIFL	liver	418.95	98.99
67	KIFL	pectoral muscle	112.16	21.14
67	KIFL	scute	641.33	486.66
68	KIFL	claw	816.78	551.97
68	KIFL	leg muscle	213.91	47.25
68	KIFL	liver	8489.48	2005.81
68	KIFL	pectoral muscle	250.95	47.30
68	KIFL	scute	2816.97	2137.60
69	KIFL	claw	930.14	628.58
69	KIFL	leg muscle	142.75	31.53
69	KIFL	liver	1755.66	414.81
69	KIFL	pectoral muscle	194.49	36.66
69	KIFL	scute	3829.36	2905.83
70	KIFL	claw	487.14	329.20
70	KIFL	leg muscle	92.27	20.38
70	KIFL	liver	1712.57	404.63
70	KIFL	pectoral muscle	94.63	17.84
70	KIFL	scute	2842.69	2157.11
71	KIFL	claw	312.47	211.16
71	KIFL	leg muscle	108.12	23.88
71	KIFL	liver	2645.43	625.03
71	KIFL	pectoral muscle	105.41	19.87
71	KIFL	scute	927.12	703.53

APPENDIX B: STABLE ISOTOPE VALUES

specimen ID #	species code	tissue	$\delta^{15}N$	$\delta^{13}C$
1	CUAM	claw	9.06	-25.32
1	CUAM	kidney	8.92	-25.61
1	CUAM	leg muscle	9.59	-25.85
1	CUAM	liver	8.58	-26.20
2	CUAM	claw	5.13	-26.56
2	CUAM	kidney	7.55	-26.76
2	CUAM	leg muscle	6.79	-26.61
2	CUAM	liver	6.93	-27.31
3	CUAM	claw	4.97	-26.63
3	CUAM	kidney	6.23	-26.90
3	CUAM	leg muscle	6.63	-26.75
3	CUAM	liver	6.66	-28.03
3	CUAM	scute	4.09	-26.78
4	CUAM	kidney	7.14	-27.66
4	CUAM	leg muscle	6.89	-27.29
4	CUAM	liver	7.38	-27.96
4	CUAM	scute	5.17	-27.12
5	CUAM	claw	8.60	-26.43
5	CUAM	kidney	9.07	-26.36
5	CUAM	leg muscle	9.73	-26.36
5	CUAM	liver	8.43	-26.61
5	CUAM	scute	7.24	-27.99
6	CUAM	claw	8.18	-25.02
6	CUAM	kidney	8.73	-24.71
6	CUAM	leg muscle	9.31	-24.37
6	CUAM	scute	8.05	-24.93
7	PECA	claw	6.26	-25.43
7	PECA	leg muscle	6.80	-29.11
7	PECA	liver	8.23	-26.12
7	PECA	pectoral muscle	6.53	-29.27
7	PECA	skin	6.41	-28.01
8	PESI	claw	7.39	-19.82
8	PESI	leg muscle	8.89	-19.58
8	PESI	liver	8.34	-22.51
8	PESI	pectoral muscle	9.35	-19.72
8	PESI	skin	9.51	-18.17
9	PECA	claw	5.85	-22.03
9	PECA	kidney	7.64	-23.63
9	PECA	leg muscle	7.16	-24.90

9	PECA	liver	8.12	-23.79
9	PECA	pectoral muscle	7.13	-25.14
9	PECA	skin	9.80	-18.11
10	PESI	claw	6.69	-21.62
10	PESI	leg muscle	10.90	-19.24
10	PESI	liver	9.83	-21.29
10	PESI	pectoral muscle	10.58	-19.94
10	PESI	skin	11.35	-15.63
11	PESI	claw	9.65	-16.44
11	PESI	leg muscle	10.86	-17.70
11	PESI	liver	11.93	-18.38
11	PESI	pectoral muscle	11.07	-17.89
11	PESI	skin	11.46	-14.78
12	SICR	claw	9.28	-22.84
12	SICR	kidney	10.97	-24.75
12	SICR	liver	10.36	-26.99
12	SICR	scute	7.29	-31.41
13	SICR	claw	9.25	-21.34
13	SICR	kidney	10.80	-23.02
13	SICR	leg muscle	9.61	-26.48
13	SICR	liver	9.40	-24.99
13	SICR	pectoral muscle	9.79	-22.81
13	SICR	scute	6.96	-25.31
14	SICR	claw	8.98	-22.03
14	SICR	kidney	10.66	-22.28
14	SICR	liver	8.12	-24.52
14	SICR	pectoral muscle	10.35	-22.48
14	SICR	scute	6.66	-23.50
15	SICR	claw	10.02	-21.70
15	SICR	kidney	11.57	-22.43
15	SICR	leg muscle	9.45	-24.29
15	SICR	liver	10.25	-23.58
15	SICR	pectoral muscle	9.99	-23.70
15	SICR	scute	7.49	-23.14
16	SICR	claw	5.42	-25.23
16	SICR	kidney	6.76	-25.98
16	SICR	leg muscle	7.13	-25.24
16	SICR	liver	6.39	-26.35
16	SICR	pectoral muscle	6.54	-25.51
16	SICR	scute	4.68	-25.37
17	LEYU	claw	5.78	-26.53
17	LEYU	follicle	5.00	-26.89
17	LEYU	leg muscle	6.05	-26.42
17	LEYU	liver	5.73	-26.75
17	LEYU	pectoral muscle	6.32	-26.53
18	LEYU	claw	6.65	-25.72

18	LEYU	kidney	7.86	-24.78
18	LEYU	leg muscle	6.10	-25.18
18	LEYU	liver	7.53	-25.29
18	LEYU	pectoral muscle	6.73	-25.02
19	LEYU	claw	6.07	-26.48
19	LEYU	egg #1	5.26	-22.63
19	LEYU	egg #2	5.74	-23.79
19	LEYU	follicle #1	5.32	-26.67
19	LEYU	follicle #2	5.82	-26.93
19	LEYU	kidney	6.49	-25.80
19	LEYU	leg muscle	5.96	-25.84
19	LEYU	liver	6.80	-26.09
19	LEYU	pectoral muscle	6.35	-26.00
20	CUAM	claw	7.61	-27.62
20	CUAM	kidney	7.62	-27.68
20	CUAM	leg muscle	7.92	-27.50
20	CUAM	liver	8.06	-28.41
20	CUAM	pectoral muscle	8.21	-27.35
20	CUAM	scute	6.56	-28.57
21	CUAM	claw	4.83	-29.84
21	CUAM	kidney	5.95	-29.95
21	CUAM	leg muscle	6.48	-29.35
21	CUAM	liver	5.65	-30.36
21	CUAM	pectoral muscle	6.68	-29.72
21	CUAM	scute	5.70	-30.42
22	LEYU	claw	6.22	-25.82
22	LEYU	follicle	2.42	-28.00
22	LEYU	kidney	5.93	-25.26
22	LEYU	leg muscle	5.45	-26.35
22	LEYU	liver	5.98	-25.82
22	LEYU	pectoral muscle	5.53	-26.18
22	LEYU	scute	2.57	-26.93
23	LEYU	claw	6.88	-25.59
23	LEYU	leg muscle	7.67	-24.69
23	LEYU	liver	7.06	-25.27
23	LEYU	pectoral muscle	8.61	-22.23
23	LEYU	scute	4.11	-26.17
24	PESI	claw	9.68	-15.40
24	PESI	follicle	10.74	-16.31
24	PESI	leg muscle	10.65	-16.11
24	PESI	pectoral muscle	10.83	-15.90
24	PESI	skin	10.50	-14.05
25	RHPM	claw	8.40	-21.55
25	RHPM	leg muscle	8.49	-21.80
25	RHPM	liver	9.06	-22.61
25	RHPM	pectoral muscle	8.41	-21.53

25	RHPM	scute	5.95	-23.26
26	RHPM	claw	9.86	-20.33
26	RHPM	kidney	10.81	-20.77
26	RHPM	leg muscle	10.02	-21.08
26	RHPM	liver	10.28	-21.46
26	RHPM	pectoral muscle	10.46	-21.10
26	RHPM	scute	6.50	-24.34
27	RHPM	leg muscle	9.53	-25.61
27	RHPM	pectoral muscle	9.80	-25.92
27	RHPM	scute	9.52	-26.35
28	CHPA	kidney	9.55	-25.51
28	CHPA	leg muscle	9.60	-27.12
28	CHPA	liver	9.37	-25.62
28	CHPA	pectoral muscle	9.70	-27.11
28	CHPA	scute	7.22	-28.27
29	CHPA	claw	10.37	-21.01
29	CHPA	kidney	11.41	-22.31
29	CHPA	leg muscle	10.54	-23.39
29	CHPA	liver	11.54	-22.61
29	CHPA	pectoral muscle	10.74	-22.93
29	CHPA	scute	8.90	-21.69
30	MATE	pectoral muscle	13.97	-18.83
30	MATE	pectoral muscle	13.98	-18.73
31	MATE	claw	10.20	-17.82
31	MATE	follicle	12.39	-18.10
31	MATE	kidney	11.45	-18.36
31	MATE	leg muscle	11.90	-17.37
31	MATE	liver	11.01	-19.05
31	MATE	pectoral muscle	12.60	-18.28
31	MATE	scute	9.73	-19.39
32	MATE	follicle	14.59	-17.57
32	MATE	leg muscle	13.97	-17.47
33	MATE	claw	9.54	-18.11
33	MATE	kidney	12.98	-17.17
33	MATE	liver	11.46	-18.46
33	MATE	pectoral muscle	12.63	-17.66
33	MATE	scute	8.09	-22.24
34	MATE	claw	9.77	-17.60
34	MATE	leg muscle	11.87	-17.84
34	MATE	liver	11.49	-18.73
34	MATE	pectoral muscle	11.91	-18.14
35	MATE	claw	12.87	-16.33
35	MATE	leg muscle	14.44	-15.92
35	MATE	liver	14.10	-16.97
35	MATE	pectoral muscle	14.54	-16.54
35	MATE	scute	12.70	-16.43

36	HESP	follicle #1	8.84	-21.10
36	HESP	follicle #2	8.90	-21.45
36	HESP	follicle #3	8.82	-21.57
36	HESP	follicle #4	8.99	-22.36
36	HESP	follicle #5	8.94	-22.17
36	HESP	follicle #6	8.82	-23.10
36	HESP	follicle #7	9.47	-21.06
36	HESP	follicle #8	9.12	-21.00
36	HESP	follicle #9	8.51	-20.74
36	HESP	pectoral muscle	8.23	-21.64
37	LEYU	claw	5.63	-26.34
37	LEYU	follicle	5.55	-26.62
37	LEYU	leg muscle	5.11	-26.31
37	LEYU	liver	6.27	-26.42
37	LEYU	pectoral muscle	5.64	-26.19
37	LEYU	scute	0.61	-27.74
38	RHPM	claw	8.85	-22.23
38	RHPM	kidney	9.11	-21.46
38	RHPM	leg muscle	9.95	-20.71
38	RHPM	liver	8.70	-23.01
38	RHPM	pectoral muscle	10.24	-21.40
38	RHPM	scute	10.40	-19.77
39	CHRE	claw	9.58	-19.83
39	CHRE	follicle	11.05	-20.12
39	CHRE	kidney	11.22	-20.49
39	CHRE	liver	10.33	-20.93
39	CHRE	pectoral muscle	11.74	-20.66
39	CHRE	scute	9.21	-19.86
40	CHRE	claw	7.72	-21.16
40	CHRE	follicle	8.01	-21.62
40	CHRE	kidney	8.05	-21.68
40	CHRE	leg muscle	8.39	-22.46
40	CHRE	liver	7.44	-23.39
40	CHRE	pectoral muscle	8.08	-24.82
40	CHRE	scute	5.88	-23.26
41	CHRE	leg muscle	8.66	-21.65
41	CHRE	liver	8.46	-22.02
41	CHRE	pectoral muscle	8.65	-22.01
41	CHRE	scute	9.92	-18.11
42	CHRE	claw	8.51	-20.82
42	CHRE	kidney	11.70	-19.69
42	CHRE	leg muscle	11.12	-20.26
42	CHRE	liver	9.77	-22.93
42	CHRE	scute	10.39	-20.17
43	HESP	claw	6.85	-23.46
43	HESP	follicle	7.41	-22.83

43	HESP	kidney	7.74	-22.83
43	HESP	liver	7.56	-23.15
43	HESP	pectoral muscle	7.41	-23.33
44	HESP	kidney	8.19	-23.90
44	HESP	leg muscle	7.24	-24.10
44	HESP	liver	8.11	-23.64
44	HESP	pectoral muscle	7.55	-23.93
44	HESP	scute	4.42	-25.53
45	HESP	claw	8.61	-20.68
45	HESP	follicle #1	9.23	-20.01
45	HESP	follicle #2	8.75	-20.21
45	HESP	follicle #3	8.91	-20.32
45	HESP	follicle #4	8.72	-20.34
45	HESP	follicle #5	8.84	-20.33
45	HESP	follicles 41	9.40	-20.51
45	HESP	kidney	9.18	-20.95
45	HESP	leg muscle	7.65	-21.70
45	HESP	liver	9.40	-21.21
45	HESP	pectoral muscle	8.12	-21.24
45	HESP	scute	3.07	-25.39
46	HESP	claw	3.83	-25.21
46	HESP	kidney	5.18	-23.74
46	HESP	pectoral muscle	4.78	-24.89
47	HESP	claw	8.56	-22.83
47	HESP	kidney	9.44	-21.48
47	HESP	leg muscle	8.96	-22.33
47	HESP	liver	8.99	-22.40
47	HESP	scute	6.95	-23.50
48	SICR	claw	6.14	-27.94
48	SICR	kidney	6.97	-27.23
48	SICR	leg muscle	6.41	-26.09
48	SICR	liver	6.56	-27.97
48	SICR	pectoral muscle	6.32	-26.61
48	SICR	scute	3.75	-26.64
49	RHPM	claw	7.33	-24.97
49	RHPM	kidney	7.98	-24.13
49	RHPM	leg muscle	6.84	-23.75
49	RHPM	liver	7.76	-24.67
49	RHPM	pectoral muscle	7.20	-24.06
49	RHPM	scute	5.75	-24.39
50	PYMO	claw	7.46	-22.99
50	PYMO	liver	9.11	-23.89
50	PYMO	pectoral muscle	8.42	-23.01
50	PYMO	scute	5.34	-23.51
51	PYMO	claw	4.62	-24.52
51	PYMO	kidney	8.21	-22.37

51	PYMO	leg muscle	7.75	-22.57
51	PYMO	pectoral muscle	7.55	-22.99
51	PYMO	scute	4.83	-22.71
52	PYMO	claw	7.58	-23.01
52	PYMO	kidney	9.41	-22.78
52	PYMO	leg muscle	8.60	-23.20
52	PYMO	liver	9.84	-23.34
52	PYMO	pectoral muscle	8.61	-23.51
52	PYMO	scute	5.57	-23.44
53	PYMO	claw	7.34	-22.94
53	PYMO	kidney	8.79	-22.48
53	PYMO	leg muscle	8.47	-22.68
53	PYMO	liver	8.82	-23.92
53	PYMO	pectoral muscle	8.57	-22.91
53	PYMO	scute	5.62	-23.35
54	PYMO	kidney	7.91	-23.32
54	PYMO	leg muscle	7.28	-23.57
54	PYMO	liver	8.16	-23.84
54	PYMO	pectoral muscle	7.10	-23.96
54	PYMO	scute	4.15	-24.81
55	PYMO	claw	7.87	-22.41
55	PYMO	kidney	8.49	-22.24
55	PYMO	leg muscle	8.57	-22.14
55	PYMO	pectoral muscle	8.54	-21.95
55	PYMO	scute	6.56	-24.25
56	GESP	claw	8.14	-22.40
56	GESP	kidney	9.76	-23.46
56	GESP	leg muscle	8.54	-23.40
56	GESP	liver	9.12	-24.43
56	GESP	pectoral muscle	8.68	-23.23
56	GESP	scute	5.60	-23.54
57	GESP	claw	8.02	-22.36
57	GESP	kidney	9.51	-22.87
57	GESP	leg muscle	8.40	-23.17
57	GESP	pectoral muscle	8.46	-23.48
57	GESP	scute	5.49	-23.47
58	GESP	follicle	9.62	-22.61
58	GESP	leg muscle	8.05	-23.33
58	GESP	liver	8.49	-24.10
58	GESP	pectoral muscle	8.31	-23.52
58	GESP	scute	4.17	-23.60
59	GESP	claw	7.92	-22.24
59	GESP	liver	9.32	-22.89
59	GESP	pectoral muscle	8.79	-23.01
59	GESP	scute	4.19	-23.26
60	GEEL	claw	7.38	-25.66

60	GEEL	liver	8.39	-26.30
60	GEEL	scute	5.52	-24.97
61	GEEL	claw	7.38	-26.52
61	GEEL	leg muscle	7.77	-25.17
61	GEEL	liver	9.51	-26.50
61	GEEL	pectoral muscle	7.97	-25.28
61	GEEL	scute	5.27	-25.87
62	GEEL	claw	5.79	-25.99
62	GEEL	liver	6.57	-25.79
62	GEEL	scute	5.21	-25.53
63	GEEL	liver	8.64	-27.01
63	GEEL	scute	5.69	-27.69
64	GEEL	scute	5.39	-25.65
65	GEEL	claw	5.83	-26.06
65	GEEL	liver	7.99	-25.81
65	GEEL	scute	3.51	-25.83
66	KIFL	claw	17.60	-14.57
66	KIFL	pectoral muscle	14.00	-18.07
66	KIFL	scute	11.58	-19.66
67	KIFL	claw	8.32	-20.46
67	KIFL	liver	10.84	-20.26
68	KIFL	claw	8.11	-20.09
68	KIFL	leg muscle	12.10	-20.15
68	KIFL	liver	10.70	-21.29
68	KIFL	pectoral muscle	11.50	-20.52
68	KIFL	scute	10.36	-19.92
69	KIFL	claw	8.68	-20.35
69	KIFL	liver	10.35	-20.92
69	KIFL	scute	11.80	-19.02
70	KIFL	liver	10.04	-21.63
70	KIFL	pectoral muscle	11.05	-21.48
70	KIFL	scute	9.93	-24.00
71	KIFL	claw	8.61	-20.98
71	KIFL	liver	10.41	-22.01
71	KIFL	pectoral muscle	11.01	-20.78
71	KIFL	scute	9.19	-19.62

APPENDIX C: TURTLE MEASURMENTS

specimen ID #	species code	sex	mass (g)	standard carapace length (cm)	plastron length (cm)
1	CUAM	F	656.0	16.7	15.9
2	CUAM	F	575.0	17.5	16.9
3	CUAM	M	884.0	17.4	16.9
4	CUAM	F	842.0	17.9	17.5
5	CUAM	F	885.0	19.2	17.7
6	CUAM	F	1182.0	19.0	18.4
7	PECA	U	969.0	21.0	16.0
8	PESI	U	479.0	14.7	11.8
9	PECA	U	586.0	18.1	14.9
10	PESI	M	419.0	14.7	12.3
11	PESI	M	462.0	15.3	11.6
12	SICR	U	783.2	18.3	14.5
13	SICR	F	762.3	18.5	14.2
14	SICR	F	664.7	18.4	14.4
15	SICR	M	586.0	16.4	13.4
16	SICR	M	786.1	18.4	14.0
17	LEYU	F	1090.5	18.9	17.0
18	LEYU	M	1354.0	22.0	18.8
19	LEYU	F	1128.2	20.0	17.3
20	CUAM	M	991.3	18.4	17.4
21	CUAM	F	1018.3	18.7	18.4
22	LEYU	F	923.7	18.7	16.0
23	LEYU	M	1615.6	20.3	18.8
24	PESI	F	391.5	14.3	11.0
25	RHPM	M	1425.8	21.6	18.4
26	RHPM	F	707.6	16.0	16.0
27	RHPM	F	505.5	14.4	13.6
28	CHPA	M	1766.0	24.5	18.7
29	CHPA	F	1696.0	23.4	18.7
30	MATE	F	1288.0	21.2	19.1
31	MATE	F	880.0	17.2	15.8
32	MATE	F	1156.0	19.4	17.7
33	MATE	F	552.0	13.8	12.6
34	MATE	M	480.0	15.4	13.3
35	MATE	M	534.0	15.4	12.9
36	HESP	F	1150.0	19.6	17.6
37	LEYU	F	1392.0	20.5	18.1
38	RHPM	F	768.0	19.2	17.1

39	CHRE	F	858.0	18.0	16.0
40	CHRE	F	704.0	17.0	14.7
41	CHRE	M	184.0	11.4	10.0
42	CHRE	M	136.0	10.0	8.5
43	HESP	F	635.0	14.9	13.7
44	HESP	F	626.0	16.1	14.9
45	HESP	F	1246.0	19.1	16.0
46	HESP	F	1260.0	19.3	16.5
47	HESP	M	274.0	12.0	12.6
48	SICR	F	824.0	18.2	14.4
49	RHPM	F	426.0	14.5	13.6
50	PYMO	F	648.0	16.6	16.0
51	PYMO	F	664.0	15.7	15.7
52	PYMO	M	553.0	16.1	15.9
53	PYMO	F	494.0	15.7	14.9
54	PYMO	M	412.0	16.0	14.0
55	PYMO	M	390.0	14.3	13.4
56	GESP	M	120.0	9.7	7.7
57	GESP	M	112.0	9.5	7.8
58	GESP	F	117.0	9.4	8.3
59	GESP	M	116.0	9.7	8.1
60	GEEL	M	234.0	10.7	9.0
61	GEEL	M	402.0	12.6	10.9
62	GEEL	F	240.0	10.8	9.5
63	GEEL	F	86.0	7.4	7.0
64	GEEL	F	94.0	7.4	6.1
65	GEEL	F	182.0	11.3	10.4
66	KIFL	M	338.0	11.7	10.0
67	KIFL	M	284.0	11.4	9.7
68	KIFL	M	428.0	13.4	10.7
69	KIFL	F	156.0	9.1	8.5
70	KIFL	M	320.0	11.9	9.8
71	KIFL	M	358.0	12.5	11.2