TRACE METAL UPTAKE AND ACCUMULATION PATHWAYS IN KEMP'S RIDLEY SEA TURTLES (*LEPIDOCHELYS KEMPII*)

A Dissertation

by

HUI-CHEN WANG

Submitted to the Office of Graduate Studies of
Texas A&M University
in partial fulfillment of the requirements for the degree of

DOCTOR OF PHILOSOPHY

May 2005

Major Subject: Wildlife and Fisheries Sciences

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Approved as to style and content by:

Andre Landry (Chair of Committee)	Gary Gill (Member)
Lee Fitzgerald (Member)	William Neill (Member)
Duncan MacKenzie (Member)	Robert Brown (Head of Department)

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ABSTRACT

Trace Metal Uptake and Accumulation Pathways in Kemp's Ridley
Sea Turtles (*Lepidochelys kempii).* (May 2005)
Hui-Chen Wang, B.S., Taipei Medical University;
M.S., National Taiwan Ocean University
Chair of Advisory Committee: Dr. André M. Landry, Jr.

Little is known of trace metal concentrations and their possible role in the mortality of critically endangered Kemp's ridley sea turtles (*Lepidochelys kempii*). Research described herein characterized concentrations of seven trace metals – Ag, Cd, Cr, Cu, Hg, Pb and Zn – in the blood and carapace tissue of captive Kemp's ridleys for use in assessing levels of these metals in wild counterparts. Concentrations of same trace metals were characterized in post-pelagic through adult life stages of 127 wild Kemp's ridleys captured from the Gulf of Mexico and southeast Atlantic during 2000 to 2002. Blood, carapace, liver, kidney, and muscle tissues from live and/or stranded Kemp's ridleys were analyzed for the aforementioned trace metals via graphite furnace atomic absorption spectrophotometer and cold vapor atomic fluorescence techniques conducted under class-100 clean laboratory conditions. Similar trace metal assessments were conducted on blue crab (*Callinectes sapidus*) prey to determine the role of food as a possible uptake pathway in Kemp's ridleys.

Overall, trace metal levels in live, captive as well as wild ridleys were higher in carapace tissue than in blood. Carapace concentrations of Ag, Cr and Hg in Kemp's ridleys across all post-pelagic life stages increased with increasing straight carapace length (SCL). Carapace tissue of wild ridleys exhibited higher Cr, Hg, and Pb levels than their blue crab prey, regardless of study area; whereas, crabs yielded higher Ag and Cu concentrations. Dead stranded ridleys yielded higher Ag, Cr, Hg, Pb, Zn levels in carapace tissue, whereas, their liver exhibited higher Cd and Cu levels. This finding suggests carapace tissue could serve as a suitable surrogate sample source for internal organs/tissues when monitoring exposure of live Kemp's ridleys to certain metals. The fact that larger, stranded ridleys exhibited higher Ag, Cd, Hg, Pb and Zn levels than did their smaller, live analogs from Texas and Louisiana implies that these older ridleys had increased opportunities to accumulate higher metal concentrations in their carapace tissue than did their younger conspecifics. This trend suggests that carapace tissue has the potential to accumulate trace metals while blood-borne concentrations reflect only recent exposure to trace metals.

DEDICATION

The dissertation is dedicated to my father for his advice and my loving husband George for his continuous support and understanding.

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I would like to thank my committee members, Drs. André Landry, Jr., Gary Gill, Lee Fitzgerald, William Neill and Duncan MacKenzie for their advice and assistance throughout the course of my graduate study. Special thanks go to my principal advisor, Dr. André Landry, Jr., who accepted me as graduate student, gave me the opportunity to study Kemp's ridley sea turtles, believed in me and guided me in accomplishing this research.

I am grateful for the financial support provided by the Texas Sea Grant College Program, the National Marine Fisheries Service and the Texas Parks and Wildlife Department. I would like to acknowledge the Marine Biology Department at Texas A&M University at Galveston for providing graduate teaching and research assistantships enabling the completion of this degree. Travel funds were provided by the Sea Turtle Symposium, Texas A&M Graduate Program Enhancement Fund, Erma Lee and Luke Mooney Travel Grant, and Galveston Graduate Student Association.

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CHAPTER I

INTRODUCTION

The goal of the Kemp's ridley (*Lepidochelys kempii*) recovery plan (USFWS/NMFS, 1992) is to provide the scientific basis for downlisting this protected sea turtle species from an endangered to threatened status. This goal would be achieved with the attainment of a nesting population of 10,000 females on the Rancho Nuevo, Mexico beach around 2020 (USFWS/NMFS, 1992). Tactics for achieving this goal include continued protection of the Rancho Nuevo nesting beach, enforcing turtle excluder device (TED) regulations in the shrimping industry, and filling information gaps prerequisite to better conservation and management (Marine Turtle Specialist Group [MTSG], 1995; Turtle Expert Working Group [TEWG], 1998). However, aspects of distribution, habitat use by all life stages, reproductive physiology and behavior, recruitment and survivorship, and marine pollution represent large voids in our knowledge and management of Kemp's ridleys. Knowledge of contaminant loads and their impacts on health of Kemp's ridleys was one of the largest information gaps identified by Kemp's Ridley Stakeholder's Meeting participants charged with developing recommendations for revising this species' recovery plan (Earl Possardt, USFWS, personal communication, April, 2004).

This dissertation follows the style and format of Marine Pollution Bulletin.

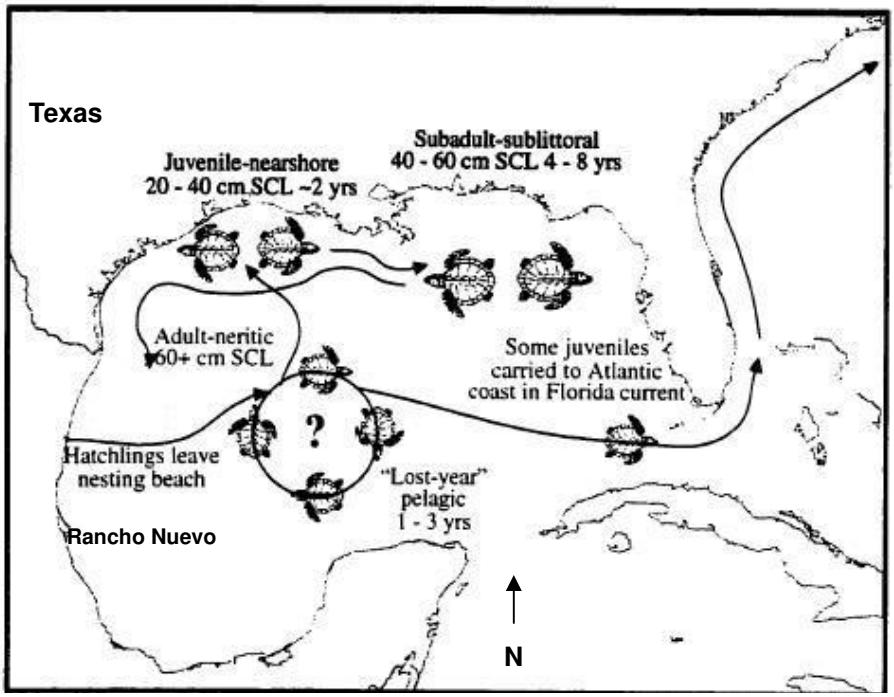


Fig. 1. Nesting beach and in-water habitats of Kemp's ridleys in the Gulf of Mexico and along the Atlantic coast (modified from Coyne, 2000).

Life History of Kemp's Ridley

Kemp's ridleys exhibit a life history typical of other sea turtle species, with the exception that their range is restricted to the Gulf of Mexico and southeast Atlantic. Detailed information on Kemp's ridley life history has been provided by Márquez-M (1994); Landry and Costa (1999); Coyne (2000); Metz (2004); and Landry *et al.* (2004). Juvenile and immature ridleys inhabit shallow, nearshore (Musick and Limpus, 1997) sandy/muddy habitats such as those along the Louisiana and upper Texas coasts (Fig. 1; Landry *et al.*, 1993 and 1995). Adult and juvenile ridleys have historically occurred in greatest abundances at Sabine Pass, Texas, Calliou Bay and Terrbonne Parish, Louisiana, and Big Gulley, east of Mobile Bay, Alabama (Ogren, 1989). They use these shallow coastal waters to feed on a large variety of benthic crustaceans, particularly portunid crabs (Shaver, 1991; Márquez-M, 1994) like the blue crab (*Callinectes sapidus*).

Role of Contaminants in Kemp's Ridley's Mortality

Kemp's ridleys' intensive use of the upper Texas and Louisiana coast as prime foraging grounds occurs in waters where the US' largest petrochemical industry discharges its effluent. Numerous petrochemical industries and intensive agricultural development along the northern Gulf coast are sources of environmental contaminants that may effect these foraging Kemp's ridleys. However, there are no data to quantify this effect (USFWS/NMFS, 1992). The petrochemical industry in Texas, especially that concentrated along its upper

coast (Galveston and Sabine-Neches estuaries), and in Louisiana's bays (Calcasieu River estuary), has increased dramatically since the 1960's to supply 60% of the US' current total production and 25% of this nation's refined oil (Petrochemical Industry, The Handbook of Texas Online).

Discharge of these petrochemical effluents as well as agricultural runoff and domestic sewage contaminate coastal receiving waters and sediment with Ag, Cd, Co, Cr, Cu, Hg, Ni, Pb, and Zn (Simon *et al*., 1994; Ravichandran *et al*., 1995a and 1995b; Sferra *et al*., 1999). Weber (1995) lamented the fact that the aforementioned contamination may have indirect effects on Kemp's ridley recovery by reducing habitat quality of its nearshore foraging grounds or health of prey communities. Direct effects, manifested by disruptions in physiological functions leading to reduced health and fitness or mortality of species constituents, also could greatly affect this species' recovery rate.

The role of marine pollution in sea turtle mortalities is not well documented (Lutcavage *et al*., 1997) and, thus, an unknown factor to resource managers charged with revising recovery plans. This is especially true for the Kemp's ridley, a species that continues to wash ashore dead on northwest Gulf beaches and whose recovery plan is outdated. Studies on the relationship between sea turtle mortality and marine pollution are generally limited to ingestion of plastics, entanglement in debris and oil toxicity (Carr, 1987; Plotkin and Amos, 1988; Stanley *et al*., 1988; Magnuson *et al*., 1990; Bjorndal *et al*., 1994; Lutcavage *et al*., 1995; Milton *et al*., 2003). Like marine mammals and sea birds, sea turtles

are long-lived, highly-mobile vertebrates occupying upper trophic levels (Meyers-Schone and Walton, 1994). As such, sea turtles represent potential indicators of contaminant uptake through the marine food chain because of their long life-span and possible bioaccumulation of pollutants.

Several studies (Magnuson *et al*., 1990; Chang, 1996; Hogstrand *et al*., 1999; Lorenzon *et al*., 2000) have shown exposure of marine animals to pollutants causes short – or long – term impacts such as increased incidence of disease and lower reproduction rate in crustaceans, fish and other animals. However, little is known about baseline values or physiological effects resulting from uptake and accumulation of environmental pollutants in sea turtles (Witkowski and Frazier, 1982; Aguirre *et al*., 1994; Godley *et al*., 1999).

Various ecological and biological factors including geographic location, diet, age, sex and distribution within organs have shown to affect trace metal concentration in vertebrates such as marine mammals (Wagemann *et al*., 1996; Das *et al*., 2003) and sea turtles (Storelli and Marcotrigiano, 2003). Many investigators reported that Hg accumulates with age in most marine mammals (Becker *et al*., 1995; Meador *et al*., 1999; Ikemoto *et al*., 2004) and loggerhead and green turtles (Gordon *et al*. 1998, Storelli *et al*., 1998b; Sakai et al., 2000a; Anan *et al*., 2002a). Variability of trace metal levels also has been observed between sexes of marine animals (Marcovecchio *et al*., 1994; Das *et al*., 2003), thus suggesting a hormonal impact.

Obstacles to Studying Trace Metals in Sea Turtles

Endangered species permit restrictions have limited previous studies of metal concentrations in sea turtles to those involving stranded carcasses, turtles killed incidentally in commercial fishing gear, and infertile eggs/dead hatchlings (Witkowski and Frazier, 1982; Davenport and Wrench, 1990; Aguirre *et al*., 1994; Sakai *et al*., 1995, 2000a and 2000b; Vazquez *et al*., 1997; Gordon *et al*., 1998; Storelli *et al*., 1998a and 1998b; Caurant *et al*., 1999; Godley *et al*., 1999; Alam and Brim, 2000; Anan *et al*., 2002a; Storelli and Marcotrigiano, 2003). While these studies have yielded useful information on Al, As, Ca, Cr, Cd, Cu, Fe, Hg, Mg, Mo, Ni, Pb, Se, Ti, Zn, and V, little is known of trace metal concentrations in live constituents. Details information on the aforementioned studies will be described in latter section.

The fact that tissue decomposition negates collection of fresh internal organs from stranded sea turtles and fosters concerns as to the chemical integrity of decaying tissue mandates that: 1) live counterparts be used in meaningful trace metal characterizations; and 2) safe and effective sample procedures be identified for such use. Pugh and Becker (2001), who compared contaminant information on sea turtles with that for marine mammals and freshwater turtles, found limited data, especially for the endangered Kemp's ridley. Their bibliography of sea turtle contamination reported that Cd, Hg and Zn levels were similar or lower than those found in marine mammals and seabirds while the former exhibited higher Cu concentrations. These workers also

recommended establishing a contaminant database for Kemp's ridleys because the potential for this species' exposure to different anthropogenic pollution sources in the Gulf of Mexico is high, with virtually nothing known regarding contaminant loading in this region.

The limited trace metal data available for live Kemp's ridleys results in part from logistical problems associated with capturing and working on an endangered species whose population level is severely depleted. The earliest study of trace metal levels in live ridleys characterized Ag, Cu, Hg, Pb, and Zn concentrations in the blood of conspecifics captured in entanglement netting operations along the Texas and Louisiana coast (Orvik, 1997; Kenyon *et al.*, 2001). This study reported that trace metal blood levels in these live ridleys were lower than tissue levels elsewhere for marine and freshwater turtles, other reptiles, fish, seabirds and marine mammals. The aforementioned workers also identified Cu, Hg and Zn concentrations as exhibiting significant positive relationships with turtle size. A later study evaluated carapace tissue as a non-invasive source for assessing Hg concentration in ridleys (Presti *et al.*, 2000).

Collection of internal tissues for trace metal analysis in live sea turtles is virtually impossible given these organisms' protected status and permit restrictions designed to reduce risks associated with invasive surgery required for said collection. Consequently, trace metal characterization in sea turtles has had to rely on analysis of samples from dead stranded individuals or the

development of non-invasive sampling techniques for use on live constituents.

Scraping of superficial carapace tissue is considered a safe, non-invasive method (Presti *et al.*, 2000) for collecting samples used in characterizing trace metal loads in endangered sea turtle species. The outer carapace layer is composed of keratinous epidermal structures growing above the bones. Keratin, a non-living tissue, has been shown useful in measuring trace metal levels of several sea turtle species (Presti *et al.*, 2000; Sakai *et al.*, 2000a). However, the transfer mechanism of trace metals into keratinous tissue of sea turtle is still unknown. Sakai *et al.* (2000a) also found Mn, Zn and Hg accumulate in carapace tissues. These carapace tissues are keratinous epidermal structures that grow above the bones. Blood, which acts as a carrier in transporting trace metal elements to organs such as the liver and kidney, is another potential source for characterizing contaminant uptake without the risk of invasive sampling of organ tissues (Chang, 1996). However, the validity of using blood to assess trace metal uptake in sea turtles will require characterization of the contaminant loading relationship between it and other body tissues.

Trace Metal Studies Involving Sea Turtles

Trace metal levels in sea turtles appear to vary with tissue source because of inter-specific differences in diets and trophic status. Food habits of sea turtles are species-and/or life-history-specific. For example, adult loggerhead turtles are carnivorous; whereas, juvenile green turtles are omnivorous while their adult

counterparts are herbivorous (Bjorndal, 1997).

Trace metal studies in stranded sea turtles have most commonly targeted non-essential Cd, Hg and Pb metals in muscle, liver, and kidney tissue (Godley *et al*., 1999; Sakai *et al*., 2000a; Storelli and Marcotrigiano, 2003). Beck *et al*. (1997) reported sea turtles contained low Hg concentrations (2,000 - 4,000 ppb in liver) when compared with those of marine mammals (< 100,000 - 400,000 ppb). Conversely, other investigators (Honda *et al*., 1990; Sakai *et al*., 2000a; Anan *et al*., 2001) found loggerhead and green turtles exhibited higher Cu levels in their livers (loggerhead: 17,700 ppb and green: 139,000 ppb) than did cetaceans (5,000 ppb) and seabirds (6,000 ppb). Like those for other marine vertebrates, highest Hg, Cd, and Pb levels in sea turtles are typically found in liver, kidney, bone/carapace, respectively (Davenport and Wrench, 1990; Godley *et al*., 1999; Sakai *et al*., 1995 and 2000a).

Investigations on essential metal concentrations in stranded sea turtles found Cu levels were highest in liver, while bone and carapace contained higher Zn concentrations (Caurant *et al*., 1999; Sakai *et al*., 1995 and 2000a). Copper and Hg were higher in liver of both loggerhead and green turtles than in their kidney while a reverse relationship was true for Cd.

The carnivorous loggerhead turtle generally exhibits a higher metal load than do herbivorous green turtles (Storelli *et al*., 1998a; Sakai *et al*., 2000a); whereas, the jellyfish-eating leatherback accumulates higher levels of Cd than do the other two species (Caurant *et al*., 1999). Trends such as these as well as

other specific studies lend strong support to the assumption that food is the main pathway of sea turtle exposure to trace metals. Sakai *et al*. (2000a) first reported that trace metal concentrations in the carapace were correlated with whole body burdens, thus indicating carapace is a potentially useful non-lethal indicator for monitoring metal levels in sea turtles.

Trace Metals and Biological Activities

Trace metals may enter the body through respiration, consumption or by absorption through the skin. Natural and anthropogenic sources (i.e. burning fossil fuel, municipal waste, industrial effluents, mining) account for a variety of trace metal inputs into the aquatic system. Seven metals including essential (Cr, Cu and Zn) and non-essential elements (Ag, Cd, Hg and Pb) were chosen for analysis because these metals are persistent in the aquatic environment and toxic to organisms at elevated levels (Cohen *et al*., 2001). Three of the aforementioned elements (Cd, Hg and Pb) are the most frequently monitored trace metals in sea turtles. In addition, Canli *et al*. (2001) mentioned that Class B metals (Ag, Cd, Cr, Hg and Zn) originating from human activities are major seawater pollutants that alter many physiological processes and biochemical parameters in blood or tissue and may cause structural deformities in some organisms.

Chromium (Cr)

Very few studies have been conducted to assess Cr-related toxicity in marine vertebrates (Eisler, 1986). What is known about Cr exposure comes from human studies. Target organs affected by Cr exposure include liver and kidney (USEPA, 1980a). Chromium can be excreted in hair and fingernails of humans while the major pathway of excretion is feces. Accidental intake of large amounts of Cr can cause kidney and liver damage and even death (ATSDR, 2000).

Copper (Cu)

Copper enters the aquatic environment through releases from mines, domestic waste water, combustion of fossil fuel and natural sources (e.g. volcanoes). Sediment seems to be the sink of Cu in the aquatic environment because it binds strongly to particles. Copper is essential for several enzymatic activities (e.g. hemoglobin formation) and is usually obtained through the diet (Chang, 1996). Excess Cu absorbed into gastrointestinal cells is bound to metallothionein after nutritional requirements are met and excreted in feces of humans (Eisler, 1998). In addition, exposure to high Cu levels can have toxic effects. Eisler (1998) reported biological variables affecting Cu accumulations in marine organisms include size, age, developmental stage, physiological adaptation and tissue specificity. Davis and Mertz (1987) suggested that organisms differ greatly in their tolerance of high Cu levels in their diets.

Zinc (Zn)

Most Zn exists in the environment as a result of mining, smelting, steel production, coal burning, and waste incineration (ATSDR, 2003). Zinc enters aquatic systems through waste streams, domestic wastewater and runoff, and it settles on the bottom. Like Cu, Zn is an essential element in maintaining cellular functions of many enzymes and protein structures, but may become toxic if accumulated at higher concentrations (Hogstrand *et al.*, 1996). Diet is the main source of Zn exposure in humans (ATSDR, 2003). After ingestion, Zn is rapidly transferred to the plasma and bound to albumin (Eisler, 1993).

Silver (Ag)

Silver, a non-essential element, enters in the aquatic environment as a result of municipal and industrial wastewater treatment releases, especially by the photographic and medical industries (Ratte, 1999; Saeki *et al.*, 2001). Upon entering the human body, Ag combines with plasma proteins and is stored in liver, skin and kidney in humans (ATSDR, 1990). Silver levels were examined because there is little known about the potential for adverse effects in marine organisms (Eisler, 1996), especially as it relates to sea turtles.

Cadmium (Cd)

Mine and agricultural runoff and wastes from metal smelting are primary sources of Cd contamination. Cadmium is a non-essential element not

metabolically regulated by vertebrates and, as such, is toxic to humans and other animals (Kostial, 1986). Food digestion rather than inhalation is the most likely uptake route of Cd in vertebrates. After ingestion, Cd is transported to the gut and is excreted in the feces. The kidney is the principal storage site for Cd, followed by the liver and other tissues (muscle, skin and bone) in humans and marine vertebrates (Kostial, 1986; Thompson, 1990; ATSDR, 1999a). Cadmium has a very long biological half-life (30 years in humans) and is one element constantly monitored in marine vertebrates because it accumulates with age by binding with metallothionein within the liver (Thompson, 1990; ATSDR, 1999a).

Mercury (Hg)

Mercury can be released in either a naturally or anthropogenic inorganic form that may be subsequently transformed into organic Hg by microorganisms in marine sediment (Thompson, 1990; ATSDR, 1999c). Mining, smelting and chloralkali plants produce Hg and emit it to the atmosphere. Mercury is of concern because of its persistence, biomagnification, and bioaccumulation through the food chain, especially in its organic methylmercury (MeHg) form in humans and marine organisms (USEPA, 1985; Mason *et al.*, 1996; Morel *et al.*, 1998). Nielsen and Grandjean (2000) mentioned kidneys are the predominant sites of inorganic Hg accumulation. Feathers have been used to monitor Hg levels in birds to a greater extend than for any other metal since Hg bonds strongly to disulfide linkages and Hg levels in feathers are not affected by various

vigorous treatments (Thompson, 1990). Mercury is excreted in the urine and feces of humans (ATSDR, 1999c).

Lead (Pb)

The primary source of Pb in the environment is from mined ores and batteries (ATSDR, 1999b). Food is the primary source of this highly toxic non-essential metal in vertebrates where it carried by blood and rapidly absorbed in soft tissues (Eisler, 1988; ATSDR, 1999b). Bone, particularly in dense cortical bone, where high levels of calcium are found, seems to be the target tissue for Pb accumulation in humans and marine organisms (Eisler, 1988; Mahaffey *et al.*, 2000).

Research Objectives and Hypotheses

Specific research objectives presented herein are to (1) develop estimates of baseline trace metal concentrations in Kemp's ridleys; (2) characterize and compare trace metal concentrations in blood and carapace tissues of live Kemp's ridleys (captive and wild) with those of internal organ and muscle tissues from stranded counterparts across their size range; and (3) assess the role of a primary prey species (blue crab) as a source of trace metal uptake by Kemp's ridleys.

These research objectives provide information on trace metal uptake and accumulation pathways in Kemp's ridley sea turtles and will allow the following

hypotheses to be tested: 1) wild ridleys exhibit higher trace metal concentrations than do their captive-reared conspecifics; 2) internal organs yield higher trace metal concentrations than do carapace tissue; and 3) trace metal concentration in ridley tissues is size/age dependent.

CHAPTER II

RESEARCH APPROACH

Homer *et al*. (1998) suggested that the study of free-ranging species like the Kemp's ridleys that inhabit potentially polluted environments would be incomplete without addressing the impact of environmental toxins on survival and recovery. Furthermore, long-lived vertebrates such as sea turtles represent useful indicators of contaminant accumulation, some of which may be age-dependent. However, no trace metal accumulation study has been conducted in live Kemp' ridleys across its constituent life stages or the foraging environments these stages may occupy. To this end, research reported herein sought to characterize trace metal concentrations in live, wild Kemp's ridleys representing post-pelagic through adult stages and, by so doing, provide valuable insight into the role of contaminants on this endangered species' health and recovery. Research summarized herein: 1) estimates baseline trace metal levels in captive Kemp's ridleys (Chapter III); 2) characterizes trace metal concentrations in wild Kemp's ridleys and assesses the role of prey as a pathway for contaminant uptake (Chapters IV and V); and 3) determines metal levels in ridleys stranded along the Texas coast (Chapter VI).

By doing so, this study generates valuable information on trace metal concentrations in Kemp's ridleys from the Gulf of Mexico and southeast Atlantic that is prerequisite to a better understanding of the role of foraging environment

on this species' health and survival. In addition, this study identifies research questions that must be addressed in revising the Kemp's Ridley Recovery Plan.

Various investigators (Witkowski and Frazier, 1982; Meyers-Schone and Walton, 1994; Law, 1996) have reported difficulties in interpreting trace metal data without adequate background data. The lack of contaminant studies on the Kemp's ridley, its severely depleted population status, and research restrictions placed on studies involving protected species have left a tremendous void in our knowledge of trace metal loads in this species. This is particularly the case involving availability of baseline data on which to assess contaminant levels across constituent life history stages and foraging environments of live ridleys. As a result, research reported in Chapter III uses Kemp's ridleys raised in captivity for 9 to 24 months as surrogates on which to approximate baseline levels used in later assessments of trace metal concentrations in wild conspecifics.

As a follow-up to establishing baseline information in captive-reared ridleys, Chapter IV provides a similar characterization and assessment of trace metal concentrations in live conspecifics captured from nearshore waters of Texas, Louisiana, and the southeast Atlantic. Recruitment and population status investigations conducted by the Sea Turtle and Fisheries Ecology Research Laboratory (STFERL) at Texas A&M University at Galveston along the upper Texas and Louisiana coasts during 2000-2002 provided an opportunity to capture juvenile and subadult turtles for this purpose. Other subadult ridleys

captured in shrimp trawls offshore South Carolina during 2001-2002 were used as additional sources upon which to assess trace metal concentrations in post-juvenile life stages. Research described in this chapter characterizes trace metal concentrations in blood and carapace tissue of live wild ridleys as well as in their blue crab prey and compares them with baseline levels reported in Chapter III to assess uptake and potential accumulation.

As a continuum to life history information presented in Chapter IV and especially that related to adult ridleys, Chapter V reports on trace metal levels in nesting females on the Rancho Nuevo, Mexico beach. In addition, trace metal levels of blue crabs collected from waters adjacent to the nesting beach were analyzed in assessing a prey's role in trace metal uptake by older constituents. Although live wild ridleys were used in the aforementioned trace metal characterizations, these assessments are limited to concentrations in blood and carapace tissue, given the restrictions on invasive sampling of a protected species like the Kemp's ridley. Internal tissues requiring invasive procedures on live constituents can be more easily obtained from dead stranded cohorts that wash onto coastal beaches. As such, Chapter VI reports on trace metals in ridleys stranded along the Texas coast during 2001-2002 as a means of characterizing concentrations in internal tissues. These latter determinations were deemed prerequisite to identifying possible correlations in trace metal levels between the carapace and liver, kidney, muscle tissue, as well as the relationship of metal loads in carapace tissue of live versus stranded ridleys.

CHAPTER III

TRACE METAL CONCENTRATIONS IN CAPTIVE-REARED KEMP'S RIDLEY SEA TURTLES

Research reported in this chapter characterizes metal concentrations in captive Kemp's ridleys used in generating baseline data upon which to assess contaminant levels in wild ridleys captured in the Gulf of Mexico and southeast Atlantic. Research restrictions listed in NMFS Endangered Species Permit (Permit # 1133), under which this study was conducted, allowed only blood and carapace tissue of ridleys to be analyzed for metal uptake in captive ridleys. Additionally, pelleted food fed to these captive ridleys was tested for trace metal composition.

The US and Mexican governments initiated a cooperative headstart program in 1978 that was designed to increase survival of hatchling and juvenile Kemp's ridleys as well as expand this species' nesting range. Approximately 2,000 Kemp's ridley eggs were transported annually from the Rancho Nuevo, Mexico nesting beach to the Padre Island National Seashore (PINS) research station between 1978 and 1988 for incubation and hatching (Owens *et al.*, 1982; Shaver, 1989; Manzella and Williams, 1992). After hatching and imprinting in Gulf waters off Padre Island, these hatchlings were then transported to Galveston for captive rearing in the NMFS Kemp's Ridley Headstart Program. By 1989, the imprinting experiment was terminated and all hatchlings were

transferred directly to the NMFS Galveston Laboratory from the Rancho Nuevo nesting beach. Approximately 25,600 live hatchings belonging to 1978 through 1992 year classes were reared between 9 -12 months and up to 2 years and tagged, with 87% of these released as yearlings into the Gulf of Mexico (Fontaine *et al.*, 1989; Caillouet *et al.*, 1995; Klima and McVey, 1995).

The NMFS Kemp's Ridley Headstart Program provided two opportunities for trace metal research. First, it provided access to post-hatchling and juvenile life stages difficult to capture in the wild. Second, captive-reared ridleys fed a controlled, artificial diet and raised in seemingly unpolluted (i.e. offshore) Gulf water served as "controls" for wild conspecifics exposed to a wide array of natural food sources and factors promoting contaminant uptake.

Research summarized herein determined baseline trace metal concentrations in blood and carapace tissue of captive-reared Kemp's ridleys. Information generated in pursuit of this objective allowed the following hypotheses for respective metals to be tested: 1) baseline trace metal concentrations in carapace tissues of captive Kemp's ridleys are higher than those in the blood; and 2) blood and carapace tissue tend to accumulate trace metals at levels higher than those in the pelleted food they eat.

Materials and Methods

Captive-Rearing Protocol

The history and description of NMFS' Kemp's Ridley Headstart Program have been summarized by Higgins (2003). Headstarted ridleys were held in individual containers (due to their aggressive nature) housed in large raceways filled with Gulf water pumped from the Galveston surf. All captive ridleys were fed dry floating pellets (AquaMax® fish diet, 5D05, Grower 500, PMI Nutrition International Inc.) formulated as a high energy, nutrient dense diet. Each 4.7-mm diameter pellet was small enough to be consumed by all sizes of ridleys (Higgins, 2003) and consisted of approximately 56% protein, 17% fat, 1% fiber, 2% nitrogen, and less 20% of ash (minerals). A new supply of AquaMax® 500 was purchased every 4 to 6 weeks, frozen and defrosted 24 hours prior feeding. A 22.5 kg (50 lb) bag lasted for approximately 1 week (Cain Bustinza, personal communication).

The feeding regime administered to captive-reared Kemp's ridleys was as follows (Higgins, 2003). Starting on day 10, each hatching was fed one pellet representing about 2% of its body weight. This feeding rate was increased during week 2 by one pellet each week, through week 24, until each turtle received 12 pellets twice a day. Beginning in week of 25, hatchings were fed twice a day according to the formula $Y = 0.12515 + [(11.502x)/1000]$, developed by NMFS for Kemp's ridleys where x = turtle weight in grams and Y = amount of feed per turtle per day in grams (Higgins, 2003). The latter feeding regime was

maintained until immediately prior to a ridley's release.

Natural seawater was pumped into the NMFS headstart facility directly from the Gulf of Mexico (Caillouet, 2000), where it was exchanged 3 to 6 times per week and monitored for water quality parameters [temperature (26 - 30 ºC), salinity (14 - 32 parts per thousand) and pH (7.5 - 8.1)] essential to growth and survival of captive Kemp's ridleys (Higgins, 2003).

Trace metal concentrations in sea water were not quantified for several reasons. Sea turtles constrict their esophagus to prevent incidental drinking (Lutz, 1997) and, in doing so, limit metal uptake via seawater ingestion. Cutaneous absorption studies have not been performed on sea turtles. However, Saeki *et al.* (2001) reported limited trace metals absorption through the skin in marine mammals that should also apply in sea turtles.

Sample Collection

Blood and carapace tissue samples were collected from captive Kemp's ridleys held at the NMFS headstart facility in 2001 and 2002. Depending upon turtle size, at least 3 to 5 mL of blood were drawn from the bilateral cervical sinus of each turtle into lithium heparinized vacutainers following the method described by Owens and Ruiz (1980). All blood samples were placed on ice and frozen until analyzed.

Approximately 0.2 g of carapace tissue was removed from the postcentral and three neighboring marginal scutes, using a plastic Teflon scraping spatula.

These scutes were cleaned with water and alcohol swabs to remove any algae or excess food debris. Latex gloves were worn when scraping carapace tissue to avoid sample contamination. All scraping tools were cleaned with an alcohol swab before and after each carapace tissue collection. Each carapace tissue sample was first placed in a whirl-pak bag that was then put in a plastic zip-lock bag. All carapace tissue samples were stored in a freezer until analysis.

Since captive ridleys were fed dry pellets manufactured with the same ingredients over the year by PMI Nutrition International Inc., food was collected only one time for analysis of metal concentrations. Approximately 0.1 - 0.2 g of dry pellets was randomly collected 10 times on the same day from one 22.5 kg (50 lb) bag of AquaMax during 2002 and used as a food source for measuring metal levels. Captive ridleys weighed on average 5.0 kg and were fed approximately 100 g of food per day at this sampling.

Sample Digestion

All digestion processes were conducted under class-100 laboratory conditions in the Laboratory for Oceanographic and Environmental Research (LOER) at Texas A&M University at Galveston. Sample digestion required the use of strong acid and taking up the residue in dilute acid. This process consisted of a standard acid digestion procedure to convert organic forms of metal to inorganic forms and minimize organic interferences (USEPA, 1986). Blood digestion followed methods developed by Orvik (1997). Frozen blood

samples were thawed in a refrigerator until liquefied before digestion. About 1 g of whole blood was added to a Teflon vial containing 5 mL of ultra pure nitric acid (Q-HNO_3). The digestion mixture was dried at ~ 60 ºC on a hot plate in a class-100 clean-air hood. After cooling, another 1 mL of Q-HNO_3 was added to the vial and the sample was digested again. Following this second digestion, 5 mL of 0.5 N Q-HNO_3 were added to the vial and held for analysis.

Carapace tissue samples were dried in an oven at ~ 50 ºC for at least 24 hours to eliminate water content prior to digestion. Approximately 0.1 g of dry carapace tissue was added to a Teflon vial containing 5 mL of Q-HNO_3. The vial was capped and digested at room temperature in a class-100 fume hood for at least 24 hours or until all residue was completely digested. Food pellets were processed following the same digestion method used for carapace tissue.

Metal Analysis

All metal concentrations were measured on a ppb (ng/g: parts per billion-ppb) with three significant figures as dry weight (d.w.) for food and carapace tissue, and wet weight (w.w.) for blood. Each sample was measured in triplicate. Cadmium, Cr, Cu, Pb, Ag and Zn levels were measured by Perkin Elmer 5100 graphite furnace atomic absorption spectrometer (GFAAS) and Zeeman background correction.

Analytical protocols and dilution methods followed those described by Orvik (1997) and refined in this study. Analysis of Ag and Pb required standard

addition procedures that combined digested samples and known concentrations of a standard solution covering a range appropriate for each metal. Cadmium analysis required a matrix modifier – monoammonium phosphate ($NH_4H_2PO_4$) + magnesium nitrate ($Mg(NO_3)_2$) solution with digested samples in order to increase the analytical signal.

Mercury concentrations were determined by the cold vapor atomic fluorescence spectrometry (CV-AFS) technique described by Gill and Bruland (1990). The detection limit was around 0.02 - 0.05 ng/L (Guentzel *et al.*, 2001). About 0.025 mL of digested sample was diluted to approximately 50 mL using distilled and deionized water that was acidified with low Hg content hydrochloric acid for analysis by CV-AFS.

Quality Controls

Quality controls ensure accuracy of analyses involving low metal concentrations. These controls were as follows. All plastic ware was soaked in a 1% Micro-90® laboratory detergent for at least 72 hours. The plastic ware was rinsed with deionized water (18 MΩ) and transferred to a 7.5 N trace metal grade nitric acid (HNO_3) solution and soaked at least 72 hours. After soaking, the plastic ware was rinsed with deionized water and soaked again in a 6 N trace metal grade hydrochloric acid (HCl) solution for another 72 hours. Subsequent to that soaking, the plastic ware was again rinsed with deionized water, filled with 1% ultra pure hydrochloric acid (Q-HCl) to preserve its acidic condition and

stored in double Ziploc bags until use.

The lithium heparinized vacutainers used to collect blood samples were also tested for trace metal contamination with blank water. This blank test measured trace metal levels in deionized water after contact with the vacutainer. These levels were below detection limits. A 0.5% of solution of Q-HNO_3 was used as a blank test solution to monitor contamination for samples analyzed by GFAAS. A standard curve was plotted for calibration before each batch sample analysis. The certified standard reference material (SRM) DOLT-2, dogfish liver, was analyzed with each batch of samples for Ag, Cd, Cr, Cu, Pb and Zn by using GFAAS. Certified values and standard deviations of DOLT-2 are shown in Table 1 (National Research Council Canada). Average percent recoveries of all elements in DOLT-2 ranged from 97.7% to 108% (Table 2).

A 0.5% Q-HCL solution prepared in 18 MΩ deionized water was used as a blank test for cold vapor atomic fluorescence for every 10 samples. Because only a few Hg reference materials exist, PACS-2, marine sediment (certified value ~ 3.04 ± 0.2 mg/kg), was chosen based on its certified Hg level and availability.

Table 1. Certified values (ppb) of elements in the standard reference material, DOLT-2.

Element	Ag	Cd	Cr	Cu	Pb	Zn
Value ± S.D.	608 ± 32	20800 ± 500	370 ± 80	25800 ± 1100	220 ± 20	85800 ± 2500

Table 2. Average percent recovery for standard reference material – DOLT-2.

Metal	Sample	% Recovery	Average ± S.D.
Ag	A	104	
	B	87	
	C	109	100 ± 11.5
Cd	A	108	
	B	108	
	C	99	105 ± 5.2
Cr	A	105	
	B	99	
	C	85	97.7 ± 10.3
Cu	A	110	
	B	102	
	C	112	108 ± 5.3
Pb	A	108	
	B	105	
	C	95	102.7 ± 6.8
Zn	A	96	
	B	101	
	C	99	98.7 ± 2.5

Statistical Analysis

Parametric (normal distribution) and non-parametric (not normal distribution) tests were used in statistical comparisons of trace metal concentrations. Range, mean and standard deviation were reported for each metal. Normal distribution was determined by the Kolmogorov-Smirnov (KS) test. There is no available method to determine the age of ridleys used in this study. Therefore, straight carapace length (SCL) was used as indicator of age. Regression analysis was utilized to characterize potential relationships between metal concentration and SCL. Paired *t*-test and Wilcoxon signed ranks test were used to detect differences in metal concentrations between blood and carapace tissue. An Independent t-test (normal distribution) and Mann-Whitney U test (not normal distribution) were applied to assess the relationship between metal levels in blood and carapace tissue with their pelleted food. The Pearson's (normal distribution) and Spearman rank (not normal distribution) correlation coefficients were calculated to determine the relationship between each metal in blood and carapace tissue as well as between metals. Results were determined significant when $p < 0.05$, unless otherwise stated.

Results

All trace metal concentrations in pelleted food were normally distributed. Trace metal concentrations (d.w.) in pelleted food (Ag = 26.6 ± 17.6, Cd = 40.3 ± 2.7, Cr = 220 ± 55.1, Cu = 4420 ± 900, Hg = 14.6 ± 3.3, Pb = 615 ± 99.9, Zn =

133,000 ± 76,200 ppb) fed to captive ridleys in 2002 are given in Appendix Table 1.

Thirty-three Kemp's ridleys (SCL = 32.4 ± 1.1 cm, range = 28.1 - 34.5 cm, Appendix Table 2) captively-reared by the NMFS Headstart Program were used in characterizing baseline trace metal values during 2001 (n = 6) and 2002 (n = 27). Straight carapace lengths of these turtles were not statistically different ($p > 0.05$) (2001: SCL = 32.8 ± 1.0 cm, range = 31.2 - 33.7 cm; 2002: SCL = 32.4 ± 1.1 cm, range = 28.1 - 34.5 cm).

Chromium levels in captive ridleys from the 2002 class were not normally distributed. Silver, Cu, Hg, and Pb concentrations in carapace tissue were significantly higher than those in the blood, regardless of year (Fig. 2). No statistical difference was detected between blood and carapace tissue in terms of Cd, Cr, and Zn levels in 2001 nor Cr in 2002. Significantly higher Cd and Zn concentrations were found in carapace tissue of 2002 captive ridleys. Mercury levels in blood were significantly higher in 2001 ($p < 0.05$) while higher blood-borne Cu concentrations were found in 2002 ($p < 0.01$; Fig. 2). Carapace-borne Pb concentration was higher in 2002 than in 2001 ($p < 0.01$). No other difference was found in metal concentrations between 2001 and 2002 year classes.

A comparison of the range and mean of trace metal levels in captive ridleys with those of their food is presented in Appendix Table 3. Trace metal concentrations in pelleted food were only compared with blood and carapace

tissue of captive ridleys from 2002 (Fig. 3). Concentration differences ranged from 1.3 (Cr) to 85 times (Cd) higher in the carapace tissue. Additionally, carapace tissue yielded higher Ag, Cu, Hg, and Zn concentrations than did those in the pelleted food ($p < 0.01$). The food did yield 275 (Cr) and 2 times (Pb) higher levels than those in the carapace tissue ($p < 0.01$). All trace metal concentrations in pelleted food were significantly higher ($p < 0.01$) than those in the blood. Concentration differences ranged from 8 (Hg) to 366 times (Cr) higher in the pelleted food.

Trace metal data from 2001 and 2002 were combined to increase sample size for concentration comparisons between blood and carapace tissue. Concentrations of Ag, Hg and Cr in blood and Cd in carapace tissue were not normally distributed. Overall, concentrations differences ranged between 1.5 (Cr) and 88 times (Cu) higher in carapace tissue (Appendix Table 3). No other statistically significant relationship was found between metal concentration and size of ridleys (Figs. 4 and 5). However, a slightly increasing trend was observed between Ag and Cr concentrations in carapace tissue and size of captive ridleys. Blood-borne Ag and Cd exhibited decreased concentrations ($p < 0.01$) with increasing size of ridleys (Fig. 4), and yielded the only statistically significant relationship found for captive conspecifics. However, this relationship may be in error given suspiciously high metal levels in a 28.1 cm SCL ridley. A similar decreasing trend also occurred in blood-borne Cu and Pb concentrations, although neither was statistically significant.

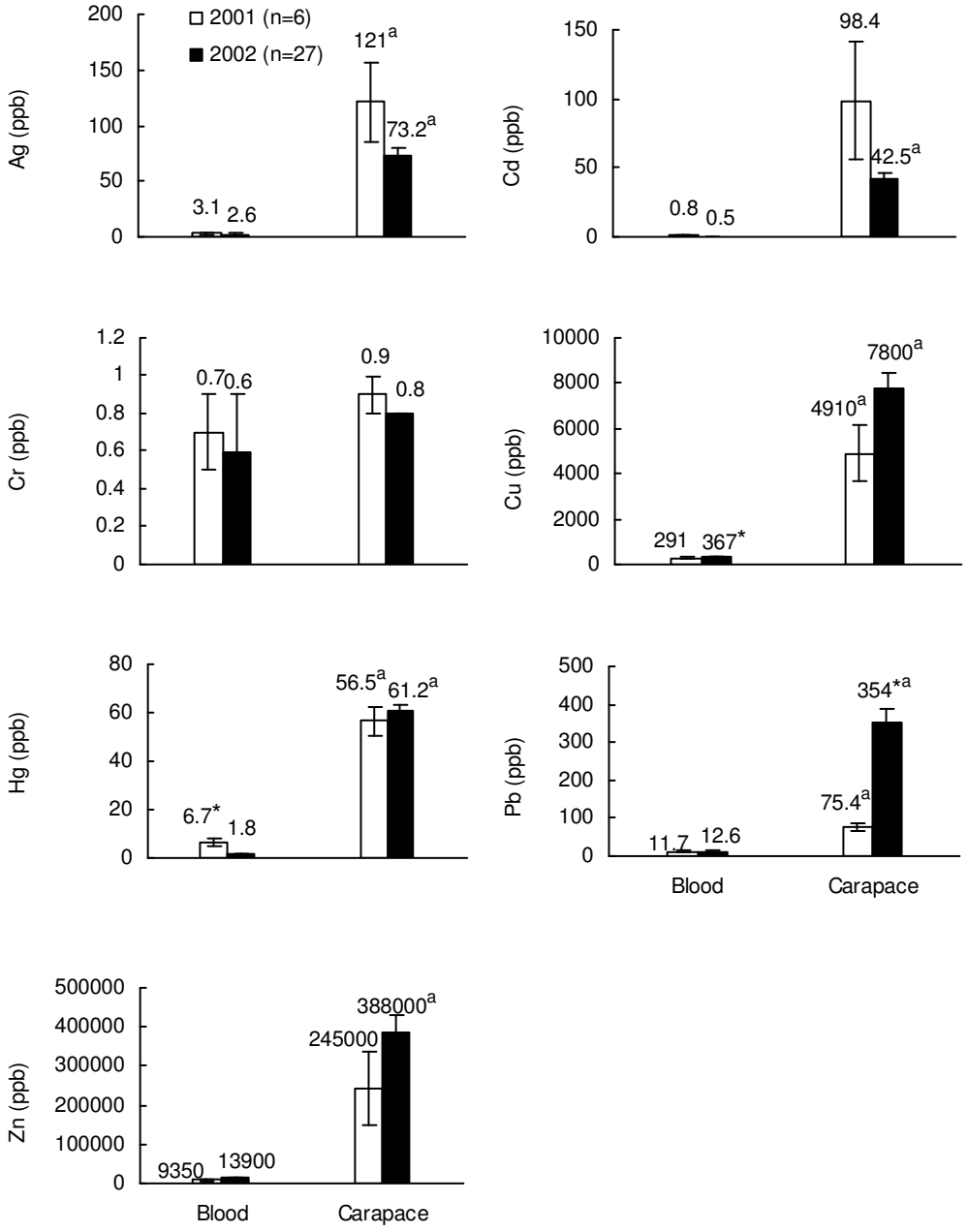


Fig. 2. Trace metal concentrations (ppb) in blood (w.w.) and carapace tissue (d.w.) of captive Kemp's ridleys from 2001 and 2002 (⌶ standard error bar; * significant difference between 2001 and 2002; [a] significant difference between blood and carapace tissue).

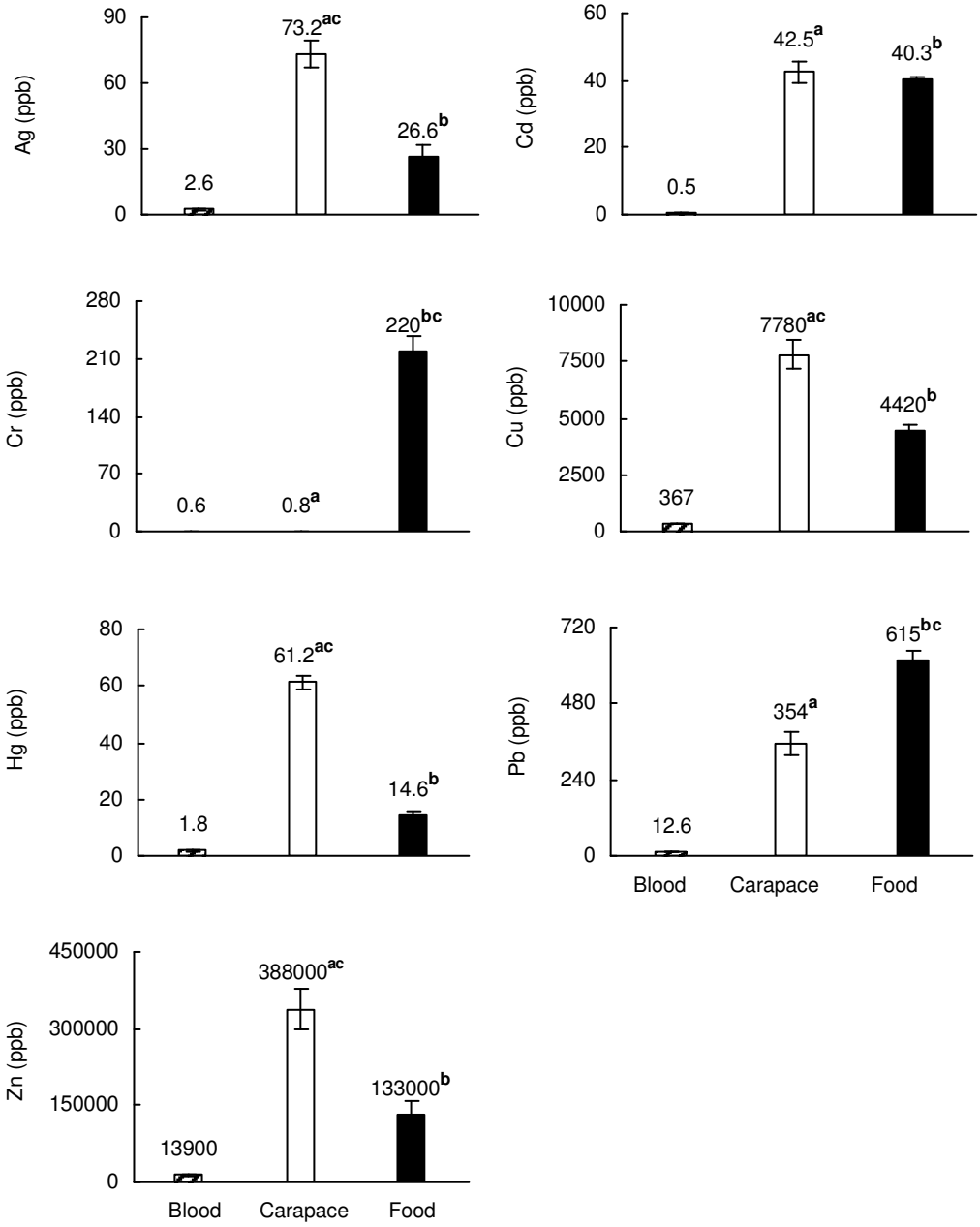


Fig. 3. Trace metal concentrations (ppb) in blood (n = 27, w.w.), carapace tissue (n = 27, d.w.) and food (n = 10, d.w.) of captive Kemp's ridleys in 2002. (significant difference between [a] blood and carapace tissue; [b] blood and food; and [c] carapace tissue and food)

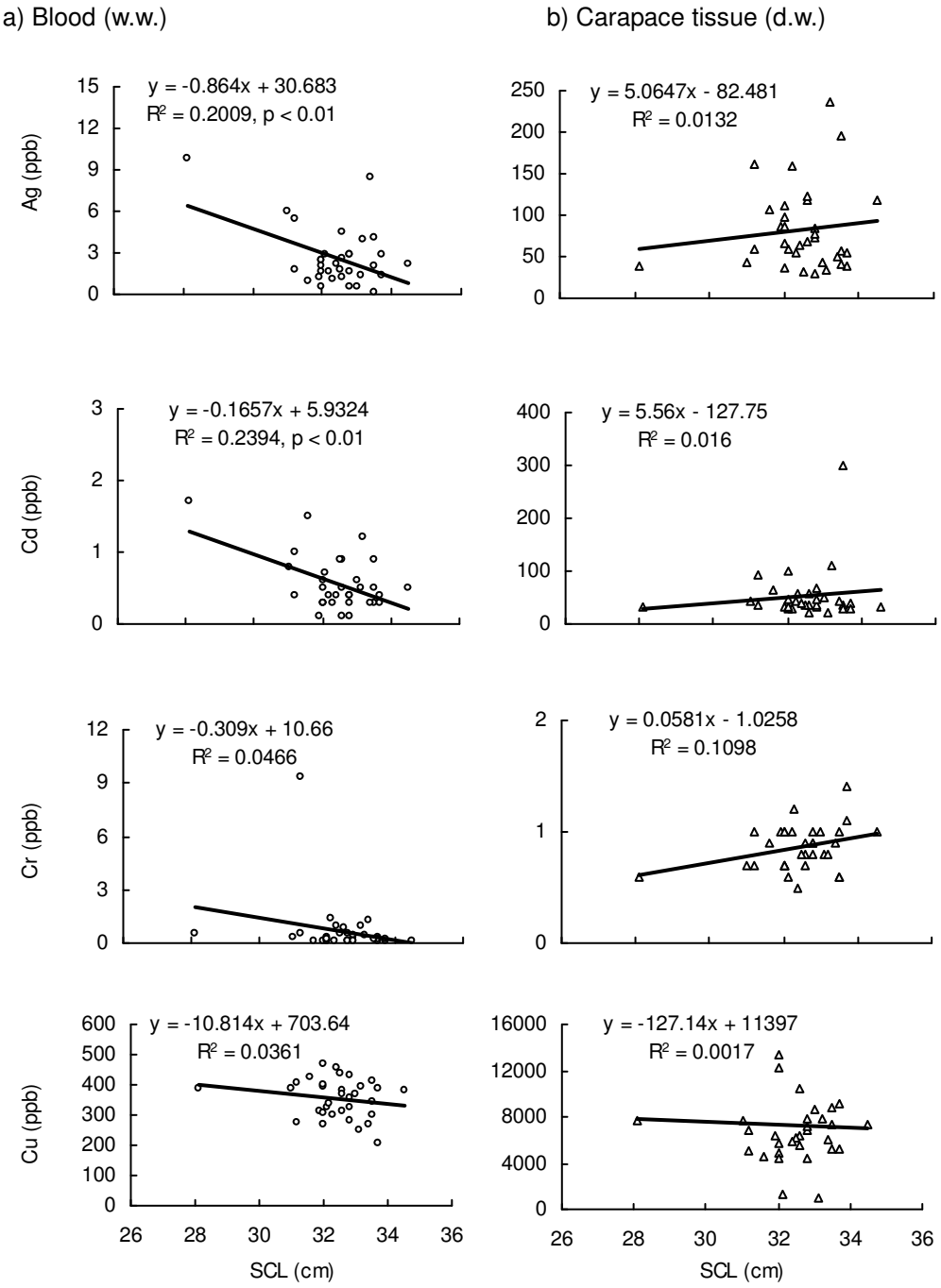


Fig. 4. Trace metal (Ag, Cd, Cr and Cu) concentrations (ppb) in blood (w.w.) and carapace tissue (d.w.) as a function of SCL (28.1 - 34.5 cm) of captive Kemp's ridleys.

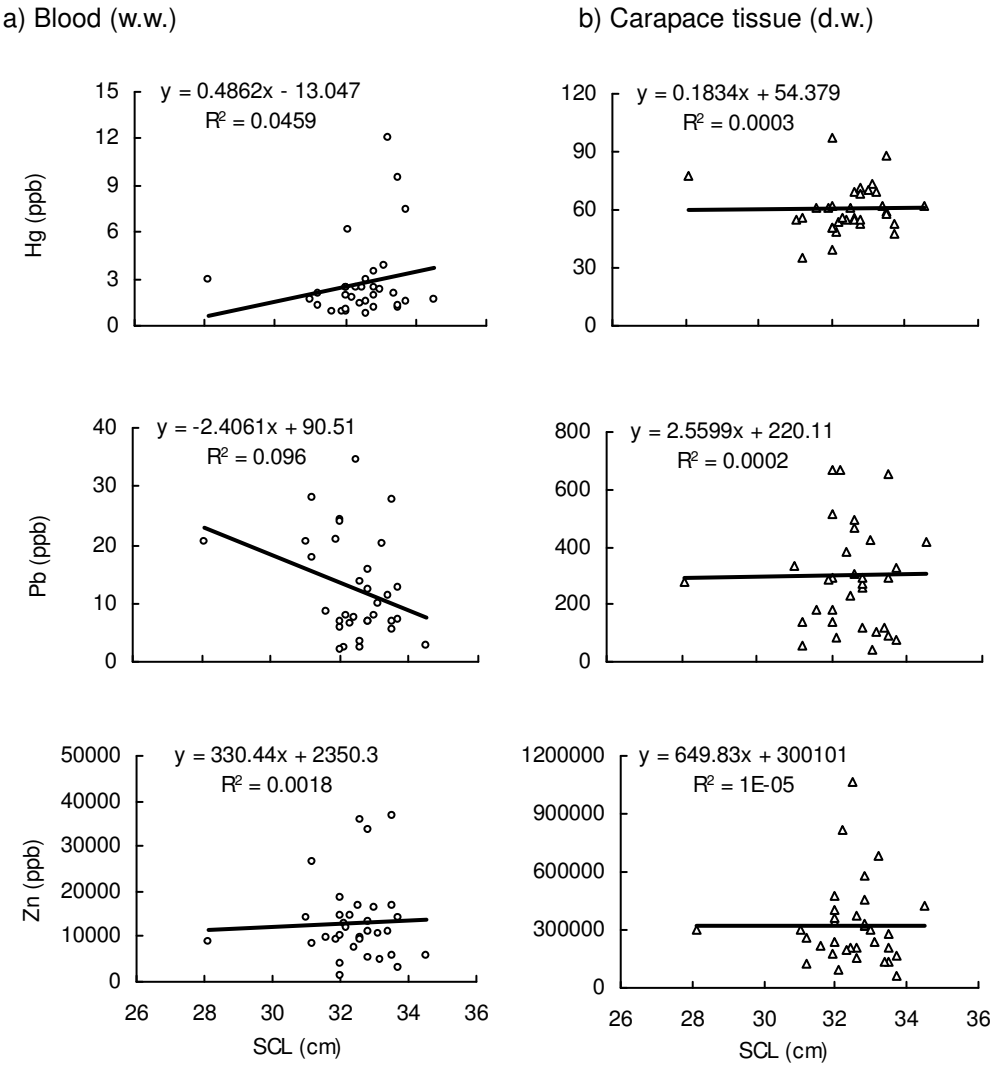


Fig. 5. Trace metal (Hg, Pb and Zn) concentrations (ppb) in blood (w.w.) and carapace tissue (d.w.) as a function of SCL (28.1 - 34.5 cm) of captive Kemp's ridleys.

Cadmium concentration in carapace tissue (Appendix Table 4) rose with increasing Ag (Pearson's $r = 0.607$, $p < 0.01$) and Cr levels (Spearman's $r = 0.399$, $p < 0.05$). A similar relationship was detected between Hg and Cr concentrations in the blood (Spearman's $r = 0.460$, $p < 0.01$). Copper in carapace tissue was positively correlated with Cr (Pearson's $r = 0.437$, $p < 0.05$) and Pb levels (Pearson's $r = 0.810$, $p < 0.01$).

Discussion

The present study is the first characterization of trace metal concentrations in captive-reared Kemp's ridleys, albeit under the prohibition of invasive sampling protocols. It also is the only study whose intent was to tackle the difficult task of developing baseline contaminant levels in sea turtle species whose prime foraging area is the petrochemical-rich shores of the Texas and Louisiana coast. Its value is heightened by the fact that other workers (Presti *et al.*, 2000; Kenyon *et al.*, 2001; Day, 2003) bemoaned the lack of baseline contaminant data in sea turtles and the resulting difficulty in rendering health assessments of wild, captive, or rehabilitated cohorts.

The aforementioned restrictions on using invasive surgery to collect strategic tissues (i.e. liver, kidney, muscle and fat) for contaminant analysis of endangered species mandated that blood and carapace tissue become the primarily sample sources for live-animal research. The decision for doing so was based on several other contaminant investigations. Trace metal concentration

studies of live ridleys conducted by Orvik (1997) and Kenyon *et al.* (2001) recommended monitoring blood and tissue concentrations of captive ridleys fed food sources with known trace metal levels as a means of examining metal uptake and bioaccumulation. Presti *et al*. (2000) reported Hg is deposited in carapace tissue of live ridleys at higher levels than in blood and hypothesized that the carapace provides a more accurate reflection of total Hg accumulation over time. The same relationship was seen in the present study. Saeki *et al.* (2001) reported limited trace metals absorption through the skin in marine mammals that should also apply in sea turtles.

This study discovered that all metal concentrations were higher in carapace tissue than in the blood and, as a result, it may better represent trace metal exposure in captive ridleys within a 28.1 to 35.0 cm SCL size range. This supports the first hypothesis that baseline trace metal concentrations in carapace tissues of Kemp's ridleys are higher than those in their blood. Evidence supporting acceptance of the second hypothesis that carapace tissue and blood accumulate trace metals at levels higher than those in the pelleted food was not nearly as strong. Only Ag, Cd, Cu, Hg, and Zn concentrations were found higher in carapace tissue than in the pelleted food. In addition, pelleted food exhibited significantly higher Cr and Pb levels when compared to those in carapace tissue. Blood-borne trace metal concentrations all failed to exceed those in the pelleted food.

Neither blood or carapace tissue provided sufficient evidence (i.e. a statistically significant linear relationship) that trace metal concentrations rose with increasing size of turtles. The small number and restricted size range of captive ridleys as well as various accumulation rates of each trace metal could attribute to lack of such a relationship.

Trace metal concentrations in pelleted food were determined because they may have impacted trace metal uptake in captive ridleys. Five of seven metal levels in pelleted food were less than those in carapace tissue of captive ridleys. These levels also were lower than those reported in other studies of blue crab prey that wild ridleys feed upon (Table 3). Consequently, these trends render captive ridleys a reasonable option in determining baseline trace metal concentrations, especially in carapace tissue, for comparison (in Chapter IV) with those of their wild conspecifics within the 28 to 35 cm SCL size range.

Table 3. Trace metal concentrations (ppb) in the pelleted food (d.w.) fed to captive Kemp's ridleys and whole blue crab.

Metal	Ag	Cd	Cr	Cu	Hg	Pb	Zn
Pelleted food*	26.6	40.3	220	4420	14.6	615	133000
Blue crab	2800[a]	170[a]	-	83500[a]	200[b]	12200[a]	103000[a]

* analyzed in the present study; [a] Park and Presley, 1997 (Swan Lake, Galveston Bay, d.w.); [b] Sager, 2002 (Lavaca Bay, w.w.), -: no data

Silver

A significantly higher mean Ag concentration (73.2 ppb, 2002) was found in carapace tissue of captive ridleys than that in their pelleted food (26.6 ppb, 2002) and blood (2.6 ppb, 2002). This may imply Ag accumulates in the carapace tissue over time. Although not significant, regression statistics applied to Ag concentrations in carapace tissue of ridleys within 28 to 35 cm SCL, by yielding a positive relationship with size, also support this possibility. Conversely, this study's finding that Ag levels in blood decreased with ridley size may indicate blood serves as a transporter of variable metal concentrations in lieu of being a source of bioaccumulation. Law's (1996) finding that blood is a likely indicator of recent exposure to contaminants tends to support this perception for Ag.

Higher Ag levels in carapace tissue than in the blood and food of captive ridleys suggest that carapace tissue represents a better baseline indicator for Ag exposure. Slightly increasing trend of Ag concentrations in carapace tissue with increasing size of ridleys indicates some potential for accumulation. Conversely, blood-borne Ag concentrations in captive ridleys failed to exhibit any trend that would suggest accumulation.

Cadmium

Like those for Ag, higher Cd concentrations in carapace tissue (42.5 ppb) than in the blood (0.5 ppb) may imply accumulation, at least over the size range of captive ridleys that were sampled. However, failure to detect a significant

difference in Cd concentration between that of carapace tissue and food (40.3 ppb) implies the former did not accumulate Cd or did so slowly. Nonetheless, there is evidence that Cd concentration did increase slightly with size of captive ridleys across the 28 to 35 cm SCL cohorts that were sampled. Additionally, food yielded higher Cd levels than that in blood (0.5 ppb), thereby indicating blood may not accumulate Cd and does not support the first hypothesis. Conversely, the large disparity between Cd concentration in pelleted food and ridleys' blood plus the inverse relationship between blood-borne Cd concentration and SCL of ridleys suggest blood transfers Cd to other organs rather than accumulates it. Examining the latter relationship across a wider size range of ridleys is needed to discern the true role of blood in regard to Cd.

The results of Cd levels in captive ridleys were similar to those found for Ag. A significantly higher Cd level in carapace tissue than in blood suggests that carapace tissue is a better baseline indicator on which to evaluate the risk associated with Cd exposure in captive ridleys. The slightly increasing trend of Cd concentrations in carapace tissue with size of ridleys, indicates some potential for accumulation. Blood-borne Cd concentrations in captive ridleys did not reflect such a possibility.

Chromium

Chromium concentrations in pelleted food fed to captive ridleys (220 ppb) were significantly higher than those in their carapace tissue (0.8 ppb) and blood

(0.6 ppb). These results, especially the disparity between Cr concentrations in carapace tissue and pelleted food, do not support the hypothesis that captive ridleys ≤ 35 cm SCL accumulate Cr concentration to a measurable degree. Although the carapace-borne Cr level of captive Kemp's ridleys was significantly higher than that in blood, these constituent levels were much lower than reported in fish (Hutchinson *et al.*, 1994).

The aforementioned higher Cr concentrations in carapace tissue suggest this tissue represents a better baseline indicator (than blood) for Cr exposure in captive ridleys. Slightly increasing Cr concentrations in carapace tissue may indicate some potential for accumulation; however, a much wider size range of ridleys must be tested for Cr to verify this possibility. Nonetheless, blood-borne levels provided no hint of Cr accumulation potential.

Copper

The relationship of Cu concentration in blood and carapace tissue of captive ridleys to that in their pelleted food mirrored the pattern for Ag. Copper concentration (7,800 ppb) in carapace tissue was significantly higher than that in pelleted food (4,420 ppb) and blood (367 ppb). Like that for Ag, these Cu data provide evidence for possible accumulation in carapace tissue, with blood an index of recent exposure. However, a mildly negative relationship between Cu levels and size of ridleys used in these analyses does provide some doubt as to the carapace's ability to store this metal over time. In addition, the fact that higher

Cu levels were found in pelleted food than in the blood diminishes support for the second aforementioned hypothesis, at least for ridleys within the 28 to 35 cm SCL size range.

Higher Cu levels in carapace tissue than in blood and pelleted food suggest that carapace tissue represents a better baseline indicator for Cu exposure in captive ridleys. However, failure to detect increasing Cu levels in blood and carapace tissue suggests that accumulation of this metal does not occur within the size range of captive ridleys used in this study.

Mercury

A significantly higher mean Hg concentration (61.2 ppb) in carapace tissue of captive ridleys than that in pelleted food (14.6 ppb) and blood (1.8 ppb) may imply the potential for this metal to accumulate in the carapace tissue over time. However, no statistically significant size-concentration relationship was found between Hg levels in carapace tissue of captive ridleys. Nonetheless, this study's finding that Hg levels in blood increased with ridley size may indicate some accumulation potential. Additional analyses involving larger ridleys are need to verify this possibility. Given these trends, there is sufficient evidence to show that Hg concentrations in captive ridleys, especially those in carapace tissue, could be used as a baseline for this metal.

Lead

Lead was one of two metals (the other one being Cr) where pelleted food (615 ppb) yielded significantly higher concentrations than did carapace tissue (354 ppb) and blood (12.6 ppb). This finding suggests the ridley's ability to bioaccumulate Pb in the samples tested is slow or undetectable over the limited size range used in this study. However, the small size range of ridleys over which these analyzes was conducted together with a slightly positive (albeit non-significant) relationship between Pb concentration and SCL mandate additional investigations, especially those involving older conspecifics. The disparity in Pb concentration between the carapace tissue and blood reinforces the perception that blood is only an index of recent exposure to this metal. As such, carapace tissue represents a better but yet to be quantified, baseline indicator for Pb exposure in captive ridleys.

Zinc

Like Ag, Cu, and Hg, Zn exhibited a significantly higher concentration in carapace tissue (338,000 ppb) than in pelleted food (133,000 ppb) and blood (13,900 ppb). The trends support the aforementioned hypotheses, at least in terms of carapace tissue and these four trace metals. However, the lower Zn level found in blood than in pelleted food does not support the second hypothesis. These trends indicate that, despite Zn concentrations exhibited little or no potential for age-dependent accumulation, the carapace tissue represents the

better baseline indicator for Zn exposure in captive ridleys.

Summary

Carapace tissue generated higher metal concentrations than did the blood (Table 4), thus suggesting some potential for accumulation or age/size relationships (e.g. Ag, Cd and Cr) in captive ridleys and support for both hypotheses in the present study. Nonetheless, a much wider size range of captive ridleys must be utilized when assessing the potential for a relationship between trace metal concentration and size of ridleys.

Overall, carapace tissue did yield higher metal (Ag, Cu, Hg, and Zn) levels than were measured in the pelleted food while the latter exhibited concentrations higher than those in the blood. Despite this finding, more research is needed to determine trace metal dynamics related to size of turtle, geographic distribution, foraging strategy and accumulation potential. Few metal-metal correlations have reported in captive Kemp's ridleys. Identifying these correlations will be important to understanding kinetics (e.g. accumulation and metabolism) and toxicity of trace metals in future studies. A comprehensive review of correlations in trace metal concentrations will be discussed in Chapter V. Although this study did not investigate various environmental parameters and biological factors (i.e. sex), the data generated here provided valuable first-hand information on trace metal concentrations in captive-reared Kemp's ridleys that will be used as a baseline for comparison with wild ridleys in later chapters.

Table 4. Mean trace metal concentrations (ppb) in blood (w.w.) and carapace tissue (d.w.) of captive Kemp's ridleys.

n = 33	Ag	Cd	Cr	Cu	Hg	Pb	Zn
Blood	2.7	0.6	0.6	353	2.7	12.4	13100
Carapace	81.8	52.6	0.9	7270	60.3	303	321000

CHAPTER IV

AN ASSESSMENT OF TRACE METAL CONCENTRATIONS IN KEMP'S RIDLEY SEA TURTLES AND THEIR BLUE CRAB PREY

Studies of trace metal accumulation in sea turtles are very limited (Gordon *et al*., 1998; Storelli *et al*., 1998b; Sakai *et al*., 2000b; Day, 2003), especially for live animals (Presti *et al*., 2000; Kenyon *et al*., 2001). Insufficient information with to which describe possible temporal and spatial trends in trace metal loads results from limited access to these protected species, small sample sizes, few historical data, and use of different analytical techniques yielding inconsistent data sets (Caurant *et al*., 1999; Sakai *et al*., 2000a; Anan *et al*., 2002a; Storelli and Marcotrigiano, 2003). This information void renders it difficult to assess cumulative effects of trace metals as well as associated toxicity risks in Kemp's ridleys. Thus, research described in this chapter characterizes trace metal concentrations in blood and carapace tissue of live, wild Kemp's ridleys, compares them with baseline data generated in captive cohorts (Chapter III), and assesses possible accumulation trends in wild Kemp's ridleys captured from Texas, Louisiana and southeast Atlantic coasts during 2000 – 2002.

The western Gulf of Mexico serves as essential habitat for three life stages of Kemp's ridleys that include: post hatchlings (< 20 cm SCL and up to 2 years old), coastal-benthic juveniles/subadults (20 - 60 cm SCL), and coastal-benthic adults (> 60 cm SCL; Orgen, 1989; TEWG, 2000). Shallow coastal waters (< 10

m) of the northwestern Gulf of Mexico serve as developmental and demersal foraging habitat for juvenile and subadult ridleys throughout the year (Ogren, 1989; Landry and Costa, 1999; Coyne and Landry, 2000; Metz, 2004; Landry *et al*., 2004). Tracking studies, stranding surveys and in-water capture operations have shown ridleys display strong seasonal fidelity to tidal passes and adjacent beachfronts of the northern Gulf (Renaud *et al*., 1994, 1995 and 1996; Landry and Costa, 1999) during March through October (Landry *et al*., 1993, 1994 and 1995), with peak densities April through August. This occurrence coincides with months when blue crab abundance and other optimum foraging opportunities (i.e. discarded by-catch from the shrimp fishery) are greatest (Landry and Costa, 1999).

The aforementioned fidelity of ridleys to foraging and development habitats adjacent to tidal passes on the upper Texas and Louisiana coast, where the US' largest petrochemical industry is housed, results in increased exposure to various levels of contaminants. The National Oceanic and Atmospheric Administration (NOAA) has characterized sediment toxicity in US coastal areas since 1991 (Long, *et al*., 1996; Hammeedi *et al*., 1999) and, in doing so, identified As, Cd, Cr, Hg, Pb, and Se as the major metals contaminating Texas waters. A well-documented example of this contamination is the Aluminum Company of America (ALCOA) Point Comfort plant's discharge of Hg into the Lavaca and Matagorda Bay estuarine system between 1966 and 1970. Biological, chemical and physical processes in these coastal waters convert Hg

to methylmercury, a highly toxic form. Methylmercury is bioaccumulated through food chains by benthic-feeding organisms and plants as they forage in contaminated water and sediment. As a result of contaminant exposure within known foraging grounds, this research focused on Kemp's ridleys captured from the Texas and Louisiana coasts so as to describe the potential for trace metal loading in this species.

Food, such as blue crabs that juveniles and adults primarily prey upon, is one likely source of trace metal uptake by Kemp's ridleys in the western Gulf (Shaver, 1991; Werner, 1994). These detritial crustaceans are scavengers and omnivores that also forage along highly industrialized coastal waters of Texas and Louisiana where they fall prey to ridleys. Total Hg accumulates in the blue crab's digestive gland while methylmercury is concentrated in its muscle (Evans and Engel, 1994). Mercury concentrations as high as 1,210 ppb have been reported for juvenile blue crabs from Lavaca Bay (Locarnini and Presley, 1996). Other studies identified metal concentrations including Ag, As, Al, Cd, Cu, Ni, Pb, Se, Ti, and Zn in tissues (e.g. gill, digestive gland) of blue crabs from different locations in Texas and Louisiana (Sims and Presley, 1976; Ramelow *et al.*, 1989; Barrera *et al.*, 1995; Locarnini and Presley, 1996; Park and Presley, 1997; Sager, 2002; Appendix Table 5).

Although many studies have measured trace metal concentrations in tissues of blue crabs (Jop *et al.*, 1997; Park and Presley, 1997; Sastre *et al.*, 1999), complementary analyses are not available for Kemp's ridleys.

Furthermore, little is known about the relationship between sea turtle health and the trophic environment as the latter influences uptake of trace metals in ridleys foraging across Texas and Louisiana waters.

Research objectives are to characterize trace metal concentrations in blood and carapace tissues of live Kemp's ridleys across their size range and compare them with baseline levels detected in captive counterparts. Achieving these objectives allows the following hypotheses for respective metals to be tested: 1) trace metal concentrations in carapace tissues of wild ridleys are higher than those in their blood; 2) trace metal concentrations in wild ridleys exceed baseline levels reported for captive ridleys; and 3) trace metal levels are higher in wild ridleys than in their blue crab prey.

Materials and Methods

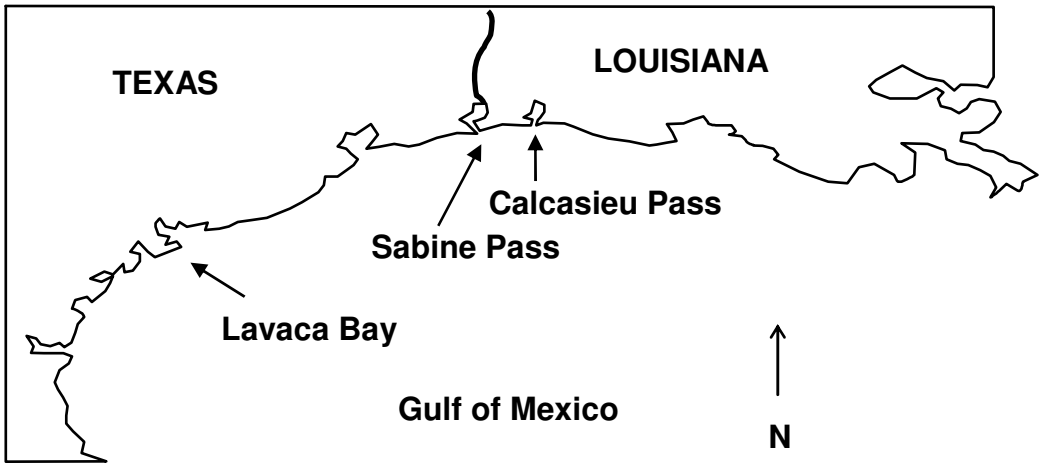
Sea Turtle Capture and Study Area

Wild Kemp's ridleys were captured in 91.4 m long entanglement nets of various mesh sizes and depths deployed along jetty, beachfront and open bay/lagoon habitat adjacent to Sabine Pass (SP) and Lavaca Bay (LB), Texas and Calcasieu Pass, Louisiana (Fig. 6a) during May to August 2000 - 2002 (Landry *et al*., 1995; Coyne and Landry, 2000; Metz, 2004). Sabine Pass extends and receives water from Sabine Lake, where a large petrochemical industrial complex associated with the "Golden Triangle" of Port Author, Beaumont, and Orange, Texas discharges its effluents (Ravichandran *et al*.,

1995a and 1995b). The Lavaca Bay study area was adjacent to the Alcoa Company Plant in Point Comfort, Texas where an EPA superfund site has been established in response to historical releases of Hg into receiving waters. The Calcasieu Pass netting site was located south of Cameron and Calcasieu Lake, Louisiana, where the latter receives industrial, agricultural and urban wastes from the Calcasieu River and its tributaries including Bayou d'Inde (Sferra *et al*., 1999), Choupique Bayou and Contraband Bayou (Ramelow *et al*., 1987).

Four to six entanglement nets were set perpendicular or parallel to the aforementioned habitats for a minimum of 6 hours each sampling day and checked every 30 minutes for sea turtles and by-catch. Entanglement netting operations were conducted monthly during May through August at selected study sites, with this effort extending for 2 to 3 weeks/month across sites (i.e., 1 week of effort at Calcasieu Pass, Sabine Pass and Lavaca Bay, respectively). Kemp's ridleys used in the present study were randomly captured in entanglement nets. Furthermore, metal concentrations in Kemp's ridleys captured from Sabine Pass and Calcasieu Pass during 1994-1995 (Kenyon *et al*., 2001) and in 1997 (Presti *et al*., 2000) were used as additional sources to assess metal concentrations in blood (Ag, Cu, Hg, Pb, Zn) and carapace tissue (Hg only).

a) Texas and Louisiana



b) Southeast Atlantic

Fig. 6. Kemp's ridley capture locations along the a) Texas and Louisiana and b) southeast Atlantic coasts.

Wild Kemp's ridleys also were captured from southeast Atlantic waters in shrimp trawls (NMFS permit # 1245) without turtle exclude devices. These turtles were used as an additional source of trace metal concentrations in slightly older life stages than were typically captured at Texas and Louisiana sampling sites. Southeast Atlantic sampling locations were randomly chosen from 600 way points between Charleston, South Carolina, and Brunswick, Georgia (Fig. 6b). These captures took place from the last week in May to the third week in July during 2001 and 2002. Trawl tows were limited to a 30-minute duration (Day, 2003).

Sample Collection and Digestion

Blood was drawn from ridleys immediately after their removal from entanglement nets following protocol described in Chapter III. Turtles captured along the Texas and Louisiana coast were taken to a land-based holding facility and held for up to 72 hours. During this time, each ridley was the subject of carapace sampling procedures that followed protocols described in Chapter III. Unlike captive-reared ridleys that were held in seawater that was changed thrice weekly, the carapace of wild conspecifics had to be cleaned with freshwater and a household sponge to remove sediment, algae and barnacles that normally accrue during life in the marine environment.

Blood and carapace tissue of Kemp's ridleys captured in trawl tows from the southeast Atlantic were collected following the protocol developed by Day (2003)

for loggerheads. Blood was drawn following the same protocol outlined for captive ridleys in Chapter III. Approximately 5 ml of blood was collected, placed in a - 10°C freezer, and eventually transferred to a -20°C storage (Day, 2003). The protocol to collect carapace tissue was as follows. Tissue was scraped from the outermost edge of the carapace within a standardized area consisting of the eight most posterior marginal scutes. This avoided scraping too deeply and causing injury to the turtle and also prevented contaminating the sample with untargeted tissues. The 2 cm of carapace dorsal and ventral to its edge were cleaned of sloughing keratin and epiphytic/epibiotic organisms using a plastic scrubbing pad, followed by a liberal rinsing with high purity distilled water and isopropanol. A cellulose based clean room paper wiper, distilled water and isopropanol were then used to remove remaining foreign matter and debris. Disposable stainless steel biopsy tools were used to obtain 0.2 – 0.5 g of superficial keratin from the prepared areas by moving the tool parallel to the edge being sampled. This yielded small shavings or splinters of keratin < 1 mm in thickness that were dropped directly into a polyethylene sample bag which was stored at - 10°C until it was transferred to - 20 °C after each trawling cruise. All non-disposable tools were rinsed with high purity water and isopropanol prior to use (Day, 2003).

The present study used a plastic Teflon scraping spatula for sampling carapace tissue of ridleys from Texas and Louisiana while a stainless steel biopsy tool was utilized for those conspecifics from southeast Atlantic. In addition,

freshwater and distilled water/isopropanol were used to clean the carapace of ridleys from Texas and Louisiana, and southeast Atlantic, respectively. However, these differences in sampling protocols did not affect measurement of trace metal levels in carapace tissue.

The Kemp's ridley's preferred food item – blue crab – was caught at Calcasieu Pass, Sabine Pass and Lavaca Bay during May through August 2001 for use in determining a potential contaminant uptake pathway. At least three adult crabs of each gender were collected in entanglement-net sets at each study site and held for subsequent chemical analyses. The relationship between contaminant uptake and size was characterized for both Kemp's ridleys and their blue crab prey. Straight carapace length and body weight were recorded for each ridley while carapace width (mm from tip to tip, CW) and weight (g) were measured for all blue crabs.

The digestive gland (DG) and muscle tissue (M) from each claw were dissected from each blue crab using a plastic Teflon knife. Muscle was chosen for analysis because it is the major tissue consumed by predators and composes a significant percentage of the organism's body mass. The digestive gland was selected because it is the major lipid storage organ, a predominant metal accumulation site, and is frequently consumed together with edible tissue (Jop *et al.*, 1997).

Digestion processes for blood and carapace tissue followed methods described in Chapter III for captive Kemp's ridleys. About 1 g of digestive gland

(w.w.) and muscle tissue (w.w.) from blue crabs was digested in Teflon vials following the same digestion method used for blood samples.

Metal Analysis

Metal determinations of blood and carapace tissue followed protocols described in Chapter III for captive Kemp's ridleys. Concentrations of Ag, Cd, Cr, Cu, Pb, and Zn were analyzed by graphite furnace atomic absorption spectrometer while Hg was analyzed by cold vapor atomic fluorescence spectrometry. All metal concentrations for muscle and digestive gland tissue were computed on a ppb w.w. basis.

Statistical Analysis

Range, mean and standard deviation were reported for each metal. Prior to any statistical analysis, all data were tested for normal distribution using a Kolmogorov-Smirnov (KS) test as well as homogeneity of variances using the Levene test. Parametric and non-parametric tests were employed, as appropriate, in statistical comparisons of metal concentrations. Regression analysis was utilized to characterize relationships between metal concentration and carapace length of ridleys and width of blue crabs, respectively. An Independent *t*-test and Mann-Whitney U test were used to analyze for metal concentration differences between wild and captive ridleys. Analysis of variance was used to test for differences in metal concentrations between blood/carapace

tissues of Kemp's ridleys and blue crab tissues. The Pearson's and Spearman rank correlation coefficients were used to assess possible relationships between metal concentrations. Results were determined significant when $p < 0.05$, unless otherwise stated.

Results

A total of 91 wild Kemp's ridleys was captured in entanglement nets along the Texas and Louisiana coasts during May to August 2000 – 2002 and used to assess trace metal levels in blood and carapace tissue. Of these, 14 and 16 ridleys were captured at Sabine Pass and Lavaca Bay, Texas, respectively, while 61 ridleys were caught at Calcasieu Pass, Louisiana. Their capture locations and respective SCL are given in Table 5. Kemp's ridleys (n = 18) captured along the southeast Atlantic coast in 2001 and 2002 were used as an additional source of blood and carapace tissue samples in the present study. These turtles were significantly larger (SCL = 46.3 cm) than individuals from the Texas (SCL = 35.7 cm, $p < 0.01$) and Louisiana (SCL = 36.3 cm, $p < 0.01$) coast, regardless of study site (Table 5).

Table 5. Straight carapace length (SCL) of Kemp's ridleys captured along the Texas, Louisiana and southeast Atlantic coasts during 2000-2002.

Site	n	SCL (cm)	Range (cm)
Texas	30	35.7 ± 6.1	27.9 – 52.8
Sabine Pass	14	36.2 ± 6.9	29.0 – 52.8
Lavaca Bay	16	35.2 ± 5.6	27.9 – 46.7
Louisiana	61	36.3 ± 8.0	22.5 – 66.3
Texas/Louisiana	91	36.1 ± 7.4	22.5 – 66.3
Southeast Atlantic	18	46.3 ± 7.1	30.9 – 57.0

Additional trace metal concentrations in 96 (blood) and 80 (blood and carapace tissue) Kemp's ridleys captured during 1994 - 1995 and in 1997, respectively, from Texas and Louisiana, where applicable, were compared with levels detected in ridleys of the current study. Straight carapace lengths of these turtles were statistically similar (1994-1995: SCL = 38 $\pm$ 10 cm, range = 21.6 – 65.8 cm; 1997: SCL = 37.6 $\pm$ 10.3 cm, range = 24.5 – 60.7 cm) with ridleys from Texas and Louisiana in the present study (SCL = 36 $\pm$ 10 cm, range = 22.5 – 66.3 cm).

A total of 25 blue crabs captured along the Texas (n = 15) and Louisiana (n = 10) coast in 2001 was used in assessing this potential source of trace metal uptake in wild ridleys from the northwestern Gulf of Mexico. Mean carapace width of blue crabs was similar between study sites (p = 0.619) and gender (male: CW = 141.5 mm, n = 11; female: CW = 121.3 mm, n = 14, p = 0.05; Table 6).

Table 6. Carapace width of blue crabs captured along the Texas and Louisiana coast in 2001.

Location	n	Carapace width (mm)	Range (mm)
Texas	15	128 ± 26.2	76.0 – 179
Sabine Pass	10	123 ± 26.3	76.0 – 158
Lavaca Bay	5	139 ± 24.9	115 – 179
Louisiana	10	133 ± 26.3	98.0 – 175
Texas/Louisiana	25	130 ± 25.8	76.0 – 179

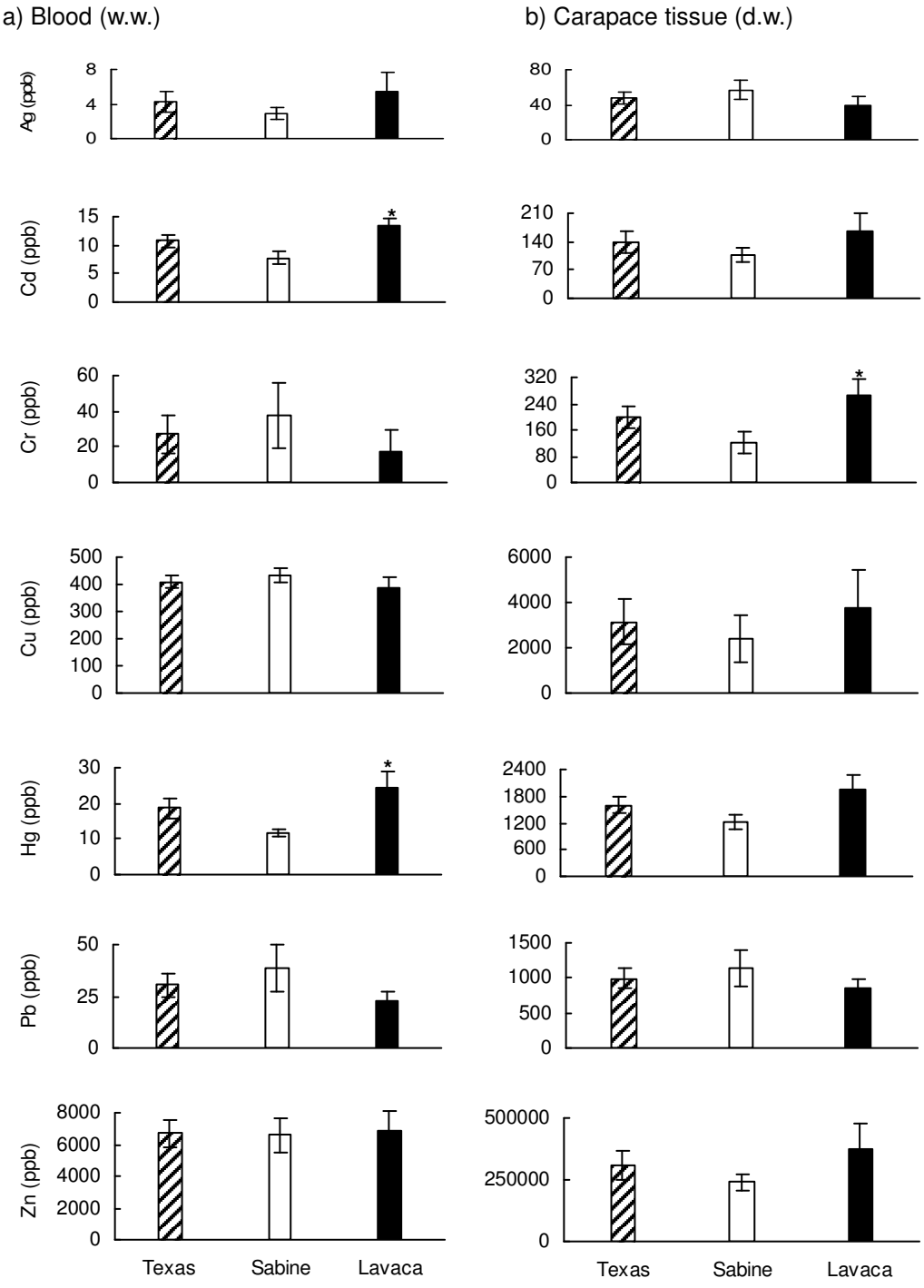


Fig. 7. Trace metal concentrations (ppb) in a) blood (w.w.) and b) carapace tissue (d.w.) of wild Kemp's ridleys captured from Sabine Pass (n = 14) and Lavaca Bay (n = 16), Texas (combined) study sites (* significant difference – $p < 0.05$, in mean concentration between Sabine Pass and Lavaca Bay).

Texas Study Sites

Kemp's ridley: trace metal concentrations

Range and mean statistics for metal levels in blood and carapace tissue of Kemp's ridleys captured from Texas sampling sites during 2000 - 2002 are given in Appendix Tables 6 and 7. Concentrations of Ag, Hg and Cr in blood and Cu in carapace tissue of these ridleys were not normally distributed. All metal concentrations in carapace tissue of ridleys were significantly ($p < 0.01$, except Hg with $p < 0.05$) higher than those in the blood (Fig. 7), regardless of sampling site. Concentration differences ranged from 7 (Cr, Cu) to 86 times (Zn) higher in the carapace tissue. Lavaca Bay ridleys yielded significantly higher Cd and Hg ($p < 0.01$) concentrations in blood, and Cr concentrations ($p < 0.01$) in carapace tissue (Fig. 7). No significant differences were found in Ag, Cu, Pb and Zn concentrations between ridleys from Sabine Pass and Lavaca Bay, regardless of sample type.

Lead concentrations in blood (Fig. 8) and Cd levels in carapace tissue (Fig. 9) increased significantly with SCL (range = 27.9 – 52.8 cm) of ridleys from all Texas study sites. A similar significant relationship also existed for Pb levels ($p < 0.01$) in blood and Cd levels in carapace tissue of ridleys from Sabine Pass and Lavaca Bay, respectively (Appendix Figs. 1 and 3). No other concentration versus size relationships were detected for ridleys from Texas study sites (Appendix Figs. 2 and 4).

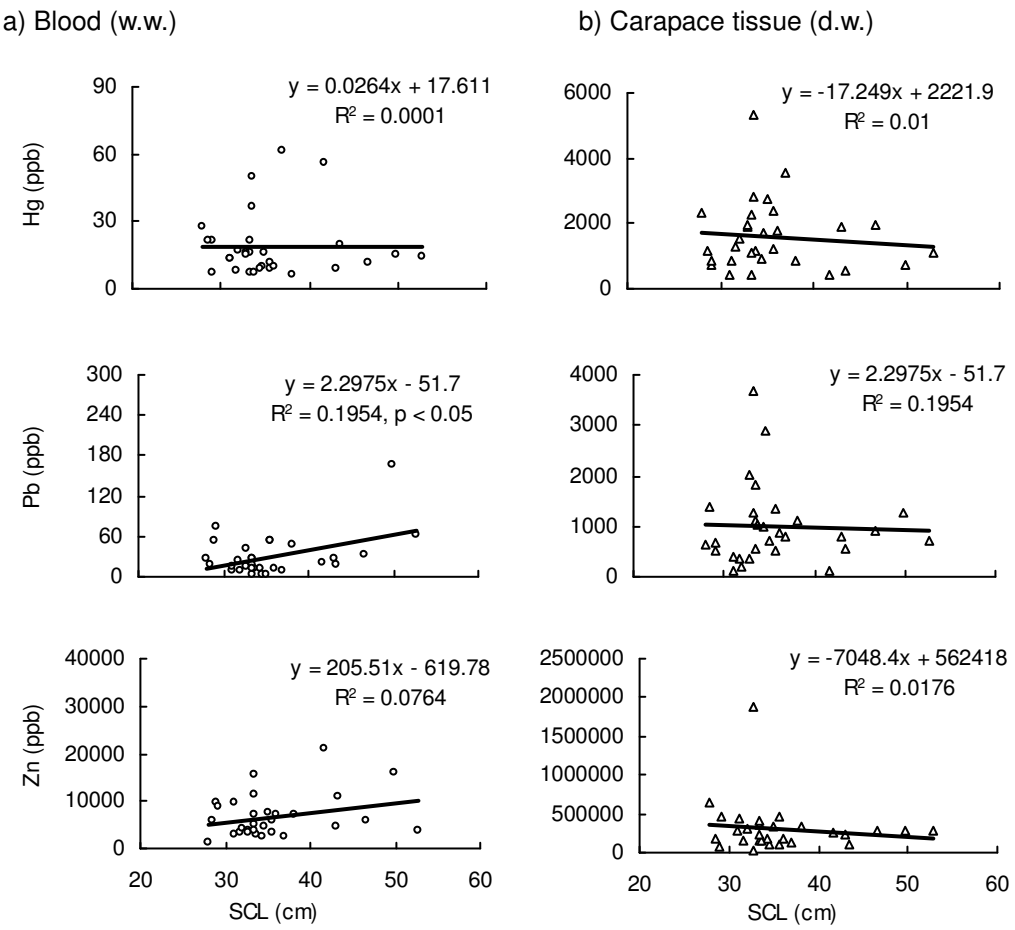


Fig. 8. Trace metal (Hg, Pb and Zn) concentrations (ppb) in blood (w.w.) and carapace tissue (d.w.) as a function of SCL (27.9 - 52.8 cm) of wild Kemp's ridleys captured from Sabine Pass and Lavaca Bay, Texas study sites combined.

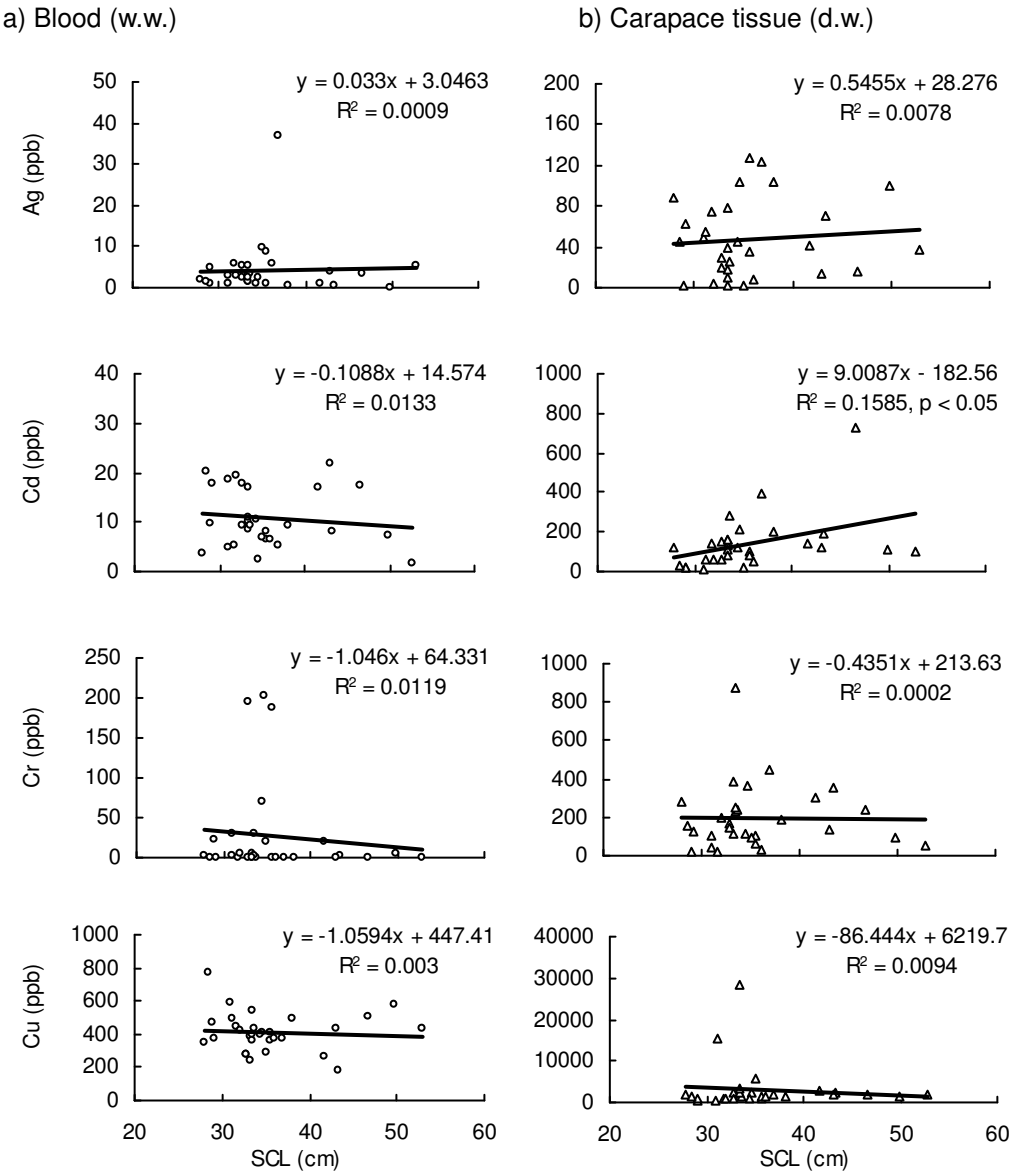


Fig. 9. Trace metal (Ag, Cd, Cr and Cu) concentrations (ppb) in blood (w.w.) and carapace tissue (d.w.) as a function of SCL (27.9 - 52.8 cm) of wild Kemp's ridleys captured from Sabine Pass and Lavaca Bay, Texas study sites combined.

Chromium concentrations rose significantly with increasing Cu (Spearman's $r = 0.469$, $p < 0.01$) and Pb levels (Pearson's $r = 0.411$) in carapace tissue from ridleys, regardless of Texas capture site (Appendix Table 8). A similar significant relationship was detected between Cd and Cr (Pearson's $r = 0.615$), Cd and Pb (Pearson's $r = 0.559$), and Cr and Pb levels (Pearson's $r = 0.924$, $p < 0.01$) in carapace tissue of ridleys from Sabine Pass (Appendix Table 9). Lead concentrations were positively correlated with Zn levels (Pearson's $r = 0.537$) in carapace tissue of ridleys from Lavaca Bay (Appendix Table 10).

Blue crab: trace metal concentrations

A total of 15 blue crabs (CW = 128.0 $\pm$ 26.2 mm) was collected at Sabine Pass (n = 10) and Lavaca Bay (n = 5) in 2001 (Appendix Table 11). Concentrations of Cr in muscle tissue of blue crabs from Texas were not normally distributed. Mean trace metal concentrations in tissues of blue crabs, regardless of study sites, were: digestive gland - Ag = 560, Cd = 166, Cr = 11.0, Cu = 19,500, Hg = 19.3, Pb = 81.5, and Zn = 45,600 ppb; muscle - Ag = 194, Cd = 40.4, Cr = 9.9, Cu = 7,910, Hg = 58.0, Pb = 44.3, and Zn = 51,100 ppb. No significant differences ($p = 0.274$) were detected among metal concentrations across carapace widths nor gender (male n = 5; female n = 10, $p = 0.149$). Significantly higher Ag (1.7 times, $p < 0.01$), Cd (4 times, $p < 0.01$), Cu (2.5 times, $p < 0.01$) and Pb concentrations (1.8 times) were found in the digestive gland of Texas blue crabs than in their muscle tissue (Fig. 10). A similar relationship

between tissues occurred for higher Ag, Cd, Cu, and Pb levels in the digestive gland of blue crabs from Sabine Pass. Conversely, muscle tissue exhibited a significantly higher Hg level in blue crabs from this site ($p < 0.01$). However, no relationship in metal concentrations between the digestive gland and muscle tissue was detected in blue crabs from Lavaca Bay.

Mercury concentrations in the muscle tissue increased with size of blue crabs from all Texas study sites (Appendix Fig. 6). A converse relationship existed between Cd levels in the muscle tissue of blue crabs from Sabine Pass (Appendix Fig. 7). No other concentration versus size relationships existed for blue crabs from across Texas study sites (Appendix Fig. 5) or within respective sites (Appendix Fig. 8).

The relationship between metal concentrations in blue crabs from all Texas study sites was as follows. Zinc concentrations significantly increased with increasing Cd in the digestive gland (Pearson's $r = 0.870$, $p < 0.01$) and muscle tissue (Pearson's $r = 0.540$; Appendix Table 12). A similar significant relationship was detected between Cr and Cu levels (Pearson's $r = 0.708$, $p < 0.01$) in the digestive gland. Silver concentrations rose significantly with increased Cr levels (Pearson's $r = 0.553$) in the digestive gland, as did Cd (Pearson's $r = 0.588$) and Cu levels (Pearson's $r = 0.599$) in muscle tissue. Lead concentrations were significant positively correlated with Hg (Pearson's $r = 0.559$) and Zn levels (Pearson's $r = 0.538$) in the digestive gland.

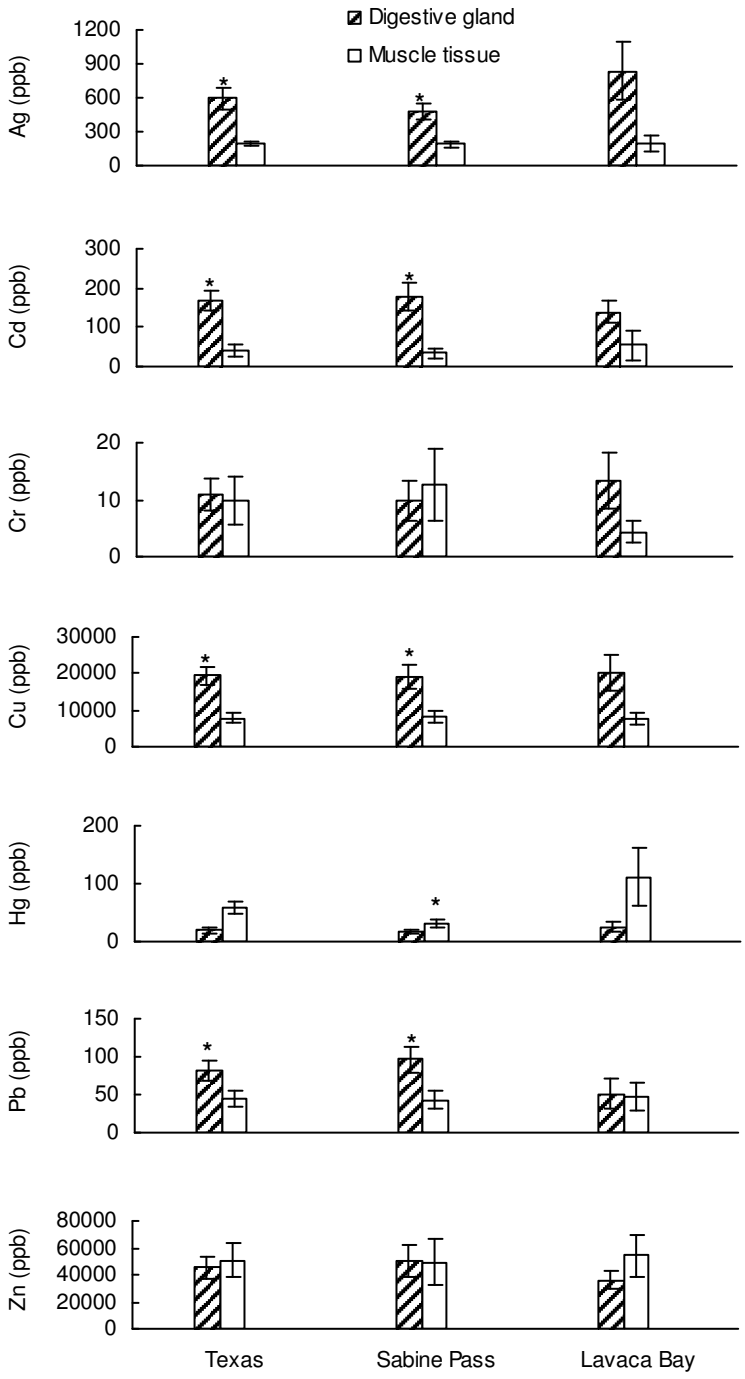


Fig. 10. Trace metal concentrations (ppb) in digestive gland (w.w.) and muscle tissue (w.w.) of blue crabs captured from Sabine Pass (n = 10) and Lavaca Bay (n = 5), Texas study sites combined (* significant difference – $p < 0.05$, in metal concentration between digestive gland and muscle tissue).

Trace metal relationships for blue crabs from Sabine Pass were as follows (Appendix Table 13): Zn concentrations rose significantly with increased Cd levels in the digestive gland (Pearson's $r = 0.900$, $p < 0.01$) and muscle tissue (Pearson's $r = 0.682$) while Ag and Cu concentrations were positively correlated in the digestive gland (Pearson's $r = 0.716$) and muscle tissue (Pearson's $r = 0.636$). A similar significant relationship also occurred between Ag and Cr (Pearson's $r = 0.636$), and Hg and Pb concentrations (Pearson's $r = 0.763$) in the digestive gland.

Cadmium concentrations rose significantly with increasing Cr levels (Pearson's $r = 0.952$) in the digestive gland, and Cu levels (Pearson's $r = 0.919$) in muscle tissue of Lavaca Bay blue crabs (Appendix Table 14). A similar significant relationship was detected between Ag and Cr (Pearson's $r = 0.922$) and Cr and Zn concentrations (Pearson's $r = 0.953$) in muscle tissue, and Hg and Pb concentrations (Pearson's $r = 0.927$) in the digestive gland.

Since metal concentrations in blood were significantly lower than those in the carapace of ridleys, the latter tissue was used in comparison with that of blue crabs to assess metal uptake in ridleys from all Texas study sites (Table 7). Significantly higher Cr, Hg, Pb, and Zn concentrations ($p < 0.01$) were found in carapace tissue than those in blue crabs, regardless of tissue type. Conversely, blue crabs yielded significantly higher Ag and Cu levels than those in carapace tissue ($p < 0.01$). A similar metal-loading trend also occurred between ridleys and blue crabs from Sabine Pass (Appendix Table 15) and Lavaca Bay

(Appendix Table 16), except no differences were detected for Cd and Zn concentrations.

Table 7. Trace metal concentrations (ppb) in Kemp's ridleys and blue crabs from Sabine Pass and Lavaca Bay, Texas study sites combined.

	Ag	Cd	Cr	Cu	Hg	Pb	Zn
Kemp's ridley							
Carapace (d.w.)	47.4	139[b]	198[ab]	3140	1610[ab]	986[ab]	311000[ab]
Blue crab (w.w.)							
Digestive gland	596[a]	166[a]	11.0	19500[a]	19.3	81.5	45600
Muscle tissue	194[b]	40.4	9.9	7910[b]	58.0	44.3	51100

[a] significant difference between digestive gland of blue crabs and carapace tissue of ridleys;
[b] significant difference between muscle tissue of blue crabs and carapace tissue of ridleys

Louisiana Study Site

Kemp's ridley: trace metal concentrations

Range and mean metal values in blood and carapace tissue of Kemp's ridleys from Louisiana (n = 61) during 2000 - 2002 are given in Appendix Table 17. Concentrations of Cu, Pb and Zn in blood and Hg and Zn in carapace tissue were normally distributed. All trace metal levels in carapace tissue were significantly higher than those in the blood ($p < 0.01$). Concentration differences ranged from 9 (Cu) to 78 times (Hg) higher in carapace tissue. Copper and Hg concentrations in carapace tissue and blood, respectively, were the only metals to increase with size of ridleys (Figs. 11 and 12).

Silver concentrations rose significantly with decreasing Cd (Spearman's r = - 0.406, $p < 0.01$) and Zn levels (Spearman's r = - 0.683, $p < 0.001$) in blood, and Hg levels (Spearman's r = - 0.299) in carapace tissue (Appendix Table 18). Cadmium and Cr concentrations were positively correlated in blood (Spearman's r = 0.279) and carapace tissue (Spearman's r = 0.583, $p < 0.01$). A similar relationship was detected between Cu and Pb levels in blood (Spearman's r = 0.271, $p < 0.01$) and carapace tissue (Spearman's r = 0.476). Zinc concentrations rose significantly with increased Cd (Spearman's r = 0.570, $p < 0.01$), and Cr levels (Spearman's r = 0.322, $p < 0.01$) in blood. Silver concentrations also increased with increasing Cr (Spearman' r = 0.467, $p < 0.01$), and Cu levels (Spearman's r = 0.452, $p < 0.01$) in carapace tissue. Cadmium concentrations were positively correlated with Cu (Spearman's r = 0.660, $p < 0.01$) and Pb levels (Spearman's r = 0.574, $p < 0.01$) in carapace tissue. A similar relationship existed between Cr and Cu (Spearman's r = 0.596, $p < 0.01$), and Cr and Pb levels (Spearman's r = 0.556, $p < 0.01$) in carapace tissue.

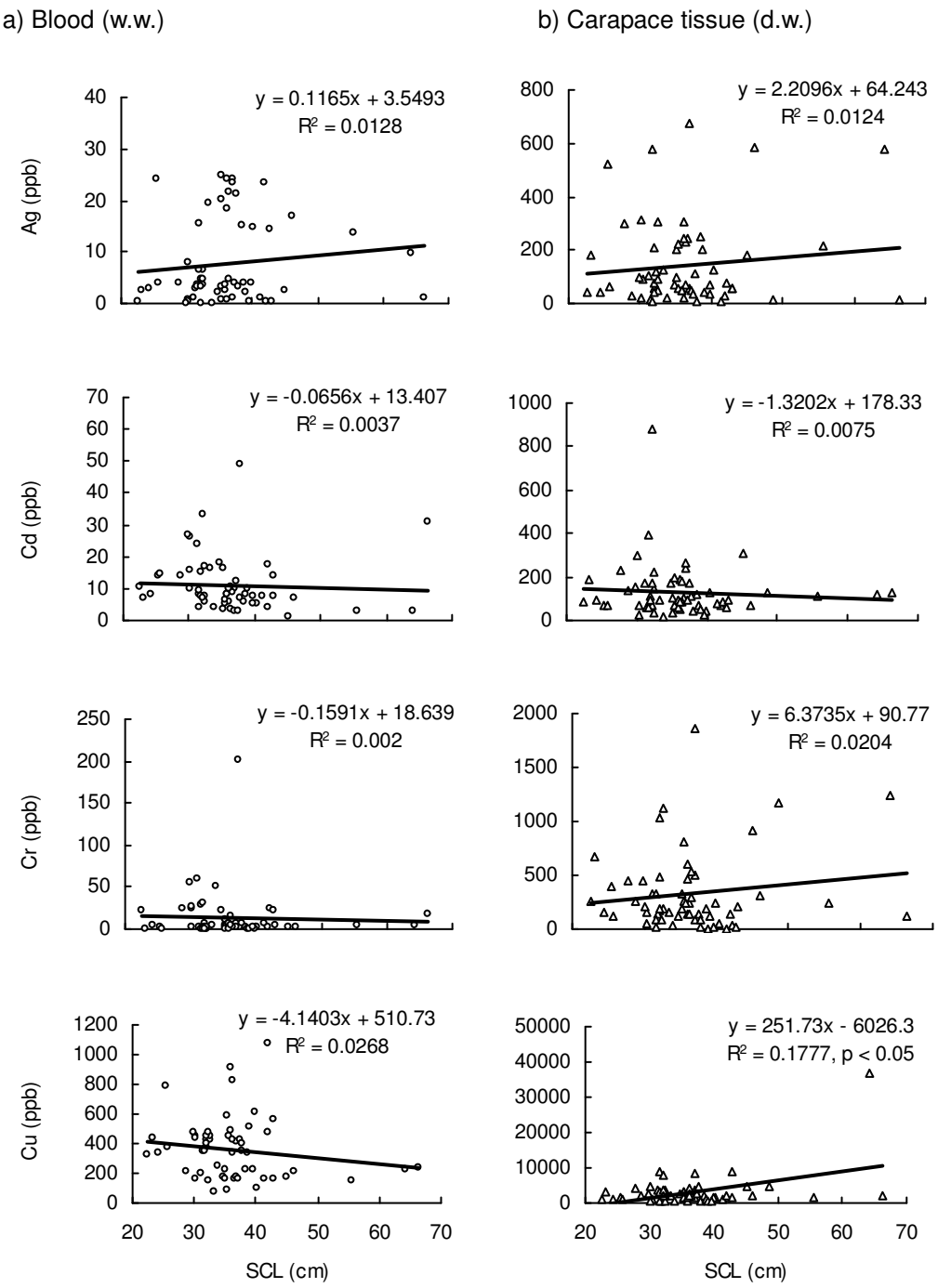


Fig. 11. Trace metal (Ag, Cd, Cr and Cu) concentrations (ppb) in blood (w.w.) and carapace tissue (d.w.) as a function of SCL (22.5 - 66.3 cm) of wild Kemp's ridleys captured from Calcasieu Pass, Louisiana.

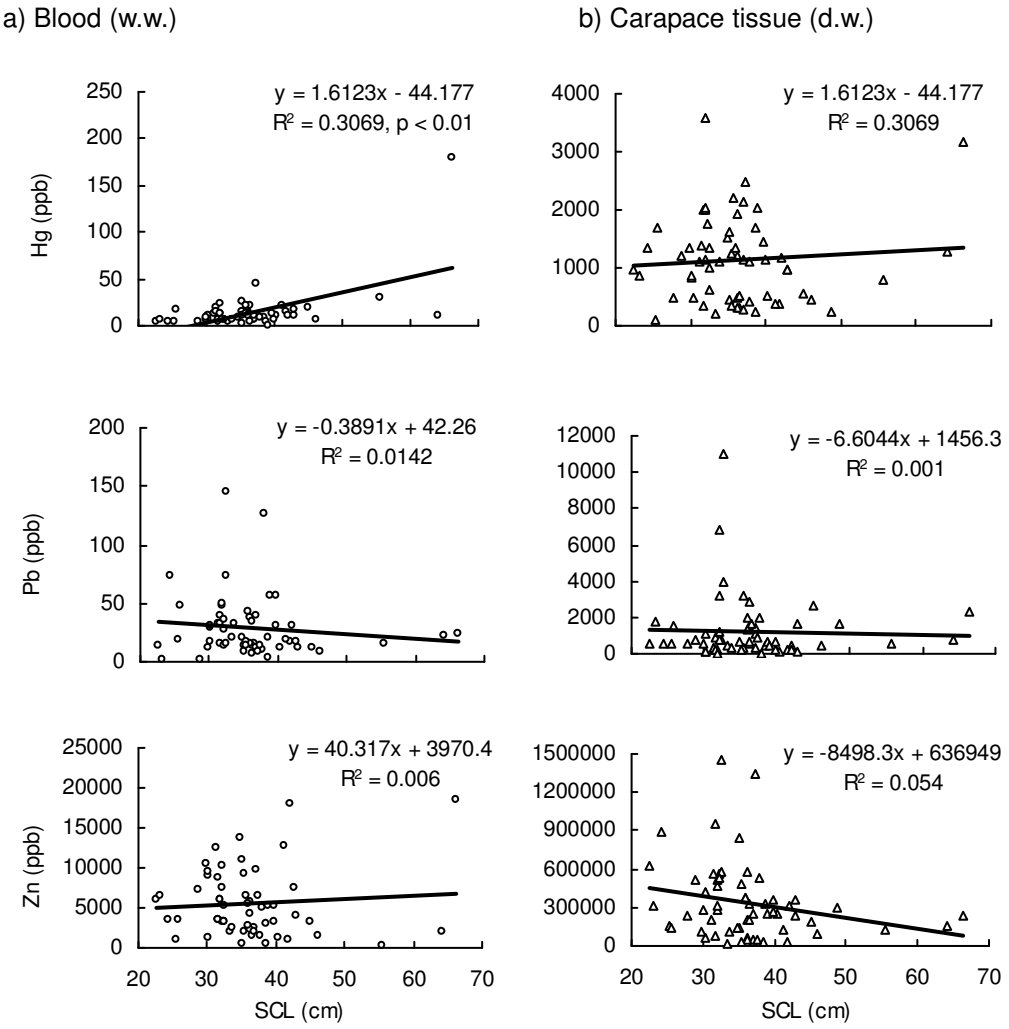


Fig. 12. Trace metal (Hg, Pb and Zn) concentrations (ppb) in blood (w.w.) and carapace tissue (d.w.) as a function of SCL (22.5 - 66.3 cm) of wild Kemp's ridleys captured from Calcasieu Pass, Louisiana.

Blue crab: trace metal concentrations

A total of 10 blue crabs (CW = 133 ± 26.3 mm) was collected at Calcasieu Pass, Louisiana in 2001. Zinc concentrations in muscle tissue of these blue crabs were not normally distributed. No significant difference ($p = 0.288$) was detected in carapace width between male (n = 6, CW = 141 mm) and female groups (n = 4, CW = 122 mm). Mean trace metal concentrations in blue crabs from Calcasieu Pass were: digestive gland - Ag = 262, Cd = 586, Cr = 13.9, Cu = 15,700, Hg = 14.1, Pb = 78.2 and Zn = 47,700 ppb; muscle - Ag = 88.9, Cd = 18.2, Cr = 1.1, Cu = 6,280, Hg = 19.4, Pb = 16.1 and Zn = 253,000 ppb (Appendix Table 19). Silver (3 times, $p < 0.01$), Cd (32 times, $p < 0.01$), Cr (12 times, $p < 0.01$), Cu (2.5 times), and Pb concentrations (5 times, $p < 0.01$) in the digestive gland were significantly higher than those in muscle tissue (Fig. 13). Mean Hg and Zn levels did not differ between tissue type nor was there a relationship between any metal concentration and blue crab size (Appendix Figs. 9 and 10).

Silver concentrations rose significantly with increased Cu (Pearson's r = 0.745) and Zn levels (Pearson's r = 0.668) in the digestive gland (Appendix Table 20). A similar relationship existed between Pb and Cd (Pearson's r = 0.873, $p < 0.01$) and Pb and Hg levels (Pearson's r = 0.639) in the digestive gland and Pb and Ag (Pearson's r = 0.647), and Pb and Zn levels (Pearson's r = 0.754) in muscle tissue. Chromium concentrations increased significantly with decreased Cu levels (Spearman's r = - 0.857) in muscle tissue.

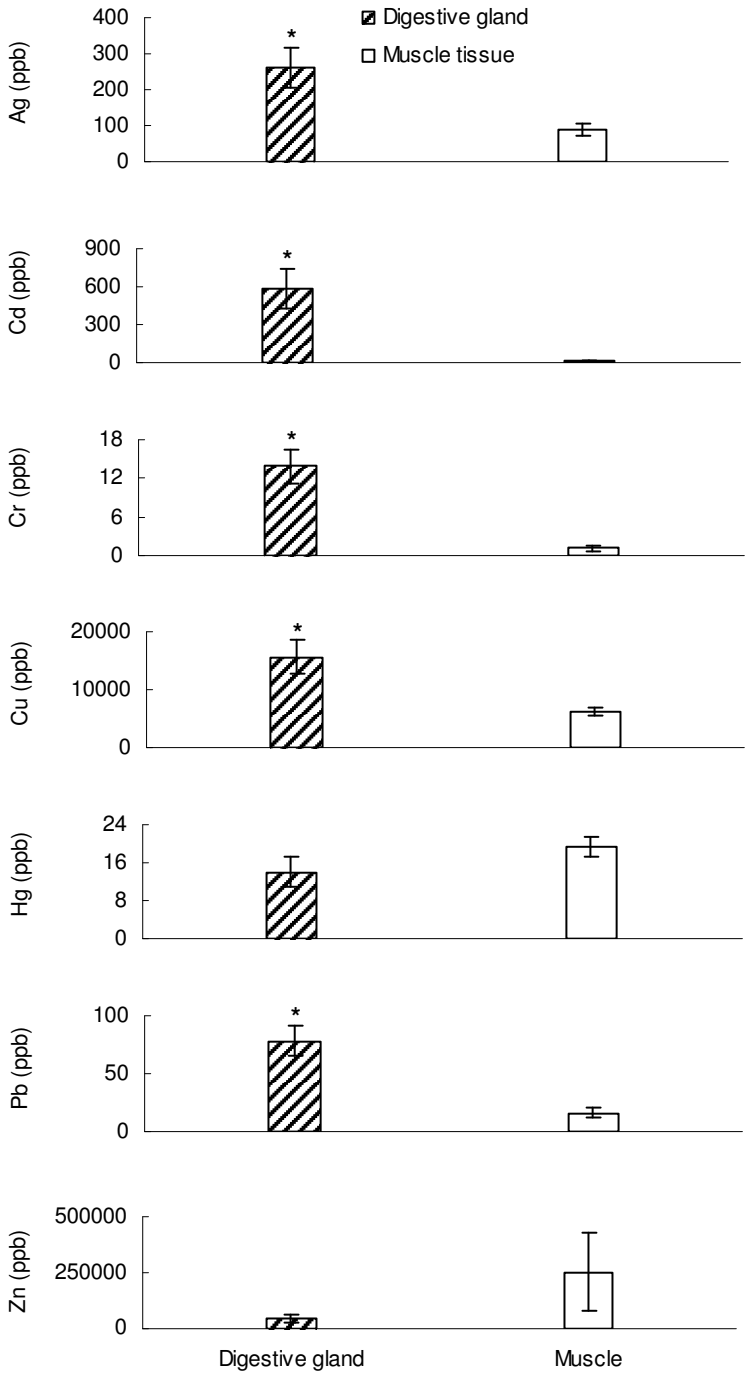


Fig. 13. Mean trace metal concentrations (ppb) in digestive gland (w.w.) and muscle tissue (w.w.) of blue crabs (n = 10) captured from Calcasieu Pass, Louisiana (* significant difference – $p < 0.05$, in metal concentration between digestive gland and muscle tissue).

Table 8. Trace metal concentrations (ppb) in Kemp's ridleys and blue crabs from Calcasieu Pass, Louisiana.

	Ag	Cd	Cr	Cu	Hg	Pb	Zn
Kemp's ridley							
Carapace (d.w.)	144	131[b]	322[ab]	3100	1120[ab]	1220[ab]	329000[ab]
Blue crab (w.w.)							
Digestive gland	262[a]	586[a]	13.9	15700[a]	14.1	78.2	47700
Muscle tissue	88.9[b]	18.2	1.1	6280[b]	19.4	16.2	253000

[a] significant difference between digestive gland of blue crabs and carapace tissue of ridleys;
[b] significant difference between muscle tissue of blue crabs and carapace tissue of ridleys

As was the case for Texas study sites, ridleys captured in Louisiana waters yielded higher Cr, Hg, Pb and Zn concentrations in carapace tissue than were detected in blue crab tissues while the latter exhibited higher ($p < 0.01$) Ag and Cu concentrations (Table 8). Silver ($p < 0.01$) and Hg concentrations were significantly higher in blue crabs from Texas than in Louisiana conspecifics, regardless of tissue type (Tables 6 and 7) while the converse was true for Cd in muscle tissue. In addition, Cr ($p < 0.01$) and Pb concentrations in muscle tissue of Texas blue crabs were significantly higher than those from Louisiana.

Southeast Atlantic Study Site

Kemp's ridley: trace metal concentrations

Range and mean statistics for trace metal values in blood and carapace tissue of Kemp's ridleys (n = 18) captured from the southeast Atlantic during 2001 - 2002 are given in Appendix Table 21. Chromium concentrations in blood

of these ridleys were not normally distributed. As was the case for ridleys captured from Texas and Louisiana, carapace tissue from the southeast Atlantic yielded higher metal concentrations than did blood of ridleys ($p < 0.05$, except Zn with $p < 0.01$). Concentration differences ranged from 2 (Cu) to 78 times (Hg) higher in carapace tissue. No relationship was detected between metal concentrations and SCL of ridleys, except Cr levels in carapace tissue decreased with increasing size (Appendix Figs. 11 and 12). In addition, Hg concentration in carapace tissue increased with increasing blood concentration (Fig. 14).

Cadmium concentrations rose significantly with increasing Hg levels (Pearson's $r = 0.561$) in blood (Appendix Table 22). Silver concentrations increased significantly with increased Cd (Pearson's $r = 0.882$, $p < 0.01$), Cu (Pearson's $r = 0.692$), Pb (Pearson's $r = 0.851$, $p < 0.01$), and Zn levels (Pearson's $r = 0.679$) in carapace tissue. Cadmium levels were positively correlated with those for Cu (Pearson's $r = 0.670$), Pb (Pearson's $r = 0.830$, $p < 0.01$), and Zn levels (Pearson's $r = 0.650$) in carapace tissue. Similar correlations were detected between Cu and Pb (Pearson's $r = 0.842$, $p < 0.01$), Cu and Zn (Pearson's $r = 0.921$, $p < 0.01$), and Pb and Zn levels (Pearson's $r = 0.841$, $p < 0.01$) in carapace tissue.

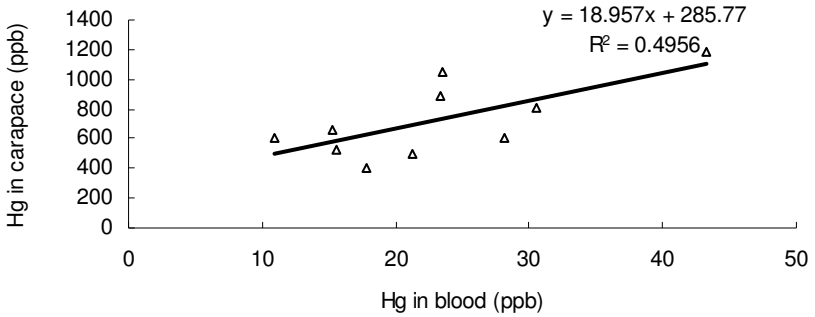


Fig. 14. Correlation of Hg concentration between blood (w.w.) and carapace tissue (d.w.) of Kemp's ridleys captured from the southeast Atlantic.

Southeast Atlantic ridleys yielded higher Cu and Hg concentrations in blood than did Texas and Louisiana conspecifics (Fig. 15). However, significantly higher Zn concentration occurred in blood of ridleys from Louisiana and Texas than those from the southeast Atlantic. No other relationship was detected for Ag, Cd, Cr, and Pb concentrations between study sites.

As was the case for blood of ridleys described above, southeast Atlantic ridleys yielded higher Ag concentration in carapace tissue, followed by that from Louisiana and Texas ($p < 0.01$), respectively (Fig. 15). Higher Cd and Cu concentrations in carapace tissue were detected in ridleys from Texas and Louisiana than in those from the southeast Atlantic ($p < 0.01$). Texas ridleys yielded higher Hg level in carapace tissue than did their conspecifics from Louisiana and the southeast Atlantic ($p < 0.01$). No other relationship was detected for Cr, Pb, and Zn concentrations in carapace tissue.

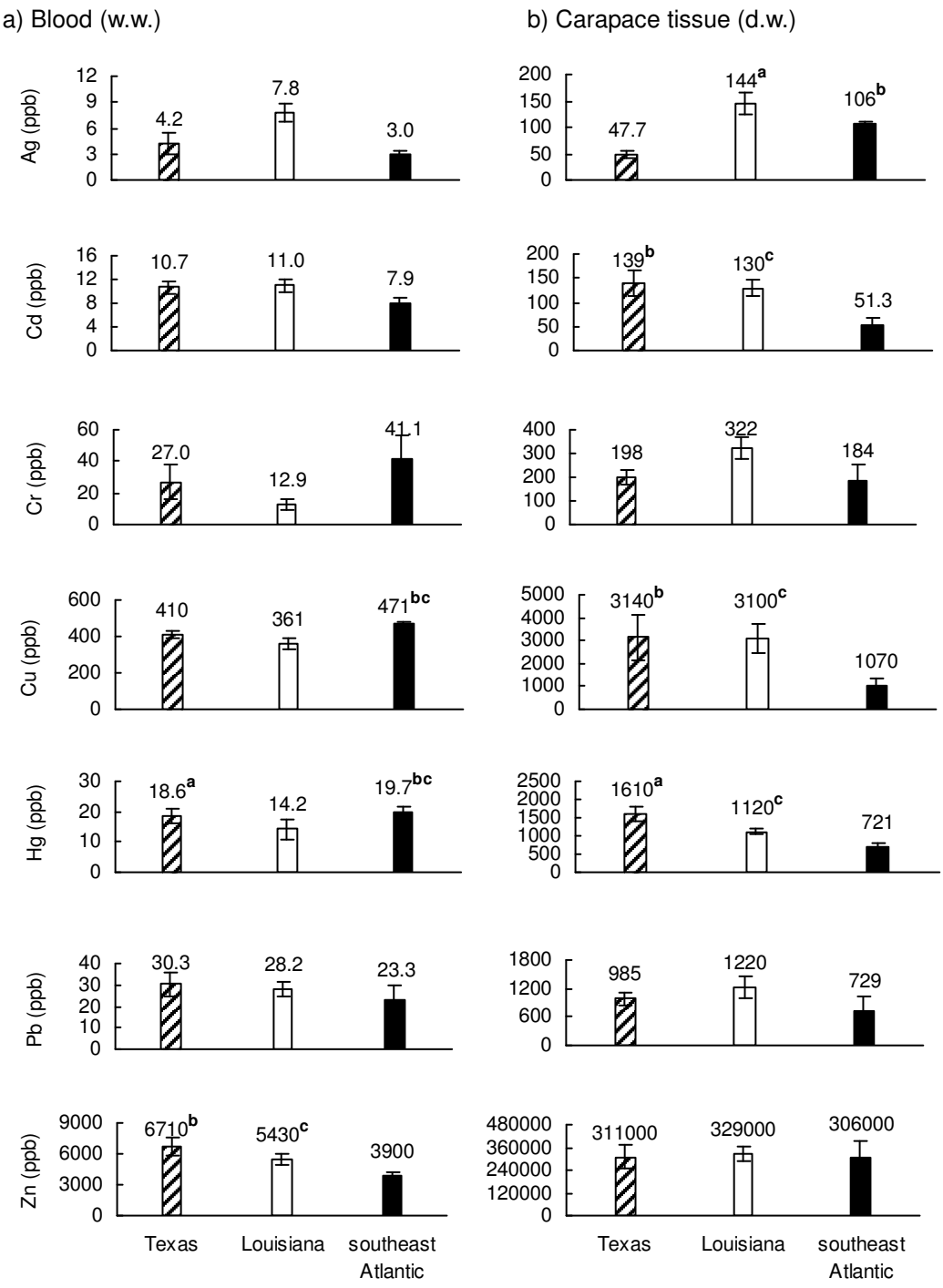


Fig. 15. Trace metal concentrations (ppb) in a) blood (w.w.) and b) carapace tissue (d.w.) of Kemp's ridleys captured from Texas (n = 30), Louisiana (n = 61) and southeast Atlantic (n = 18) coasts (significant difference – $p < 0.05$, between [a] Texas and Louisiana, [b] Texas and southeast Atlantic; [c] Louisiana and southeast Atlantic).

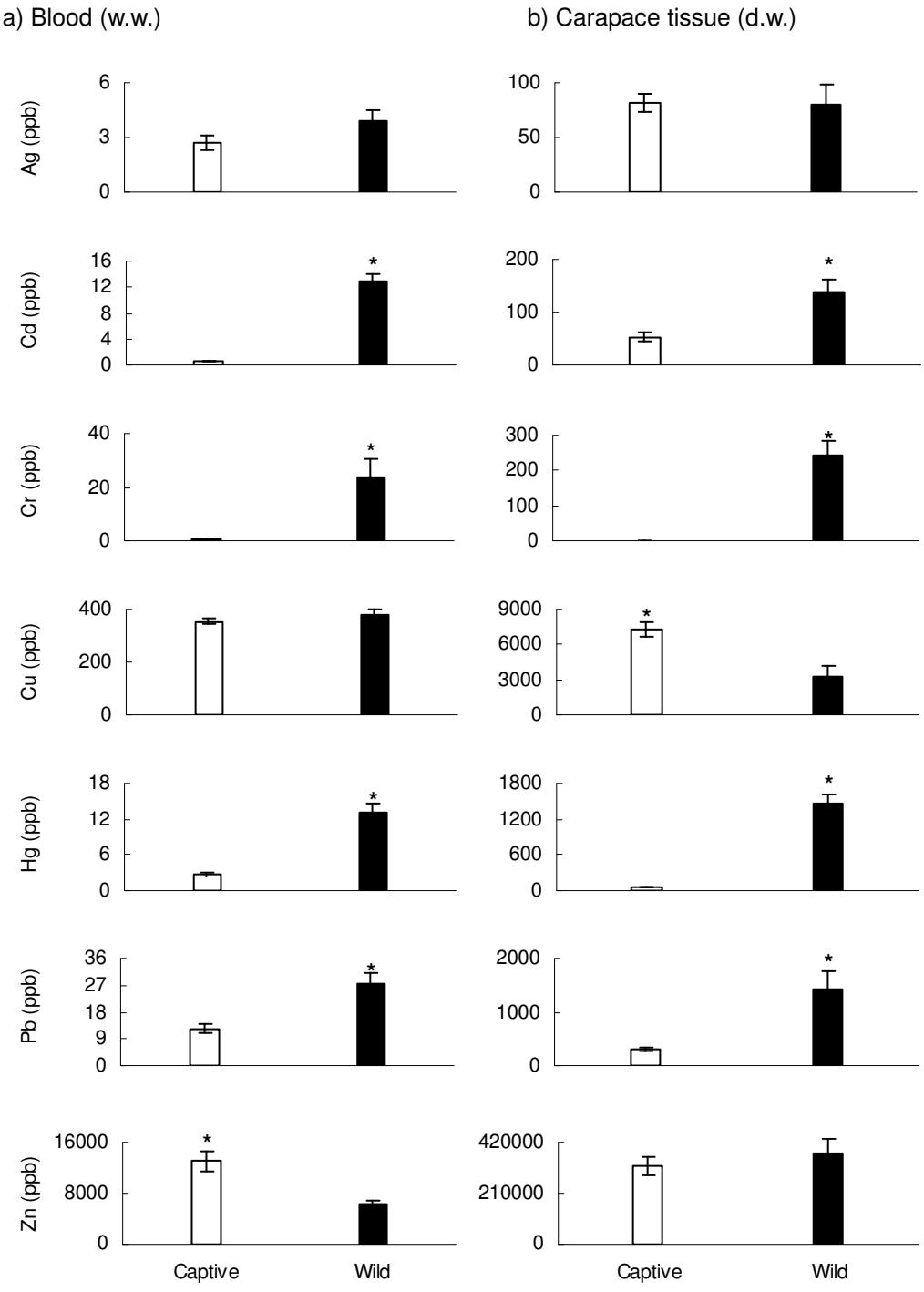


Fig. 16. Trace metal concentrations in captive (n = 33) and wild (n = 40) Kemp's ridleys from 28 to 35 cm SCL (* significant difference – $p < 0.05$, in mean metal concentrations between captive and wild Kemp's ridleys).

Wild vs. Captive Kemp's Ridleys: Trace Metal Concentrations

Trace metal levels from 28.0 to 35.0 cm SCL Kemp's ridleys captured along the Texas and Louisiana coast were compared to those of similar-sized captive conspecifics (Fig. 16). Straight carapace length of wild ridleys (32.0 ± 1.7 cm SCL, n = 40) used in this comparison did not exhibit significant size differences from that of captive ridleys (32.4 ± 1.1 cm SCL, n = 33). Mean trace metal levels of wild ridleys are shown in Table 9. Cadmium, Cr, Hg and Pb concentrations were significantly ($p < 0.01$) higher in wild ridleys than those in captive conspecifics, regardless of sample type. Concentration differences ranged from 2.6 (Cd) to 270 times (Cr) higher in carapace tissue of wild ridleys.

Conversely, Cu and Zn concentrations in carapace tissue and blood, respectively, of captive ridleys were significantly higher (~ 2 times; $p < 0.01$) than those in wild ridleys. No statistical significance was detected for Ag and Cu in blood and Ag and Zn in carapace tissue between captive and wild ridleys. However, trace metal levels in wild ridleys were generally higher than those in captive cohorts, except for Ag concentrations in carapace tissue.

Table 9. Mean trace metal concentrations (ppb) in blood (w.w.) and carapace tissue (d.w.) of wild Kemp's ridleys (28 to 35 cm SCL) from the Texas and Louisiana coast.

n = 40	Ag	Cd	Cr	Cu	Hg	Pb	Zn
Blood	3.9	12.8	24.5	374	12.9	28.2	6320
Carapace	80.7	138	243	3330	1450	1410	374000

Discussion

Wild Kemp's ridleys, like their captive-reared counterparts discussed in Chapter III, exhibited higher trace metal concentrations in carapace tissue than in their blood, regardless of study area or study site. This finding supports the predication that trace metal concentrations in carapace tissue of wild Kemp's ridleys should be higher than those in their blood. As a result, carapace tissue will be used as the basis on which to compare trace metal concentrations in Kemp's ridleys across study sites and in relation to those in their blue crab prey in remaining sections of this discussion. Available trace metal concentrations in live Kemp's ridleys reported by other investigators (Presti *et al*., 2000; Kenyon *et al*., 2001) also will be discussed within their respective metal sections.

A possible age-related relationship between trace metal concentrations and size of ridleys also was examined in the present study. Concentrations of Pb in blood and Cd in carapace tissue of ridleys captured from Texas significantly increased with increasing turtle size. In addition, Hg and Cu levels in blood and carapace tissue, respectively, of ridleys captured from Louisiana also exhibited a similar positive relationship with turtle size. A much wider size range of ridleys in Chapter V will provide a more complete assessment of trace metal accumulation in this species.

Identifying the various mechanisms of contaminant uptake and potential impacts of trace metals on an organism is extremely important in assessing its health (Phillips, 1995). Thus, metal concentrations in blue crabs, the ridley's

preferred prey, were examined in the present study. Although blue crab tissues analyzed in this chapter (muscle tissue and digestive gland only) may not be representative of trace metal concentrations within the entire body, they still provide insight into the possible contaminant relationship between Kemp's ridleys and their prey. Overall, blue crabs captured from Texas and Louisiana exhibited higher Ag and Cu concentrations while carapace tissue generated higher Cr, Hg, Pb and Zn levels, regardless of tissue type or study area. These findings did not fully support the aforementioned hypothesis that trace metal levels are higher in wild ridleys than in their blue crab prey. Trace metal concentrations in blue crabs from different locations in Texas and Louisiana are summarized in Appendix Table 5.

Silver

Wild ridleys (28 to 35 cm SCL) yielded lower Ag concentrations in their carapace tissue (80.7 ppb) compared to those of their captive conspecifics (81.8 ppb; Fig. 16). This finding supports the rejection of the hypothesis that Ag concentrations in wild ridleys exceed baseline levels reported for captive ridleys. Based on differences between metal concentrations in food sources of captive versus wild conspecifics, the latter could potentially accumulate higher Ag levels from their blue crab prey (DG - 596 ppb, M - 194 ppb) than from the pelleted food (26.6 ppb) fed to headstart counterparts. However, this potential was not realized given similar Ag concentrations in carapace tissues of wild and captive ridleys. It

is possible that Ag is not bioaccumulated in Kemp's ridleys or that carapace tissue does not represent the main accumulation site. Nonetheless, these tissues did exhibit the potential for trace metal accumulation within the size range of ridleys sampled, given the results of their comparison with blood-borne levels.

Silver combines with plasma proteins and accumulates in the liver of higher level aquatic organisms (ATSDR, 1990; Zeisler *et al*., 1993; Eisler, 1996; Saeki *et al*., 2001). An invasive biopsy to analyze trace metal levels in internal organs, such as liver, would likely provide much needed information on this metal and its accumulation potential in Kemp's ridleys.

Kenyon *et al*. (2001) reported the only published Ag concentrations in blood of live Kemp's ridleys (0.9 ppb) captured from Texas and Louisiana. A comparison of the present study with that investigation indicates significantly higher blood-borne Ag levels in Texas and Louisiana wild ridleys from the present study (6.6 ppb). The fact that blood often represents recent exposure (Chang, 1996) to contaminants may explain this finding as well as the difference between carapace and blood-borne levels of Ag in the present study.
Saeki *et al*. (2001) reported the only mean blood Ag level (0.6 ppb) in marine mammals that was similar to those in captive (2.7 ppb) and wild ridleys from Texas (4.2 ppb), Louisiana (7.8 ppb) and southeast Atlantic (3.0 ppb). Silver concentrations in hairs of marine mammals collected from the north Pacific ranged from 2.0 to 47.5 ppb (Saeki *et al*., 2001; Ikemoto *et al*., 2004). Data in

these aforementioned studies were at least two times lower than those in carapace tissue of ridleys, regardless of captive or wild as well as geographic locations, in the present study.

Age-related relationships between trace metal concentrations and size of animals reported for pinnipeds and marine mammals (Becker *et al*., 1995; Saeki *et al*., 2001) were not observed for Ag in ridleys during the present study. Nonetheless, southeast Atlantic ridleys did exhibit significantly higher Ag levels in carapace tissue than did their smaller juvenile conspecifics from Texas. Moreover, quite variable growth rates of sea turtles within the same cohort and population (Zug *et al*., 1997) may explain this accumulation relationship between Ag levels and size of ridleys.

Silver concentrations in blue crabs captured from Sabine Pass and Lavaca Bay were at least 5 times lower than those reported for the Galveston Bay (Park and Presley, 1997; Appendix Table 5). Conversely, Ag levels in blue crabs from Calcasieu Pass were similar to those in the Calcasieu River. Blue crabs captured in the present study yielded higher Ag concentrations than did ridleys and, as such, the hypothesis that Ag levels are higher in wild ridleys than in their blue crab prey must be rejected.

Bioaccumulation is an important consideration in trace metal studies that must be addressed as is relates to Ag uptake in ridleys. Bioaccumulation is the uptake of a substance via body surface (bioconcentration) or by means of food uptake (biomagnification). It is generally acknowledged that the

digestive gland is the major repository of Ag in crustaceans and that food chain biomagnification of Ag in the aquatic system is unlikely (Eisler, 1996). Ratte (1999) reported that Ag bioaccumulation in marine fish is generally lower than that in crustaceans. Rouleau *et al*. (2000) provided evidence that biological half-life of Ag is much longer in crustaceans than in fish. These findings may explain the fact that the relative Ag distribution for Kemp's ridleys and blue crabs was digestive gland > muscle > carapace tissue across Texas and Louisiana study sites. Further investigations involving invasive biopsy of internal organs in live ridleys are needed to assess whether this relationship holds true for all potential accumulation sites in Kemp's ridleys.

Blue crabs captured from Sabine Pass and Lavaca Bay exhibited significantly higher Ag levels than those from Louisiana blue crabs, regardless of tissue type. This finding does not explain why higher Ag concentrations were found in carapace tissue of Louisiana ridleys compared to that of their Texas conspecifics. This variability in Ag concentrations between ridleys and their blue crab prey may be due to a relatively small sample size used in assessing the latter's metal levels and the variations in metal loads within tissues.

No clinical studies have used blood or carapace tissue to assess presence of trace metals in Kemp's ridleys. Therefore, more research on trace metal metabolism is needed for a more accurate evaluation of the

impact of Ag accumulation and potential toxic effects in Kemp's ridleys. There is evidence indicating that Ag can induce a toxic response in marine mammals at elevated concentrations (19,300 ppb) through its effect on radical-scavenging enzyme systems and its possible synergism with other more toxic metals (Becker *et al*., 1995). Therefore, correlations between metals across life stages of Kemp's ridleys will be discussed in Chapter V.

In summary, only one hypothesis that Ag concentrations in carapace tissues of wild ridleys are higher than those in their blood could be accepted. Failure to detect a significant difference in Ag concentrations between wild and captive ridleys as well as those of wild ridleys and blue crabs casts some doubt on the accumulation potential for Ag and/or the role food plays in the uptake of this metal in Kemp's ridleys.

Cadmium

Wild ridleys within the 28 to 35 cm SCL size range exhibited significantly higher Cd concentration (137 ppb) in carapace tissue than did their captive conspecifics (52.6 ppb; Fig. 16). Hence, the hypothesis that Cd concentrations in wild ridleys exceed baseline levels reported for captive cohorts is accepted. A significantly higher Cd level was detected in blue crab prey (DG – 166 ppb) than that in the pelleted food (40 ppb) of captive counterparts. This concentration difference could account for the disparity in Cd accumulation between wild and captive ridleys.

Available information on Cd concentrations in sea turtles is limited to that for stranded carcasses and reproductive products (Sakai *et al*., 1995; Vazquez *et al*., 1997; Gordon *et al*., 1998; Caurant *et al*., 1999; Sakai *et al*., 2000a and 2000b), with no reports for live constituents. The current study is the first attempt to characterize Cd levels in blood and carapace tissue of Kemp's ridleys. A comprehensive comparison in metal concentrations between tissues of live ridleys and stranded conspecifics will be presented in Chapter VI.

Nielsen *et al*. (2000) reported blood Cd levels in marine mammals range from 17 to 10,800 ppb. These exceed those in captive (0.6 ppb) and wild ridleys from Texas (10.7 ppb), Louisiana (11.0 ppb) and the southeast Atlantic (7.9 ppb). In addition, approximately 10 times higher Cd concentrations were found in hairs of marine mammals (Yediler *et al*., 1993; Medvedev *et al*., 1997; Wiig *et al*., 1999) than for ridleys in the present study.

Cadmium concentrations in carapace tissue rose with increasing size of ridleys captured from Texas and, by doing so, were in contrast to similar investigations involving kidney tissue of green turtles (Gordon *et al*., 1998; Anan *et al*., 2002a). These variations may be due to differences in diets between ridleys (carnivores) and green (herbivores) turtles. Conversely, the trend for juvenile ridleys from Texas and Louisiana to exhibit higher Cd level in carapace tissue than their larger southeast Atlantic counterparts may reflect geographic differences wherein there are disparities in contaminant loading between ridley capture sites.

Cadmium is an element of particular concern in the current study because it concentrates in the digestive gland of blue crabs and can be passed onto consumer organisms (Engel and Brouwer, 1984). Blue crabs captured from Texas and Louisiana yielded higher Cd concentrations in the digestive gland than in their muscle tissue. This disparity was probably due to the presence of hymocyanin, oxygen-binding protein of blue crabs binding with Cd in the digestive gland (Brouwer *et al*., 1983).

The relative distribution of Cd in Kemp's ridleys and its blue crab prey was digestive gland > carapace tissue > muscle. This finding does not fully support the hypothesis that trace metal levels are higher in wild ridleys than in their blue crab prey. As was the case for Ag, it leaves doubt as to the role that carapace tissue may play as a potential accumulation site for this metal in Kemp's ridleys. Eisler (1986) reported evidence for Cd transfer through various trophic levels but only among lower organisms (e.g. blue crab) was biomagnification exhibited. Marine vertebrates accumulate Cd largely in the liver and kidney (ATSDR, 1999a). Nonetheless, reports that Cd levels tend to increase with age of an organism are not available for sea turtles (Sakai *et al*., 2000a). As such, this lack of a size-concentration relationship for Cd in Kemp's ridleys may be due to Cd accumulation in the liver and not in tissue analyzed during the present investigation.

In summary, the hypotheses that Cd concentrations in carapace tissues of wild ridleys are higher than those in their blood and exceed baseline levels reported for captive ridleys are both accepted. The fact that carapace tissue of wild ridleys exhibited higher Cd levels than did their blood may indicate the potential for accumulation in carapace tissue. Food sources may have effected Cd levels between wild and captive ridleys. The fact that higher Cd levels were in ridleys from Texas and Louisiana than in their larger cohorts from the southeast Atlantic also implies that respective contaminant loads in food sources may play an important role in uptake pathways. Higher Cd levels in the digestive gland of blue crabs was grounds for rejecting the hypothesis that Cd levels are higher in wild ridleys than in their blue crab prey. Nonetheless, the fact that carapace tissue did yield higher Cd concentrations than did muscle tissue of blue crabs may be explained by small sample size and/or accumulation disparities between tissue types in this crustacean.

Chromium

Chromium has not been routinely monitored in marine organisms since it is an essential element in vertebrates for which no biomagnification has been reported in aquatic food chains (Eisler, 1986). The few available studies of Cr concentrations in sea turtles have been based on stranded carcasses and eggs (Stoneburner *et al*., 1980; Storelli *et al*., 1998a; Alam and Brim, 2000), with none of these being Kemp's ridleys. Thus, the present study is the first report of Cr

concentrations in Kemp's ridleys. These levels were at least 5 times less than those reported in marine mammal hair (Ikemoto *et al*., 2004).

Neither size-dependent relationships nor differences in Cr concentration were detected for wild ridleys between study areas. Like that for Cd, wild ridleys within the 28 to 35 cm SCL size range exhibited significantly higher Cr levels (243 ppb) in carapace tissue than did their captive counterparts (0.9 ppb; Fig. 16). This finding runs counter to the fact that higher Cr levels were found in pelleted food (220 ppb) than in blue crab prey (DG - 11 ppb). Nonetheless, this result supports the hypothesis that Cr concentrations in wild ridleys exceed baseline levels reported for captive ridleys. However, this disparity may be due to other factors such as variations in metabolic regulation of Cr concentration between wild, very active ridleys and their captive, activity-restricted counterparts or diet-dependent element correlations that facilitate the uptake of this metal in wild conspecifics at much higher levels than in captive cohorts. More research is needed to explain this disparity.

Ridley carapace tissue did yield higher Cr concentrations than either blue crab tissue type, thus providing support for accepting the hypothesis that Cr levels are higher in wild ridleys than in their blue crab prey. It also may imply that carapace tissue serves as an accumulation site for this metal.

In summary, hypotheses outlined in the introduction of this chapter that Cr concentrations in carapace tissues of wild ridleys are higher than those in their blood, exceed baseline levels reported for captive ridleys, and are higher in wild ridleys than in their blue crab prey are all accepted.

Copper

Captive ridleys exhibited a significantly higher Cu level (7170 ppb) in carapace tissue than did their wild conspecifics (3,330 ppb) within 28 to 35 cm SCL. Hence, this finding serves to reject the hypothesis that Cu concentrations in wild ridleys exceed baseline levels reported for captive cohorts. A significantly higher Cu concentration was detected in blue crab prey (DG – 19,500 ppb) than that in the pelleted food (4,420 ppb) of captive counterparts. It could not explain the disparity in Cu accumulation between wild and captive ridleys because of limited information on Cu metabolic regulation. A relatively small sample size (blue crab) could be another reason for this concentration difference. Copper accumulation in blue crabs has been studied extensively but future research involving physiological function of Cu is needed for a better understanding of Cu accumulation in live ridleys.

Copper measurements have been included in most of metal studies of sea turtle carcasses (Aguirre *et al*., 1994; Sakai *et al*., 1995, 2000a and 2000b; Caurant *et al*., 1999; Anan *et al*., 2002a). These studies include one available investigation on live Kemp's ridleys from 1994 and 1995 (Kenyon *et al*., 2001).

Blood-borne Cu levels (524 ppb) in wild ridleys of the aforementioned study were significantly higher than those in the present study (379 ppb). The significance of this difference is lessened by the assumption that blood-borne levels reflect recent exposure (e.g. metal loading in prey) to possible Cu sources.

Studies by Yediler *et al*. (1993), Medvedev *et al*. (1997), and Ikemoto *et al*. (2004) reported that Cu levels in marine mammal hair ranged from 5400 to 33,800 ppb. These Cu concentrations are at least two times higher than those of wild ridleys in the present study.

Copper concentration in carapace tissue increased with increasing size of Louisiana ridleys, a trend mirrored by Kenyon *et al*.'s (2001) investigation of live Kemp's ridleys. This finding suggests Cu accumulates with growth of ridleys. However, Texas and Louisiana ridleys exhibited higher Cu levels in carapace tissue than did their larger southeast Atlantic conspecifics. The latter finding implies that geographic differences in diet or ambient contaminant loads may play a role in trace metal uptake in ridleys through subadult life stages.

Diet appears to be a critical factor influencing Cu levels in aquatic organisms (Eisler, 1998). Like that for Cd, blue crabs' hemocyanin has a large number of high-affinity Cu-binding sites (Brouwer *et al*., 1982). Significantly higher Cu levels found in blue crabs, regardless of tissue type (digestive gland > muscle tissue), than those in carapace tissue of wild ridleys mandates that the hypothesis that Cu levels are higher in wild ridleys than in their blue crab prey be rejected. Further investigation of Cu levels in the internal organs (i.e. liver) may

provide better information for potential accumulation sites in Kemp's ridleys.

In summary, the present study found evidence only to support the hypothesis that Cu concentrations in carapace tissues of wild ridleys are higher than those in their blood. More investigations are needed on Cu metabolism and regulation within ridleys before assuming this metal is accumulated to a measurable degree.

Mercury

Wild ridleys (28 to 35 cm SCL) exhibited significantly higher Hg concentration (1450 ppb) in carapace tissue than did their captive conspecifics (60.3 ppb; Fig. 16). This finding supports the hypothesis that Hg concentrations in wild ridleys exceed baseline levels reported for captive ridleys. This acceptance and significantly higher Hg levels in blue crab prey (M – 58.0 ppb) than in pelleted food (14.6 ppb) implies that food plays an important role in Hg uptake in live ridleys.

Mercury levels have been studied extensively in stranded sea turtles (Sakai *et al*., 1995, 2000a and 2000b; Storelli *et al*., 1998a and 1998b; Gordon *et al*., 1998; Godley *et al*., 1999), with these including a few investigations of live Kemp's ridleys captured from Texas and Louisiana (Presti *et al*., 2000; Kenyon *et al*., 2001) and loggerheads trawled from the southeast Atlantic (Day, 2003). Significantly higher blood-borne Hg concentrations in live ridleys within a similar size range of that used in this study were reported by Presti *et al*. (24.1 ppb),

followed by those in the present study (15.6 ppb) and an investigation by Kenyon *et al*. (13.2 ppb). Like that for Cu, the significance of this difference relates only to the assumption that blood-borne levels reflect more recent exposure to possible Hg sources. Mercury levels in blood (29 ppb) of loggerheads (Day, 2003) were higher than those in the present study. Additionally, the current study exhibited significantly higher carapace-borne Hg concentrations (1,280 ppb) than that reported by Presti (1,070 ppb) for ridleys and by Day (461 ppb) in loggerheads. This finding implies sizable variability in contaminant loads across different prey species as well as sea turtle species.

Nielsen *et al*. (2000) reported blood Hg levels in marine mammals (250 – 1,000 ppb) were at least one order of magnitude higher than those in wild ridleys. In addition, Hg concentrations in blood of captive marine mammals (Nigro *et al*., 2002) were 52 times higher than those of captive (2.7 ppb) ridleys in the present study. Various Hg levels (1,400 – 11,000 ppb) have been reported in marine mammal hair (Yediler *et al*., 1993; Watanabe *et al*., 1996; Medvedev *et al*., 1997; Beckmen *et al*., 2002; Ikemoto *et al*., 2004). In general, carapace-borne Hg concentrations in wild ridleys were lower than those reported by forementioned studies of marine mammals.

Mercury concentration has been found to increase with age/length in live sea turtles (Presti *et al*., 2000; Kenyon *et al*., 2001; Day, 2003). A similar relationship was detected in carapace tissue of ridleys from Louisiana. Ridleys captured in Texas exhibited significantly higher Hg level in carapace tissue than

did their southeast Atlantic and Louisiana conspecifics. It implies that geographic differences in diets or ambient contaminant loads may play a role in Hg uptake in ridleys. For example, higher Hg levels found in carapace tissue of ridleys from Lavaca Bay than those from Sabine Pass probably reflects the concentration disparity between their blue crab prey from respective study areas. Food is most likely a significant route for Hg intake in sea turtles (Kenyon *et al*., 2001).

Ridley carapace tissue did yield higher Hg concentrations than did either blue crab tissue type, thus providing support for accepting the hypothesis that Hg levels are higher in wild ridleys than in their blue crab prey. It also may imply that carapace tissue serves as an accumulation site for this metal.

In summary, hypotheses outlined in the introduction of this chapter that Hg concentrations in carapace tissues of wild ridleys are higher than those in their blood; exceed baseline levels reported for captive ridleys; and are higher in wild ridleys than in their blue crab prey are all accepted.

Lead

Wild ridleys within the 28 to 35 cm SCL size range exhibited significantly higher Pb concentration (1,410 ppb) in carapace tissue than did their captive counterparts (303 ppb; Fig. 16). Hence, the hypothesis that Pb levels in wild ridleys exceed baseline levels reported for captive conspecifics is accepted. This finding runs counter to the fact that higher Pb levels were found in pelleted food (615 ppb) than in blue crab prey (DG - 81.5 ppb; M - 44.3 ppb). This

concentration difference may be due to a small sample size used in analyses and variations in metabolic regulation of Pb levels between wild (i.e. very active) and captive (i.e. reduced physical activity) ridleys. Nonetheless, that fact that carapace tissue of wild ridleys exhibited higher Pb levels than did blood may indicate potential accumulation in said tissue. Lead has been reported to bioaccumulate in aquatic animals (ATSDR, 1999) and, as such, this may account for the disparity reported herein.

Lead measurements have been included in most metal studies of sea turtle carcasses (Godley *et al.*, 1999; Sakai *et al.*, 2000a and 2000b; Anan *et al.*, 2002a). These studies include one available investigation on live Kemp's ridleys from 1994 and 1995 (Kenyon *et al.*, 2001). Blood-borne Pb concentrations (11.1 ppb) in wild ridleys from the aforementioned study were significantly lower than those in the present study (28.9 ppb). The significance of this difference is likely due to blood-borne levels reflecting only recent exposure to possible Pb sources. No differences in Pb concentration were detected between wild ridleys from Texas and Louisiana study areas. However, Pb concentrations in carapace tissue increased with increasing size of ridleys from Texas, a trend mirrored by other marine organisms (Eisler, 1988; Medvedev *et al.*, 1997).

Similar blood-borne Pb levels were detected between marine mammals (10 - 40 ppb; Nielsen *et al.*, 2000) and wild ridleys (23.3 - 30.3 ppb) in the present study. In addition, Pb concentrations in carapace tissue of these wild ridleys (728 - 1,220 ppb) were lower than those in marine mammal hair (1,400 - 134,000 ppb;

Medvedev *et al*., 1997; Ikemoto *et al*., 2004).

Blue crabs in the present study yielded higher Pb concentrations in the digestive gland than in their muscle tissue. These levels were lower than those for blue crabs reported by (Park and Presley, 1997) in Galveston Bay (Appendix Table 5). Nonetheless, ridley carapace tissue did yield higher Pb concentrations than either blue crab tissue type, thus providing support for accepting the hypothesis that Pb levels are higher in wild ridleys than in their blue crab prey. It also may imply that carapace tissue serves as an accumulation site for this metal. Eisler (1988) reported that Pb concentrations in aquatic vertebrates tend to localize in hard tissue, such as bone or carapace tissue.

Like that for Cr and Hg, the hypotheses of this chapter that Pb concentrations in carapace tissues of wild ridleys are higher than those in their blood; exceed baseline levels reported for captive ridleys; and are higher in wild ridleys than in their blue crab prey are all accepted.

Zinc

Wild ridleys (28 to 35 cm SCL) did not yield a statistical difference between Zn concentrations in their carapace tissue (374,000 ppb) and those of their captive conspecifics (321,000 ppb; Fig. 16). Therefore, the hypothesis that Zn concentrations in wild ridleys exceed baseline levels reported for captive ridleys is rejected. Based on differences between metal concentrations in food sources,

captive ridleys could potentially accumulate higher Zn levels from the pelleted food (133,000 ppb) than in blue crab prey (DG – 45,600 ppb; M – 51,000 ppb) fed to wild ridleys. However, this potential did not explain the fact that similar Zn concentrations in carapace tissue existed between wild and captive ridleys. It is possible that carapace tissue does not represent the main accumulation site for Zn in ridleys. Another possible reason may be the fact that crustaceans can regulate body-borne Zn concentrations (Eisler, 1993).

Neither size-dependent relationships nor differences in Zn concentration were detected for wild ridleys between study areas. Zinc concentrations also have been studied extensively in sea turtle carcasses (Aguirre *et al*., 1994; Sakai *et al*., 1995, 2000a and 2000b; Gordon *et al*., 1998; Caurant *et al*., 1999; Anan *et al*., 2002a). Kenyon *et al*. (2001) reported the only published Zn concentrations in blood of live Kemp's ridleys (7,550 ppb), with these being significantly higher than those in the present study (5,870 ppb). The significance of this difference is lessened by the assumption that blood-borne levels reflect recent exposure to possible Zn sources. In addition, carapace-borne Zn levels in wild ridleys of the present study were about two times higher than those reported in marine mammal hair (125,000 – 186,000 ppb; Watanabe *et al*., 1996; Medvedev *et al*., 1997; Ikemoto *et al*., 2004).

Zinc concentrations in blue crabs from Texas were similar to those of conspecifics from Louisiana in the present study. Ridley carapace tissue did yield significantly higher Zn concentrations than either blue crab tissue type, thus

providing support for accepting the hypothesis that Zn levels are higher in wild ridleys than in their blue crab prey. In addition, carapace tissues also exhibited some potential for accumulating Zn when one compares these results to blood-borne levels.

Overall, the hypotheses that carapace tissue of wild ridleys exhibited higher Zn levels than did blood and their blue crab prey are both accepted. Doing so seems to indicate accepting the potential for Zn accumulation by carapace tissue. Nonetheless, failure to detect a significant difference in Zn concentrations between wild and captive ridleys casts some doubt on the role food plays in the uptake of this metal.

Summary

Carapace tissue of wild ridleys generated higher metal concentrations than did the blood, which suggests the potential for accumulation in carapace tissue. Age/size relationships in Cd, Cu, Hg, and Pb concentrations were detected in carapace tissue of wild ridleys. However, a much wider size of ridleys will be tested in Chapter V to verify such as a relationship between trace metal level and size. In addition, variability of growth rate and sex of ridleys also must be taken into consideration when attempting to explain trace metal accumulation. It is unclear whether carapace tissue is the main accumulation site for trace metals within the size range of ridleys sampled by the present study. Invasive sampling techniques should be used to generate information on trace metal accumulation

within the body of live ridleys and to compare it with levels in stranded carcasses as a means of evaluating accumulation potential. A feeding experiment involving isotope-labeled food may help determine the transfer route and dynamics of trace metal needed to identify accumulation sites in live sea turtles.

Differences in contaminant loads in food sources may explain the concentration disparities (Cd and Hg) in carapace tissue between wild and captive ridleys. Chromium and Pb levels were the only two metals where pelleted food yielded significantly higher concentrations than did blue crabs. This finding runs counter to the fact that higher metal levels were found in wild ridleys than in their captive conspecifics. Much more research is needed on trace metal metabolism and regulation in live ridleys to explain this disparity. Nonetheless, ridley carapace tissue did yield higher Cr, Hg, Pb and Zn concentrations than either blue crab tissue type while the latter exhibited higher Ag, Cd and Cu levels. Concentration differences between Kemp's ridleys and their blue crab prey are most likely related to geographic regions, life stages and bioaccumulation capacity in specific tissues of blue crabs.

Pugh and Becker (2001) and Milton and Lutz (2003) indicated age, gender, diet, and geographic location are important factors in the potential for animals to be affected by accumulated persistent pollutants. Although this study did not investigate gender of wild ridleys, the data generated herein provided information on possible metal uptake from food sources and potential for accumulation of trace metals. Trace metals are not equally distributed in the

marine environment and, as such, some variations are expected to be seen in wild Kemp's ridleys captured from different geographic regions. The current study recommends long-term monitoring to provide better information on spatial and temporal variability in trace metal uptake pathways.

Metal toxicity is directly dependent upon its bioavailability (i.e. chemical forms) and bioaccumulation in aquatic organisms (Fisher and Wang, 1998). Therefore, information on trace metal speciation is needed to more accurately evaluate accumulation potential in live ridleys. In addition, data on the role of abiotic factors (i.e. salinity, temperature) also are needed because of their potential for modifying trace metal concentrations in aquatic organisms. Overall, this chapter provided useful information on trace metal levels in live Kemp's ridleys that could be used to assess the potential for contaminant accumulation in sea turtles.

CHAPTER V

TRACE METAL CONCENTRATIONS IN NESTING KEMP'S RIDLEY SEA TURTLES AND THEIR BLUE CRAB PREY

Trace metal investigations of live adult sea turtles are limited, with those targeting nesting females and their reproductive products (i.e. eggs and dead hatchlings) even rarer (Vazquez *et al.,* 1997; Sakai *et al.,* 1995 and 2000a; Alam and Brim, 2000). Research described herein characterizes trace metal levels in blood and carapace tissue of female ridleys nesting on Rancho Nuevo, Mexico. In so doing, it continues Chapter IV's assessment of the potential for a size-dependent relationship in trace metal concentration among Kemp's ridleys from the Gulf of Mexico and the southeast Atlantic. In addition, this chapter provides additional information on the role blue crab prey may play in trace metal uptake in Kemp's ridleys.

Adult sea turtles spend all their life in the ocean, with only mature females coming ashore to nest. Mature Kemp's ridleys are restricted mainly to the Gulf of Mexico (Márquez-M *et al.*, 2001), where they primarily nest along the northeastern Mexico coast on or near Rancho Nuevo in southern Tamaulipas, approximately 322 km (200 miles) south of Brownsville, Texas. Ridley females reach sexual maturity as early as 6 years old (Márquez-M, 1994) while most new nesters average 9 to 13 years old (Zug *et al.*, 1997). An adult ridley is defined as a > 60 cm SCL ridley whose nesting season usually extends from April to August

(Miller, 1997). Female ridleys emerge to nest in synchronized aggregations termed *arribadas* (Spanish for arrival) that provide opportunities for scientists to study their biology, physiology, and toxicology.

Satellite tracking studies (Byler, 1989; Byler and Plotkin, 1994) have shown that adult ridleys generally travel in continental shelf waters less than 50 m deep. Márquez-M (1994) reported these females migrate northward to feeding areas off Mississippi and Alabama and southward to the Campeche Sound. These are also areas of the Gulf of Mexico where a high density of offshore oil extraction has historically precipitated chronic and/or low level spills that could facilitate metal uptake in migrating ridley females (Pugh and Becker, 2001). In addition, coastal waters within highly industrialized areas, such as those in Texas and Louisiana (Appendix Table 23), serve as feeding grounds where these adult ridleys may ingest contaminants.

Research objectives summarized herein are to characterize trace metal levels in blood and carapace tissues of adult Kemp's ridley females and to compare these concentrations with those of younger cohorts to complete the assessment of the potential for a size-dependent relationship among selected trace metals. This information allows the following hypotheses for respective metals to be tested for an older, reproductively mature sector of the ridley population: 1) trace metal concentrations in carapace tissues are higher than those in the blood; 2) trace metal levels are higher in ridleys than in their blue crab prey; and 3) trace metal concentrations in ridleys across all post-pelagic life

stages are age/size-dependent.

Materials and Methods

Study Site

The Rancho Nuevo Tamaulipas, Mexico (Fig. 1) nesting beach was visited in May 2002 to collect blood and carapace tissues from nesting females as well as blue crabs from an adjacent lagoon.

Sample Collection and Digestion

Four wheelers were used in daily patrols of the Rancho Nuevo beach for nesting females during May 2002. Approximately 5 mL of blood was drawn from nesting females that were encountered during these surveys following protocol described in Chapter III. A stainless steel biopsy tool was used to collect about 0.2 g of carapace tissue from each female. Prior to scraping, carapace tissue was cleaned with an alcohol swab to clean off excess sand. These blood and carapace tissue samples were initially placed on dry ice and were kept frozen until analysis.

To increase the sample size of mature conspecifics, two additional ridleys captured from Calcasieu Pass, Louisiana and classified as adult females due to lack of a male secondary sexual characteristic (i.e. longer tail that extends well beyond the carapace) also were analyzed for trace metal concentrations by the aforementioned protocols. Trace metal data generated on these ridleys were

combined with those of their Mexico conspecifics to compare within the adult size class as well as across other size classes.

A total of 10 blue crabs also was collected from the lagoon adjacent to the nesting beach. Blue crabs were stored in double zip-lock bags, initially placed on dry ice and kept frozen until analysis. Digestion methods for both blood and carapace tissue are described in Chapter III. The dissection and digestion procedures used for blue crabs followed those outlined in Chapter IV.

Metal Analysis

Trace metal determinations of blood, carapace tissue and blue crabs followed protocols described in Chapters III and IV. Concentrations of Ag, Cd, Cr, Cu, Pb, and Zn were analyzed by graphite furnace atomic absorption spectrometer while Hg was analyzed by cold vapor atomic fluorescence spectrometry. All trace metal concentrations were measured on a ppb dry/wet weight basis.

Statistical Analysis

Parametric and non-parametric tests were employed, as appropriate, in statistical comparisons of metal concentrations. Range, mean and standard deviation were reported for each metal. Normal distribution was determined for these data by the Kolmogorov-Smirnov test. Regression analysis was utilized to characterize relationships between metal concentration and carapace length of

ridleys across all life stages to assess possible accumulation with size. Paired *t*-test and a Wilcoxon signed rank test were used to test for differences in metal concentrations between Kemp's ridley blood and carapace tissue and blue crab tissues. The Pearson's and Spearman's rank correlation coefficients were used to assess possible relationships between metal concentrations. An Independent *t*-test and Mann-Whitney U test were used to detect differences in metal concentrations across constituent size classes for both blood and carapace tissue in ridleys. Results were determined significant when $p < 0.05$, unless otherwise stated.

Results

Eighteen ridleys nesting at Rancho Nuevo, Mexico in May 2002 were sampled to characterize trace metal concentrations in their blood and carapace tissue. Curved carapace length (CCL) recorded on these ridleys by Mexican biologists surveying the beach had to be transformed to straight carapace length (SCL) by using the following equation (Coyne, 2000): SCL = 0.346 + 0.948 x CCL. This conversion enabled their comparison with size data on live ridleys captured from Texas, Louisiana and southeast Atlantic. Mean SCL of nesting ridley females (65.0 cm) was significantly larger ($p < 0.01$) than that for juveniles and subadults from Texas (35.7 cm), Louisiana (36.3 cm), and the southeast Atlantic (46.3 cm).

Nesting Ridley Females: Trace Metal Concentrations

Range and mean of metal concentrations in blood and carapace tissue of nesting ridley females are given in Appendix Table 24. Concentrations of Ag, Cr and Zn in blood of these ridley females were not normally distributed. As was the case for ridleys captured from Texas, Louisiana and the southeast Atlantic, carapace tissue from these Mexican conspecifics yielded significantly higher ($p < 0.01$) metal concentrations than did their blood. Concentration differences ranged from 1.8 (Cu) to 112 times (Cr) higher in carapace tissue.

Cadmium concentration rose significantly with increasing Cu level in blood (Pearson's $r = 0.559$), and Cu (Pearson's $r = 0.673$, $p < 0.01$) and Pb levels (Pearson's $r = 0.683$, $p < 0.01$) in carapace tissue (Appendix Table 25). A similar significant relationship existed between Ag and Pb (Pearson's $r = 0.612$, $p < 0.01$), and Ag and Zn (Pearson's $r = 0.663$, $p < 0.01$) in carapace tissue, and Ag and Cr in blood (Spearman's $r = 0.693$). Chromium concentration was positively correlated with Cu (Pearson's $r = 0.485$) in carapace tissue.

Metal concentrations in a total of 127 Kemp's ridleys from the southeast Atlantic, Louisiana, Texas, and Mexico were combined to assess the potential for accumulation of metal concentrations across all life stages. These ridleys were classified as follows: smaller juveniles - 20 – 40 cm SCL; larger juveniles/subadults - 41 – 60 cm SCL; and adults - > 60 cm SCL (Fig. 17). Trace meal concentrations in these adult turtles are given in Table 10.

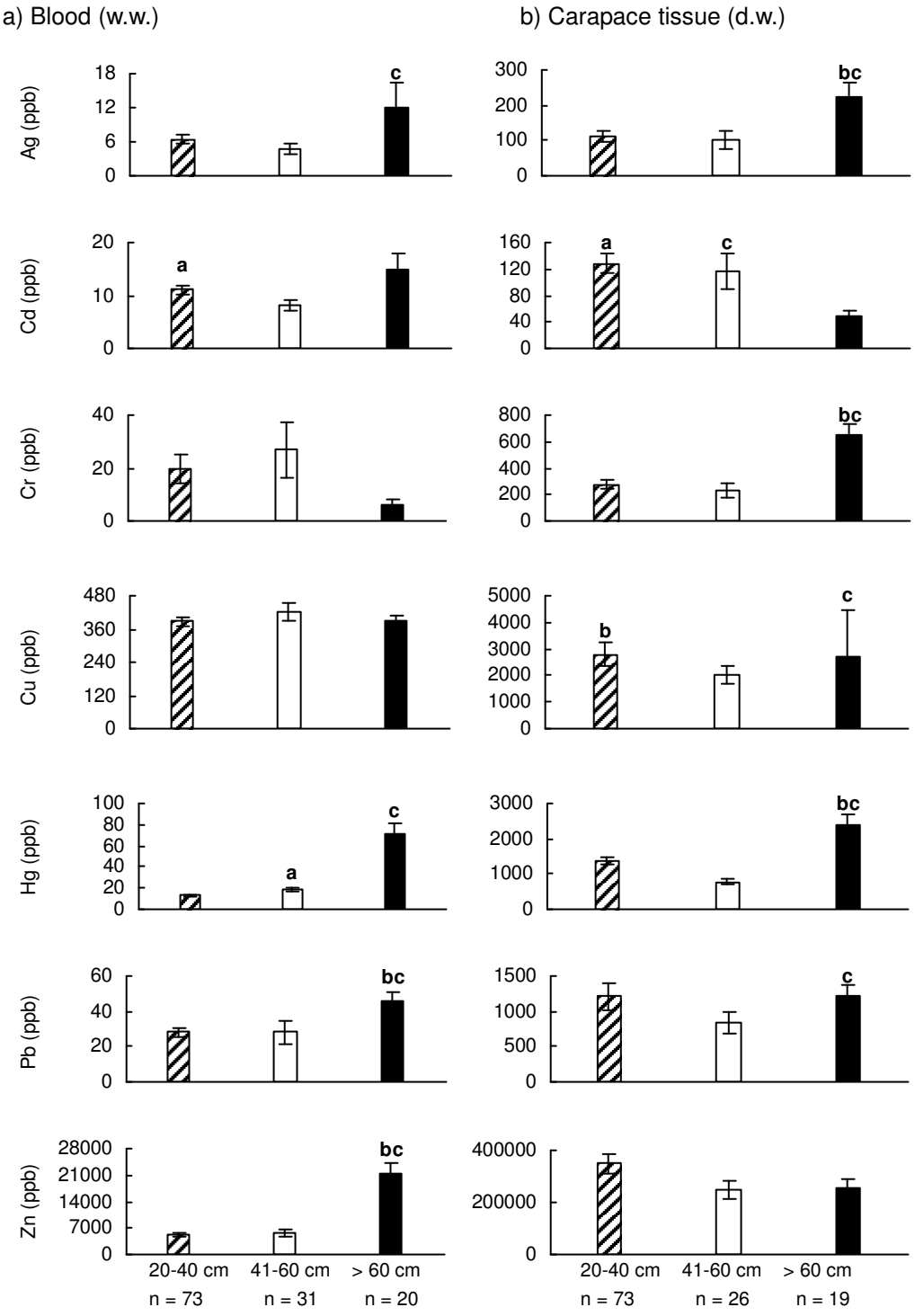


Fig. 17. Trace metal concentrations (ppb) in size classes (cm) of Kemp's ridleys from the southeast Atlantic, Louisiana, Texas and Mexico study sites (significant difference – $p < 0.05$, between [a] 20-40 and 41-60 cm; [b] 20-40 and > 60 cm; and [c] 41-60 and > 60 cm).

Table 10. Trace metal concentrations (ppb) in carapace tissue (d.w.) of adult Kemp's ridleys from Louisiana and Mexico.

	Louisiana (n = 2)	Mexico (n = 18)
SCL (cm)	65.2 (64.2 – 66.3)	65.0 (61.4 – 75.2)
Ag	296 (13.7-578)	216 (69.5-634)
Cd	124 (121-126)	40.5 (14.7-124)
Cr	680 (114-1240)	641 (289-1770)
Cu	19300 (2020-36600)	738 (230-2340)
Hg	2230 (1270-3180)	2410 (518-5400)
Pb	1560 (804-2320)	1180 (193-2630)
Zn	195000 (154000-237000)	260000 (87210-817000)

Adult ridley females yielded significantly higher ($p < 0.01$) Pb and Zn levels in blood and Ag, Cr, Cu and Hg levels in carapace tissue than did their juvenile and sudadult cohorts (Fig. 17). These adult ridleys also exhibited higher Ag, Cd and Hg concentrations in blood and Pb concentration in carapace tissue than did their younger conspecifics. In addition, Cd levels in blood and carapace tissue of smaller juveniles were higher than those of their larger conspecifics. Mercury, Pb, and Zn concentrations in blood increased with increasing size of ridleys across all post-pelagic life stages (Fig. 18). A similar relationship also existed between Ag, Cr and Hg concentrations in carapace tissue (Fig. 19), while an inverse relationship occurred between Cd concentration in carapace tissue and size of ridley. No other trend was detected for Cu, Pb and Zn concentrations in carapace tissue.

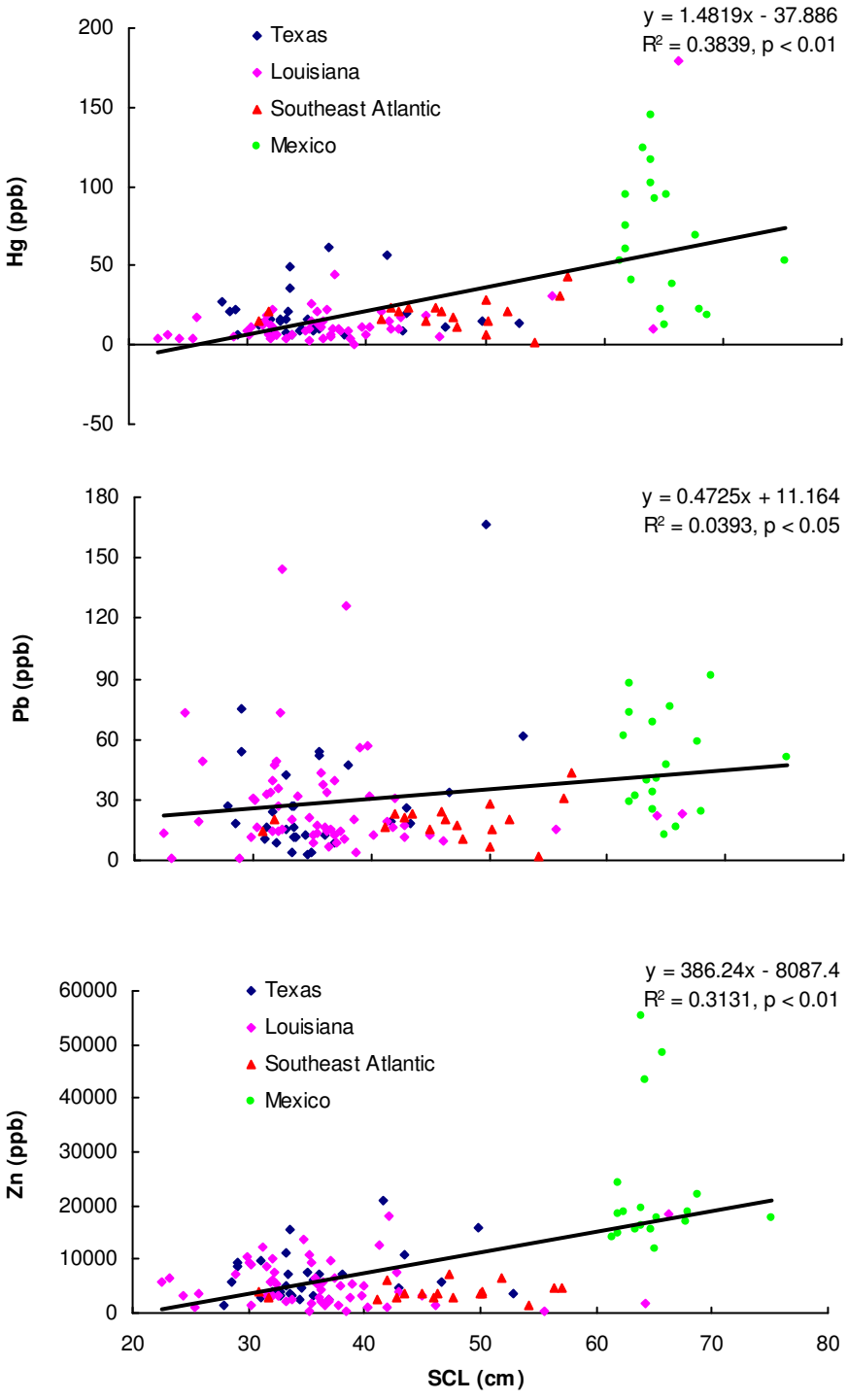


Fig. 18. Trace metal (Hg, Pb, Zn) concentrations (ppb) in blood (w.w.) as a function of SCL of wild Kemp's ridleys captured from the southeast Atlantic, Louisiana, Texas and Mexico.

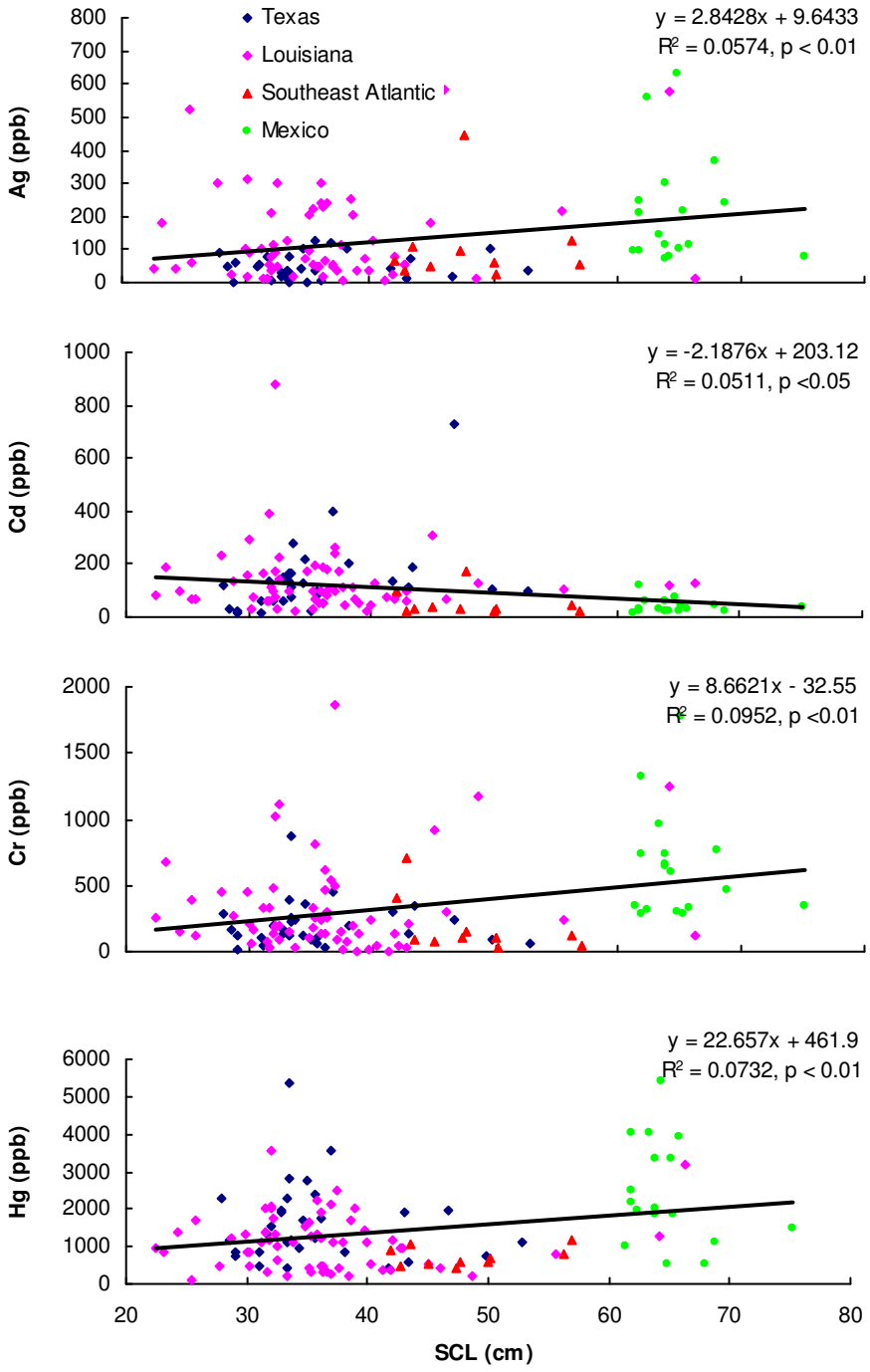


Fig. 19. Trace metal (Ag, Cr, Cd, Hg) concentrations (ppb) in carapace tissue (d.w.) as a function of SCL of wild Kemp's ridleys captured from the southeast Atlantic, Louisiana, Texas and Mexico.

In addition, correlation analyses were used to detect any metal-metal interactions for live Kemp's ridleys across post-pelagic life stages (Table 11). Silver concentrations decreased significantly with increasing Cd (Spearman's $r = -0.284$, $p < 0.01$) and Zn levels (Spearman's $r = -0.384$, $p < 0.01$) in blood. Conversely, Zn concentrations rose significantly with increasing Cd (Spearman's $r = 0.414$, $p < 0.01$), Hg (Spearman's $r = 0.417$, $p < 0.01$), and Pb levels (Spearman's $r = 0.295$, $p < 0.01$) in blood. A similar positive correlation was found between Ag and Cr (Spearman's $r = 0.449$, $p < 0.01$), Ag and Pb (Spearman's $r = 0.223$), and Cr and Cu levels (Spearman's $r = 0.212$) in carapace tissue. Cadmium concentrations rose significantly with increasing Cr (Spearman's $r = 0.247$, $p < 0.01$), Cu (Spearman's $r = 0.650$, $p < 0.01$), and Pb levels (Spearman's $r = 0.421$, $p < 0.01$) in carapace tissue. Lead concentrations were positively correlated with Cr (Spearman's $r = 0.419$, $p < 0.01$), Cu (Spearman's $r = 0.234$, $p < 0.01$), and Hg levels (Spearman's $r = 0.239$) in carapace tissue. A similar relationship existed between Hg and Zn (Spearman's 0.265, $p < 0.01$) and Pb and Zn levels (Spearman's $r = 0.207$) in carapace tissue.

Table 11. Pearson's correlation coefficients for trace metal relationships in blood and carapace tissue of Kemp's ridleys of all life stages.

Blood		Ag	Cd	Cr	Cu	Hg	Pb
	Cd	-0.284*					
	Cr	-0.124	0.055				
	Cu	-0.114	0.112	-0.111			
	Hg	-0.042	-0.019	0.013	0.147		
	Pb	-0.077	0.136	-0.127	0.160	0.081	
	Zn	-0.348**	0.414**	0.144	0.112	0.417**	0.295**
Carapace		Ag	Cd	Cr	Cu	Hg	Pb
	Cd	0.100					
	Cr	0.449**	0.247**				
	Cu	0.105	0.650**	0.212*			
	Hg	-0.175	0.015	0.169	-0.036		
	Pb	0.223*	0.421**	0.419**	0.342**	0.239*	
	Zn	-0.058	0.158	-0.041	0.177	0.265**	0.207**

* Correlation coefficient is significant at the 0.05 level
** Correlation coefficient is significant at the 0.01 level

Blue Crabs: Trace Metal Concentrations

A total of 10 blue crabs (5 male, 5 female) was collected from water adjacent to Rancho Nuevo, Mexico and used in assessing this prey's role as a metal uptake pathway in nesting females (Appendix Table 26). No difference ($p = 0.146$) was found in blue crab carapace width between males (CW = 138 mm) and females (CW = 125 mm). All trace metal concentrations in these blue crabs were normally distributed. Mean trace metal concentrations in these blue crabs were digestive gland - Ag = 196, Cd = 52.1, Cr = 4.7, Cu = 11,400, Hg = 8.9, Pb = 40.1, Zn = 41,400 ppb; muscle - Ag = 75.5, Cd = 5.1, Cr = 1.6, Cu = 3,300, Hg = 23.2, Pb = 17.8, Zn = 58,400 ppb (Fig. 20). Higher Ag (2.5 times, $p < 0.01$), Cd (10 times, $p < 0.01$), Cr (3 times, $p < 0.01$), and Cu concentrations (3 times, $p < 0.01$) were found in the digestive gland. Conversely, higher Hg levels were found in the muscle tissue ($p < 0.01$). In addition, Ag ($p < 0.01$) and Hg ($p < 0.05$) concentrations in the digestive gland rose with increasing carapace width (Appendix Figs. 13 and 14).

Mercury concentrations rose significantly with increased Ag (Pearson's $r = 0.917$, $p < 0.01$), Cd (Pearson's $r = 0.678$), and Cr levels (Pearson's $r = 0.759$) in the digestive gland (Appendix Table 27). A similar relationship was found between Cd and Cu (Pearson's $r = 0.920$, $p < 0.01$), and Cr and Pb levels (Pearson's $r = 0.759$) in the digestive gland. In addition, Ag concentration rose significantly with increasing Cr (Pearson's $r = 0.784$, $p < 0.01$) and Pb levels (Pearson's $r = 0.642$) in muscle tissue.

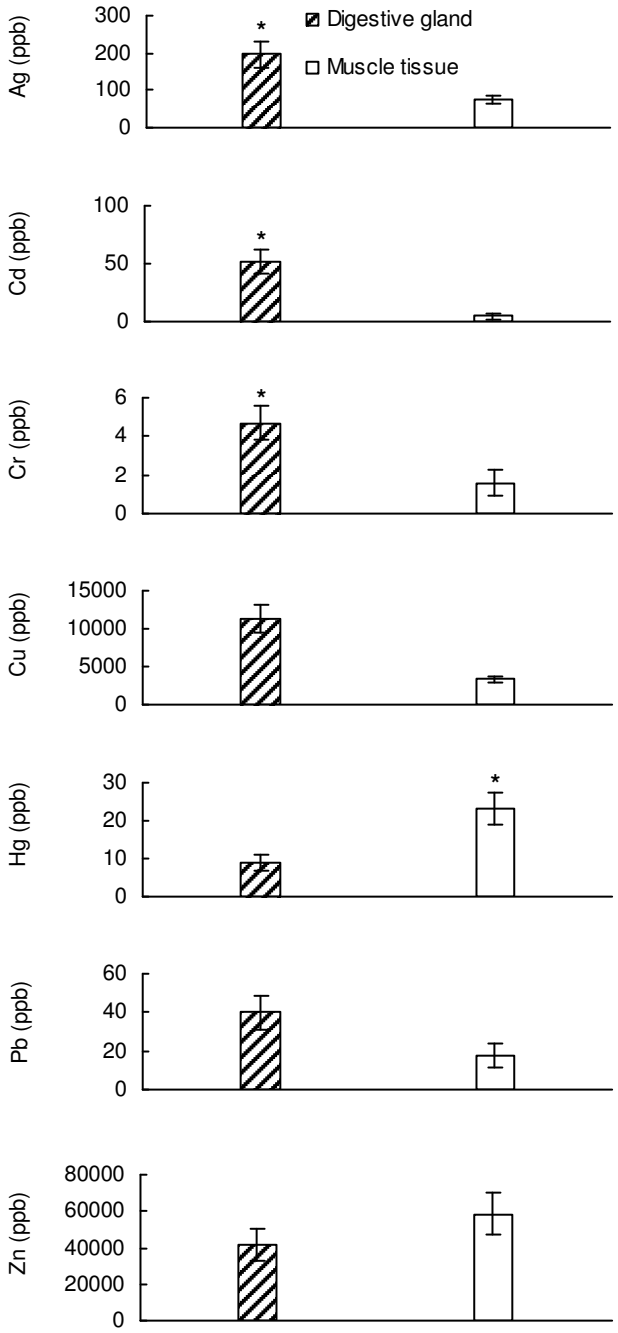


Fig. 20. Mean trace metal concentrations (ppb) in digestive gland (w.w.) and muscle tissue (w.w.) of blue crabs (n = 10) captured from near the Kemp's ridleys nesting beach at Rancho Nuevo, Mexico (* significant difference – $p < 0.05$, in mean metal concentrations between digestive gland and muscle tissue).

A comparison of metal concentrations in ridleys and blue crabs from Mexico is shown in Table 12. Carapace tissue yielded higher Cr, Hg, Pb and Zn concentrations than those in their blue crab prey, regardless of tissue type. Silver and Cd levels were higher in carapace tissue than in muscle tissue of blue crabs. In addition, blue crabs exhibited higher Cu concentrations than those in carapace tissue. This finding was similar to that described in Chapter IV.

Discussion

The present study is the first to characterize trace metal concentrations in adult ridley females and blue crabs from the Rancho Nuevo, Mexico nesting beach. These adult females, like their juvenile/subadult cohorts in Chapter IV, exhibited higher metal concentrations in carapace tissue than in their blood. This finding supports the aforementioned hypothesis that trace metal concentrations in carapace tissue of wild ridleys are higher than those in heir blood.

Trace metal concentrations in blue crabs were examined in the present study to assess metal uptake pathways in adult ridley females and as a means of completing the evaluation of any size-dependent metal concentrations across all post-pelagic life stages.

Table 12. Trace metal concentrations (ppb) in Kemp's ridleys and blue crabs from Mexico.

	Ag	Cd	Cr	Cu	Hg	Pb	Zn
Kemp's ridley (n = 17)							
Carapace (d.w.)	216[b]	40.5[b]	641[ab]	738	2420[ab]	1180[ab]	260000[ab]
Blue crab (w.w., n = 10)							
Digestive gland	196	52.1	4.7	11400[a]	8.9	40.1	4140
Muscle tissue	75.5	5.1	1.6	3300[b]	23.2	17.8	58400

[a] significant difference between digestive gland of blue crabs and carapace tissue of ridleys;
[b] significant difference between muscle tissue of blue crabs and carapace tissue of ridleys

This comparison was partially limited by the fact that no blue crab data were available for ridleys captured from the southeast Atlantic for comparison with those from Texas, Louisiana and Mexico in determining metal uptake pathways. Nonetheless, higher Ag, Cd, Cr, Hg, Pb, and Zn concentrations in carapace tissue of nesting females supported the hypothesis that trace metal levels tend to accumulate at levels higher than those in their blue crab prey. Conversely, blue crab prey, regardless of tissue type, exhibited higher Cu levels than those in carapace tissue of Kemp's ridleys.

Additionally, data generated on live Kemp's ridleys in this chapter were combined with those reported in Chapter IV to provide a complete size range of ridleys across all post-pelagic life stages with which to compare and evaluate potential trace metal accumulation. Interestingly, variations in size-concentration relationships between blood (Hg, Pb, Zn) and carapace tissue (Ag, Cd, Cr, Hg) resulted in not all trace metals of the present study accumulating with increasing size of Kemp's ridleys (Figs. 18 and19). This finding supports the third

hypothesis that trace metal concentrations in ridleys across all post-pelagic life stages are age/size-dependent, but only for specific metal assessments. The only trace metal that appeared to accumulate in both blood and carapace tissue with increasing size of ridleys was Hg. This result is similar to that of other studies on metal concentration versus size relationships in Kemp's ridleys (Presti *et al*., 2000) and loggerheads (Days, 2003).

Silver

Carapace tissue of adult ridley females exhibited significantly higher Ag concentrations than did their blood and, thus, provided support for accepting the hypothesis. Significantly higher Ag levels in carapace tissue were found in adult females (224 ppb) rather than in their juvenile (110 ppb) and subadult conspecifics (103 ppb; Fig. 17). This size-dependent relationship (Fig. 19) appears to strengthen the assumption that carapace tissue accumulates Ag over time. There are several probable factors facilitating the uptake of Ag in these adult females. It can be assumed that, as adults, these females cumulatively fed on more blue crab prey across their life than did their smaller constituents and, in doing so, exhibited increased potential to accumulate higher Ag concentrations. Another factor that may play a role in these positive relationships is the strong possibility these adults, like the younger conspecifics captured in this study, used the Texas and Louisiana coast as a foraging ground. As such, these adults have probably accumulated trace metals like Ag since their juvenile life history stage.

The fact that carapace-borne Ag concentrations rose significantly with increasing blood-borne levels (Appendix Fig. 15) suggests carapace tissue could be considered a useful surrogate tissue (to internal organs and tissues) for monitoring Ag exposure in Kemp's ridleys. However, verification of carapace tissue serving in this surrogate role must include a comparison of this tissue's Ag levels with those in internal organs (i.e. liver, kidney, muscle and fat). Such a comparison will be presented in Chapter VI.

Silver concentrations in blue crabs from Mexico, regardless of tissue type, were significantly lower than those in their Texas and Louisiana constituents. This fact could be explained by the latter's blue crab foraging in highly industrialized areas that were not available to Mexican counterparts.

Carapace tissue of adult ridley females did exhibit significantly higher Ag levels than did blue crab muscle tissue (Table 12) as well as higher, yet non-significant, concentrations when compared to that in the digestive gland. Therefore, the hypothesis that Ag levels are higher in ridleys than in their blue crab prey is accepted, at least on a conservative basis, assuming relationships between concentrations in ridley carapace tissue and those in blue crab tissues continue to exhibit the aforementioned trends. Although it is easy to reach this conclusion, the small sample size upon which this assessment is based mandates further study with which to remove any doubts as to the accumulation potential of carapace versus digestive gland. Additional research characterizing trace metal concentrations in internal organs and muscle tissue in live Kemp's

ridleys also is needed to elucidate the body burden in different tissues.

Relationships may exist between metals that can impact the absorption, distribution, and excretion of one or more metals (ATSDR, 1990). Knowledge of these interactions will important in determining toxic effects of trace metals on Kemp's ridleys. The body burden of a trace metal may be the result of exposure from more than one source over an extended time frame. Furthermore, the correlation between metals could have additive or antagonistic effects that need to be addressed.

Overall, a variety of metal-metal correlations were found in Kemp's ridleys across all post-pelagic life stages (Table 11) and between study areas. A positive correlation was found between Ag and Pb in carapace tissue of Kemp's ridleys of all post-pelagic life stages. The cause of these two metal interactions is uncertain but the correlation is more likely related to similar aquatic biogeochemistry. In addition, positive correlation was detected between Ag and Cr in carapace tissue of ridleys of all post-pelagic life stages. This is the first report on interaction between Ag and Cr in sea turtles. These complex interactions still need further investigation to understand their impact on metal dynamics within a Kemp's ridley's body.

In summary, the present study supports the hypotheses as they relate to Ag, with these being: concentrations in carapace tissues are higher than those in the blood and these levels are size-dependent across all post-pelagic life stages (carapace tissue only). Silver appears to accumulate in carapace tissue.

However, failure to detect a significant difference in Ag concentrations between ridleys and the digestive gland of their blue crab prey casts some doubt on the accumulation potential for Ag and/or the role food plays in the uptake of this metal.

Cadmium

Like that for Ag, carapace tissue of adult ridley females exhibited significantly higher Cd levels than did their blood and, in doing so, this trend supports the hypothesis. Significantly higher Cd levels in carapace tissue were found in juvenile (128 ppb) and subadult ridleys (116 ppb) than in their adult conspecifics (49.2 ppb; Fig. 17). An inverse relationship existing between Cd levels in carapace tissue and size of ridleys (Fig. 19) rejects the third hypothesis that Cd concentrations in ridleys across all post-pelagic life stages are age/size-dependent. These findings suggest that Cd concentrations in carapace tissue of ridleys appear not to be influenced by size of ridleys. Cadmium concentrations decreased with increasing size, a finding similar to that of other investigations on stranded sea turtles (Gordon *et al.*, 1998; Sakai *et al.*, 2000b). Sakai *et al.*'s (2000a) report of limited excretion of Cd in greens and loggerheads (via egg laying process) remains inconclusive for the Kemp's ridley because of the paucity of pertinent data. Therefore, further investigations to characterize trace metal concentrations in ridley eggs and hatchlings are needed to determine possible excretion of Cd by the carapace tissue during the

reproductive cycle.

Blue crabs, regardless of tissue type, collected from the Rancho Nuevo, Mexico nesting beach exhibited lower Cd levels than did counterparts from Texas and Louisiana. The Rancho Nuevo nesting beach is far removed from any industrialized coastal areas (Márquez-M, 1994) and probably has better water quality that would likely result in lower Cd levels found in its blue crabs.

Carapace tissue of adult ridley females yielded significantly higher Cd levels than did their blue crab muscle tissue (Table 12) but statistically lower concentrations than did the digestive gland of their prey. Thus, only part of the hypothesis can be accepted. This disparity leaves substantial doubt as to the role that carapace tissue may play as an accumulation site for this metal in Kemp's ridleys. In addition, uncertainty as to the accumulation potential of specific tissues in blue crabs and the sample size used in this assessment could play a large role in the validity of this concentration disparity.

Interactions of Cd with other metals (e.g. Cr, Cu, Pb, and Zn) could affect the absorption potential of an organism's body (ATSDR, 1999a). Cadmium is frequently obtained as a by-product of Cu, Pb and Zn production. It is most likely the first explanation to the positive correlations detected between Cd and Zn, and Cd and Cu in carapace tissue of Kemp's ridleys across all post-pelagic life stages. Sakai *et al*. (2000b) suggested that the correlation between Cd and Cu in green turtles may be related to metallothionein synthesis. This same correlation, although its origin was not tested, may hold true for Kemp's ridleys as well.

Barrera *et al.* (1995) reported that Cd uptake in aquatic organisms appears to be regulated by the Cd to Zn ratio in the environment. Zinc negates the toxic effects of Cd in many aquatic organisms (Eisler, 1993). For example, Zn can protect embryos of amphibians against Cd-induced developmental deformation or countertract adverse effects of Cd on limb regeneration and growth of crabs. The presence of Cr and Zn also could decrease Cd uptake. As a result, positive relationships existing between Cd and Cr, and Cd and Zn in carapace tissue may be an indication of Kemp's ridley's health when exposed to Cd. Higher Cd and Zn levels reported in seabirds may be due to the induction of metallothionein synthesis (Kim *et al*., 1996). Moreover, positive correlation between Cd and Pb was detected in carapace tissue across ridleys of all life stages that has not been reported for other sea turtles. Further investigation of the role metallothionein synthesis plays in metal relationships is needed for live Kemp's ridleys to determine possible additive or beneficial effects from Cd.

Overall, the hypothesis that Cd concentrations in carapace tissues are higher than those in the blood is accepted. In addition, carapace tissue of adult ridleys females did yield higher Cd levels than found in blue crab muscle tissue and, in doing so, partially justifies accepting the hypothesis that Cd levels are higher in ridleys than in their blue crab prey. The fact that Cd levels in carapace tissue decreased with increasing size of ridleys diminishes support for the hypothesis that Cd concentrations in ridleys across all post-pelagic life stages are age/size-dependent.

Chromium

Carapace tissue of adult ridley females exhibited significantly higher concentrations than did their blood and, thus, the hypothesis is accepted. This finding also suggests the potential for Cr accumulation in carapace tissue. Significantly higher Cr levels in carapace tissue were found in adults (644 ppb) than in their juvenile (273 ppb) and subadult conspecifics (236 ppb; Fig. 17). Although no significant metal level versus size relationship was found in carapace tissue for Cr, there is evidence that higher Cr levels existed in carapace tissue of larger ridleys.

Blue crabs, regardless of tissue type, collected from the Rancho Nuevo exhibited significantly lower Cr levels than those from Texas. Like that for Ag and Cd, highly industrialized areas along the Texas coast could account for this concentration disparity in blue crabs.

Carapace tissue of adult ridley females did yield significantly higher Cr concentrations than either tissue type in their blue crab prey (Table 12). This finding supports acceptance of the hypothesis that Cr levels are higher in wild ridleys than in their blue crab prey. It also implies that carapace tissue serves as a potential accumulation site for this metal.

Generally, Cr toxicity increases with the presence of Cd and Zn in marine organisms (Eisler, 1986). The current study found a positive correlation between Cr and Cd in carapace tissue of ridleys across all post-pelagic life stages (Table 11). Chemical similarity between Cr and Cu (ATSDR, 2000) may account for the

positive correlation in carapace tissue of ridleys. A similar relationship also was found in marine mammals (Watanabe *et al*., 1996; Kunito *et al*., 2002). The positive correlation existing between Cr and Pb in carapace tissue of ridleys has not been reported for other sea turtles.

In summary, hypotheses outlined in the introduction of this chapter that Cr concentrations in carapace tissues of ridleys are higher than those in their blood; these levels are higher than those in their blue crab prey; and Cr concentrations in ridleys are age/size-dependent (carapace tissue only) are all accepted.

Copper

Carapace tissue of adult ridley females exhibited significantly higher Cu concentrations than did their blood and, in doing so, the hypothesis is accepted. Although significantly higher Cu levels were found in carapace tissue of adults (2,690 ppb) than in their subadult conspecifics (2,020 ppb), those of juveniles (2,780 ppb) were even greater (Fig. 17). The fact that most subadults analyzed in this study were captured from the southeast Atlantic where contaminant loading may not have been as great as that along northwestern Gulf shores could explain at least part of this finding. Various questions as to Cu requirements and regulation during the reproduction process also must be answered before a complete understanding of this disparity is reached.

Higher Cu concentrations have been reported for small green sea turtles (Sakai *et al*., 2000b), a trend mirrored by the present study. Size-dependent

relationships between Cu concentration and size of ridleys were not observed in the present study. Failure to detect size-concentration relationships casts some doubt on the accumulation potential for Cu in ridleys. Nonetheless, there is evidence for potential Cu accumulation in carapace tissue, given the results of their comparison with blood-borne levels and the fact that higher levels were found in adult females.

The hierarchy for Cu concentration in adult ridley females and its blue crab prey was digestive gland > muscle > carapace tissue (Table 12) and, given this result, the hypothesis is rejected. This finding is similar to that for conspecifics from Texas and Louisiana. Questions arise as to the role that carapace tissue may play as a potential accumulation site for this metal. Further investigation involving metabolism in a ridley's body is needed to determine potential accumulation for this essential metal.

Eisler (1998) reported that Cu interacts with other metals, such as Ag, Cd, Hg, Pb, and Zn, in ways that may be either beneficial or harmful to organisms. Excessive levels of Zn decrease Cu absorption. Additionally, toxicity, metabolism and accumulation of Cu may differ in the presence of other metals. In addition to the aforementioned interactions between Cd and Cu, and Cr and Cu, a positive relationship of Cu and Pb was detected in carapace tissue of ridleys across post-pelagic life stages (Table 11). A further study on metallothioneins is needed to determine their impact on Cu dynamics in Kemp's ridleys.

In summary, only the hypothesis that Cu concentrations in carapace tissues

are higher than those in the blood is accepted. Neither size-dependent relationships nor differences in Cu concentration between adult ridley females and their blue crab prey were detected in the present study. These findings cast some doubt on the role food plays in the uptake of this metal and whether carapace tissue truly represents a potential accumulation site for Cu in ridleys. As such, biopsy sampling of the internal organs or muscle tissue in live ridleys is needed to determine whether Cu accumulates in different tissues.

Mercury

Carapace tissue of adult ridley females exhibited significantly higher Hg concentrations than did their blood and, thus, the hypothesis is supported. Significantly higher Hg concentrations in carapace tissue were found in adults (2,400 ppb) than in their juvenile (1,360 ppb) and subadult conspecifics (801 ppb; Fig.17). This size-dependent relationship (Fig. 19) appears to strengthen the assumption that carapace tissue accumulates Hg over time. As a result, the third hypothesis that Hg concentrations in ridleys across all post-pelagic life stages are age/size-dependent is also accepted. A similar relationship was detected in other investigations on Kemp's ridleys (Presti *et al*., 2000; Kenyon *et al*., 2001), loggerheads (Day, 2003) and other marine vertebrates (Becker *et al*., 1995; Endo *et al*., 2002).

Like that for Ag, carapace-borne Hg concentrations rose significantly with increasing blood-borne levels (Appendix Fig. 15). This finding may imply that

carapace tissue could be considered a useful surrogate tissue (to internal organs and tissues) for monitoring Hg exposure in Kemp's ridleys. Carapace tissue of adult ridley females did exhibit significantly higher Hg concentrations than either blue crab tissue type, thus providing justification for accepting the hypothesis.

Limited correlations were detected in Hg concentrations with other trace metals in the present study (Table 11). Mercury concentrations appear to be positively correlated with Pb and Zn levels in ridleys across all post-pelagic life stages. Since elevated toxicity of Hg has been reported to relate with the presence of Pb and Zn (Eisler, 1987; Barrera *et al*., 1995; ATSDR, 1999c), a further investigation on toxic effects of these correlations is needed.

In summary, hypotheses outlined in the introduction of this chapter that Hg concentrations in carapace tissues are higher than those in the blood; these levels are higher in ridleys than in their blue crab prey; and Hg concentrations are age/size-dependent (both blood and carapace tissue), are all accepted.

Lead

Carapace tissue of adult ridley females exhibited significantly higher ($p < 0.01$) Pb concentrations than did their blood and, thus, the hypothesis is accepted. Significantly higher Pb levels in carapace tissue were found in adults (1,220 ppb) than in their juvenile (1,210 ppb) and subadult conspecifics (832 ppb; Fig. 17). No size-dependent relationship was detected in carapace tissue of ridleys across all post-pelagic life stages. However, blood-borne Pb

concentrations did increase with increasing size of ridleys (Fig. 18). Thus, the third hypothesis is accepted. Nonetheless, carapace tissue did exhibit some degree of accumulation, given the results of their comparison with blood-borne levels

Lead concentrations in Mexican blue crabs, regardless of tissue type, were lower than those of conspecifics from Texas and Louisiana. The Rancho Nuevo nesting beach is far removed from industrialized areas and probably had better water quality that resulted in lower Pb levels in its blue crabs.

Carapace tissue of adult ridley females did yield significantly higher ($p < 0.01$) Pb concentrations than either blue crab tissue type (Table 12), thus providing support for accepting the hypothesis that Pb levels are higher in ridleys than in their blue crab prey. It also implies that carapace tissue serves as a potential accumulation site for this metal.

The toxicokinetic and toxicological effects of Pb could be affected by interactions with other metals (ATSDR, 1999b). Lead accumulates at 10 times greater concentrations in fish in the presence of Zn (Eisler, 1993). Lead-Zn mixtures appear to be more additive effects in the toxicity in marine organisms (Eisler, 1993). A further investigation on physiological and toxicological studies is needed to determine their toxic effects in Kemp's ridleys.

Overall, the hypotheses outlined in this chapter that Pb concentrations in carapace tissues are higher than those in the blood; these levels are higher in ridleys than in their blue crab prey; and Pb levels are age/size-dependent (blood

only), are all accepted. It also indicates some potential for Pb accumulation in carapace tissue of ridleys.

Zinc

Carapace tissue of adult ridley females exhibited significantly higher Zn concentrations than did their blood and, in doing so, the hypothesis is accepted. This finding is similar to all trace metal concentrations discussed in the present study. Neither a size-dependent relationship nor a concentration difference was detected in carapace tissue of ridleys across all post-pelagic life stages (Fig. 17). However, blood-borne Zn concentrations did increase with increasing size of ridleys (Fig. 18). Thus, the hypothesis that Zn concentrations in ridleys across all post-pelagic life stages are age/size-dependent is not fully accepted. A similar age-related relationship in Zn concentrations was found in the blood of Kemp's ridleys investigated by Kenyon *et al*. (2001) and liver of loggerhead sea turtles (Gordon *et al*., 1998).

Carapace tissue of adult ridley females did exhibit significantly higher ($p < 0.01$) Zn levels then either blue crab tissue type (Table 12) and, consequently, the hypothesis that Zn levels are higher in ridleys than in their blue crab prey is accepted. This finding provides evidence for potential Cu accumulation in carapace tissue, as do results of its comparison with blood-borne levels.

The correlations of Zn and other trace metals could have influenced in the toxicity, but little information is available on the consequences of these

interactions (Barrera *et al*., 1995). Knowledge of these interactions may provide information to understand Zn accumulation, metabolism and toxicity (Eisler, 1993). Zinc transport may be diminished in the presence of other trace metals (e.g. Cd, Cu, Hg; ATSDR, 2003).

Like that for Pb, the aforementioned hypotheses that Zn concentrations in carapace tissues are higher than those in their blood; these levels are higher in ridleys than in their blue crab prey; and Zn concentrations are age/size-dependent (blood only), are all accepted.

Summary

Carapace tissue exhibited higher trace metal concentrations than did the blood and, thus, may suggest: 1) blood-borne concentrations are probably indicative of recent exposure to respective trace metals; and 2) the potential for accumulation of various metals in carapace tissue of Kemp's ridleys. Size-dependent relationships were detected for certain metals in blood (Hg, Pb, Zn) and carapace tissue (Ag, Cr and Hg) of ridleys across all post-pelagic life stages. These relationships, especially in the case of carapace tissue, would seem to support the potential for the accumulation of particular trace metals in ridleys. This finding further implies that continued accumulation of certain metals may reach concentrations that would prove harmful to ridley health. Conversely, Cd was the only metal whose concentration decreased with increasing size of ridleys. The fact that blood-borne levels of Ag and Hg increased with increasing

levels in carapace tissue suggests that the latter tissue source could be considered a candidate surrogate (to internal organs and tissues) for monitoring Ag and Hg exposure in Kemp's ridleys. The utility of carapace tissue in this regard must be evaluated in live ridleys via a thorough comparison of its metal concentrations with those of internal organs and tissues. Such an evaluation will require a special permit to do so.

Higher metal concentrations were detected in adult ridleys than in their juvenile and subadult cohorts, except for Cd and Cu. The bulk of this finding is likely explained by the fact that adult ridleys cumulative consume more prey over time than do smaller conspecifics, thus the greater potential for uptake and possible accumulation of larger metal quantities**.** Additional characterizations of distribution and uptake of trace metals by internal organs and muscle tissue in live ridleys are needed to determine accurate accumulation sites as well as assess the potential of carapace tissue as a non-invasive surrogate sample source. Moreover, analysis of trace metal concentrations in eggs and dead hatchlings from females nesting at Rancho Nuevo is needed to determine possible contaminant transfer through the reproductive cycle.

Blue crabs collected from Mexico exhibited lower metal levels (Ag, Cr, Cd, Cu and Pb) than did Texas and Louisiana constituents. This concentration difference is expected given the less industrialized coastal waters along the Rancho Nuevo, Mexico nesting beach. Significantly higher Ag, Cd, Cr, Hg, Pb and Zn concentrations found in carapace tissue of adult ridley females than in

their blue crab prey were probably an artifact of the potential for prolonged accumulation in these females as well as the fact that they likely used the highly industrialized Texas and Louisiana coasts as a prime foraging ground. This concentration disparity between ridleys and blue crab prey was mirrored by their Texas and Louisiana conspecifics.

Various metal-metal interactions were detected in carapace tissue of ridleys across all post-pelagic life stages. A majority of these correlations are the first to be reported in live sea turtles. Detailed study of these correlations will help to determine more accurately the potential for toxic effects from trace metals. Research on metallothioneins in live ridleys is needed to provide information for reducing the potential for metal toxicity. In addition, more research on the health impacts (i.e. immune function) of trace metal concentrations is needed, specifically as it pertains to effects on Kemp's ridley's recovery rate.

CHAPTER VI

TRACE METAL CONCENTRATIONS IN KEMP'S RIDLEY SEA TURTLES STRANDED ALONG THE TEXAS COAST

Permit restrictions on invasive sampling of live protected species such as the Kemp's ridley limited characterizations of trace metal concentrations in Chapters III through V to blood and carapace tissue of live conspecifics. In so doing, no information was generated in those chapters for trace metal loads in internal organs/tissues that are commonly used in contaminant investigations and are known bioaccumulation sites. Lack of this information negates any meaningful assessment of the significance of trace metal measurements for the blood and, especially, carapace as indices of contaminant loading in the Kemp's ridley. In an effort to fill this information void and render the aforementioned assessment possible, this chapter summarized trace metal concentrations in liver, kidney, muscle, and carapace tissue of dead stranded Kemp's ridleys that washed onto Texas beaches during 2001-2002.

The aforementioned research approach is based on the fact that the vast majority of information on contaminant loads in protected sea turtles, including Kemp's ridleys, has come from studies on dead, stranded specimens. Liver, kidney, pancreas and muscle tissues have been analyzed for trace metal concentration in stranded carcasses of different sea turtle species (Edmonds *et al.*, 1994; Aguirre *et al.*, 1995; Gordon *et al.*, 1998; Sakai *et al.*, 2000a and

2000b), with the Kemp's ridley a very minor contributor to these investigations. Over 6,000 sea turtles have stranded on Texas shores since 1980, with loggerheads and Kemp's ridleys comprising 50 and 30%, respectively, of this total (Shaver, 1998; TEWG 2000). The western Gulf of Mexico, especially the Texas and Louisiana coast (zones 17 to 21), where high shrimping effort occurs, accounts for the highest proportion of stranded Kemp's ridleys (Shaver, 1998; TEWG, 2000). The 430 stranded ridleys found on Gulf beaches in 1994 was the highest on record since 1986. From 1986 to 1997, zones 17 to 21 accounted for 44.8 % of all Kemp's ridleys stranded over the Gulf of Mexico and southeast Atlantic (TEWG, 2000).

Thousands of sea turtles have been necropsied to determine cause of death, with little information generated on possible mortality scenarios as a result of these examinations. While strandings have great utility as an index of population status and mortality, their value in determining cause of death is often compromised. By the fact that 3 to 5 days are required for dead sea turtles to bloat, float, and wash ashore to strand. This post-mortem interval typically results in substantial tissue decomposition and a considerable loss of forensic information on cause of death. Although this decomposition results in a loss of aromatic hydrocarbons and volatile organics, trace metal loads can be characterized in most stranded carcasses as long as target tissues are not in an extreme state of decay (Sis and Landry, 1992; Keller, 2003; O'Hara *et al*., 2003).

Research described herein characterizes trace metal concentrations in

carapace tissue, internal organs and muscle tissue of Kemp's ridleys stranded along the Texas coast. Information generated by this research allowed the following hypotheses for respective metals to be tested: 1) trace metal concentrations in the internal organs of stranded Kemp's ridleys are higher than in their carapace tissue; and 2) trace metal concentrations in internal organs and/or muscle tissue of stranded ridleys are correlated with those in their carapace tissue, such that latter could be used as surrogate sample source from which to characterize trace metal loading in live Kemp's ridleys.

Materials and Methods

Stranding Locations

Kemp's ridleys stranding along the Texas coast during May 2001 through April 2002 were collected by National Marine Fisheries Service's Sea Turtle Stranding and Salvage Network from the following locations: Mustang Island, North Padre Island, and South Padre Island. Where appropriate, these ridleys were used as additional sources of blood, carapace and internal organs (i.e. liver, kidney) and muscle tissue in assessing trace metal levels. Stranding locations as well as size and condition code of ridleys are given in Appendix Table 27. Physical condition of stranded turtles was classified by the following codes: 0 - live turtles; 1 - fresh dead carcasses; and 2 - moderately decomposed carcasses.

Sample Collection and Digestion

Blood was drawn from code 0 ridleys and carapace tissue was collected from codes 0, code 1 and code 2 counterparts. Internal organ samples, including those from the liver, kidney and muscle tissue, were collected, when possible, from code 1 and code 2 carcasses. In order to compare data for soft (liver, kidney, muscle) versus hard (carapace) tissues, conversion factors reported for stranded green turtles (Anan *et al*., 2002a) were used to convert wet-weight basis to a dry-weight basis. These factors were 3.33 for liver and 5.0 for kidney and muscle tissue.

Code 1 and 2 carcasses had a wedge/plug of carapace tissue removed from one of their postcentral or marginal scutes via the same cleaning and sampling protocol outlined for live ridleys in Chapter III. About 10 g wet weight of liver, kidney and muscle tissue were removed from code 1 and code 2 carcasses with plastic knives to avoid contamination during the necropsy procedure. Samples were stored in double zip-lock bags and frozen until analysis. Approximately 1 g wet weight (w.w.) of liver, kidney and muscle tissue and 0.1 g dry weight (d.w.) of carapace tissue samples were digested, respectively, in Teflon vials following the same digestion method used for blood and carapace samples described in Chapter III.

Statistical Analysis

Parametric and non-parametric tests were used in statistical comparisons of metal concentrations. Range, mean and standard deviation were reported for each metal. Regression analysis was utilized to characterize potential relationships between metal concentration and carapace length. The Pearson's and Spearman rank correlation coefficients were used to compare metal concentrations between different tissue sample types from stranded ridleys. A Student *t*-test and Independent *t*-test were used to detect differences in metal concentrations in carapace tissue from live and dead ridleys. Results were determined significant when $p < 0.05$, unless otherwise stated.

Results

Kemp's Ridleys: Trace Metal Concentrations

Trace metal concentrations (all normally distributed) were determined for 10 dead, stranded carcasses (code 1, n = 3; code 2, n = 7) and 1 live Kemp's ridley collected along the Texas coast during 2001 - 2002 (Appendix Tables 28 - 34). Four of these stranded ridleys were collected from Mustang Island, 3 from South Padre Island, and 4 from Padre Island National Seashore. Mean SCL of these stranded ridleys was 49 cm while they ranged from 33.9 to 64.0 cm.

Generally, carapace tissue (Fig. 21) of dead, stranded ridleys exhibited significantly higher Ag, Cr, Hg and Zn levels than those in liver (Cr, Zn), kidney

(Ag, Cr, Zn), and muscle tissue (Ag, Hg, Zn; Fig. 21). Although non-significant, Pb levels in carapace tissues greatly exceeded those in other tissues.

Conversely, significantly higher Cd and Cu levels were found in kidney and liver, respectively, of stranded ridleys. Blood-borne trace metal levels in the only live ridley sampled were: Ag = 19.2, Cd = 38.2, Cr = 6.6, Cu = 4,560, Hg = 40.0, Pb = 47.0 and Zn = 51,700 ppb. These levels were higher than mean Cu concentrations in carapace tissue and muscle of dead, stranded conspecifics.

Liver and kidney were positively correlated with Ag (Pearson's $r = 0.874$, $p < 0.01$), Cr (Pearson's $r = 0.855$, $p < 0.01$), and Cu (Pearson's $r = 0.797$) concentrations (Appendix Table 36). A similar relationship was found for Hg concentrations in liver and carapace tissue (Pearson's $r = 0.896$, $p < 0.01$).

Mercury concentrations in liver increased significantly ($p < 0.01$) with increasing size of stranded ridleys in the present study (Appendix Fig. 18). No other statistically significant relationship was found between metal concentration and size of ridleys (Appendix Figs. 16 - 19). However, a slightly increasing trend was observed between Ag, Cd, Cu, and Pb concentrations in liver and kidney; and Pb and Hg levels in carapace tissues and size of stranded ridleys. In addition, a slightly inverse size-concentration relationship was observed in Cr levels of all tissues and Cd level in carapace tissue of stranded ridleys.

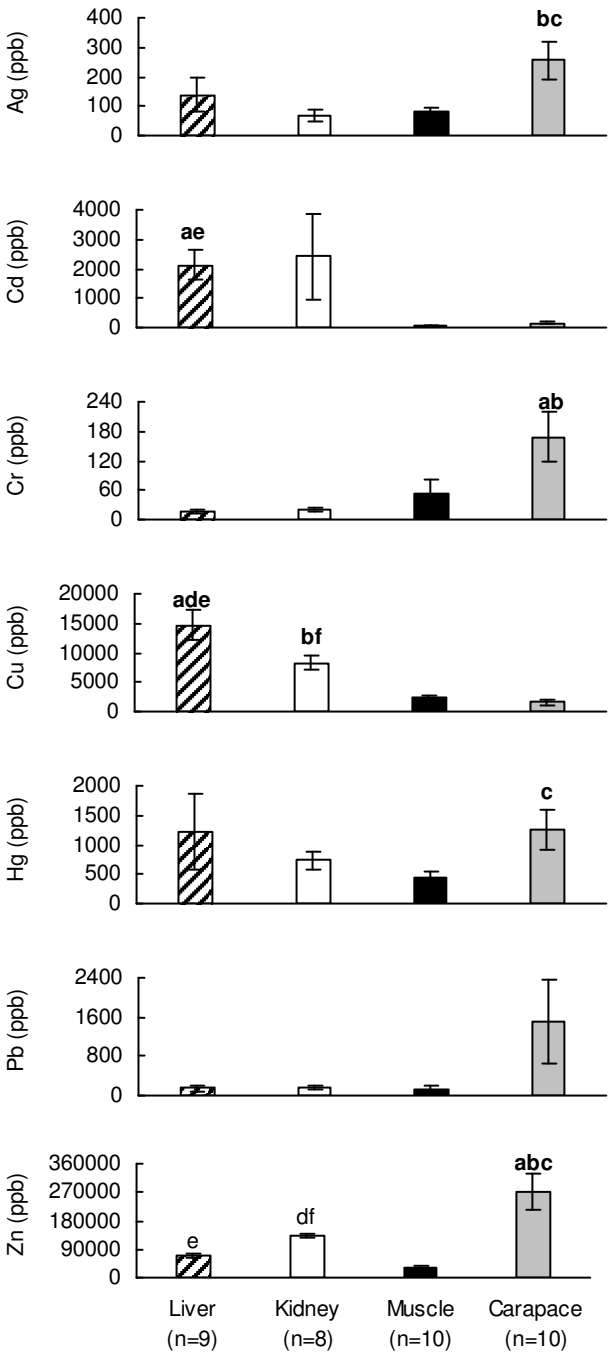


Fig. 21. Mean trace metal concentrations (ppb) in liver (d.w.), kidney (d.w.), muscle (d.w.) and carapace tissue (d.w.) of dead Kemp's ridleys stranded along the Texas coast during 2001-2002 (significant difference – $p < 0.05$, between [a] carapace and liver; [b] carapace and kidney; [c] carapace and muscle; [d] liver and kidney; [e] liver and muscle; and [f] kidney and muscle).

Silver concentrations were positively correlated with Pb levels in liver (Pearson's $r = 0.774$, $p < 0.05$) and muscle tissue (Pearson's $r = 0.794$, $p < 0.01$; Appendix Table 37). A similar correlation existed between Ag and Cd (Pearson's $r = 0.844$, $p < 0.01$) in the liver; Ag and Cu (Pearson' $r = 0.722$), Ag and Zn (Pearson's $r = 0.933$, $p < 0.01$) in muscle tissue; and Ag and Hg (Pearson's $r = 0.643$) in carapace tissue. Cadmium levels in the liver rose significantly with increasing Cu (Pearson's $r = 0.723$) and Pb (Pearson's $r = 0.885$, $p < 0.01$). A similar relationship was found between Cd and Pb in kidney (Pearson's $r = 0.725$), Cd and Cu in carapace tissue (Pearson's $r = 0.978$, $p < 0.01$), and Cd and Zn in carapace tissue (Pearson's $r = 0.660$). Copper concentrations were positively correlated with Pb (Pearson's $r = 0.825$, $p < 0.01$), and Zn (Pearson's $r = 0.830$, $p < 0.01$) in the kidney; and Zn (Pearson's $r = 0.705$) in carapace tissue. A similar relationship also was found between Hg and Pb (Pearson's $r = 0.874$, $p < 0.01$) in the carapace tissue and Pb and Zn (Pearson's $r = 0.751$) in muscle tissue.

Stranded vs. Live Kemp's Ridleys: Trace Metal Concentrations

Trace metal levels in carapace tissue from stranded Kemp's ridleys collected from the Texas coast were compared to those of a similar size range of live ridleys captured from Texas and Louisiana (Figs. 22 and 23). Straight carapace lengths of stranded ridleys (49.0 ± 11.0 cm SCL, range = 33.9 - 64.0 cm) used in this comparison were slightly larger than those of live ridleys (39.5 ± 5.0 cm SCL, range = 34.4 - 55.6 cm). Except for Cr and Cu, higher metal concentrations were found in stranded ridleys rather than in their live counterparts. Silver concentrations in carapace tissue of stranded ridleys were significantly higher than those for live cohorts ($p < 0.01$). However, live ridleys exhibited higher Cu levels ($p < 0.01$). No other statistically significant concentration difference was found between live and stranded ridleys.

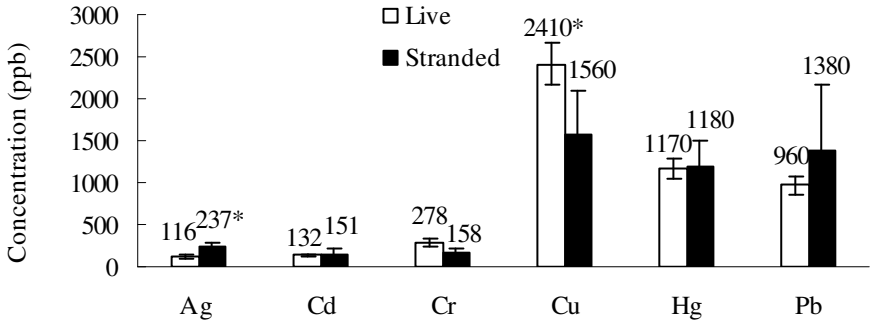


Fig. 22. Trace metal concentrations (ppb) in carapace tissue (d.w.) of live (n = 44) Kemp's ridleys from Texas and Louisiana and stranded (n = 11) Kemp's ridleys from Texas (*significant difference in carapace tissue between live and stranded ridleys).

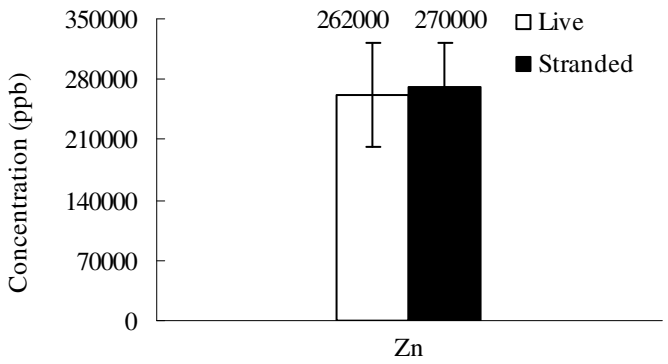


Fig. 23. Zinc concentration (ppb) in carapace tissue (d.w.) of live (n = 44) Kemp's ridleys from Texas and Louisiana and stranded (n = 11) Kemp's ridleys from Texas.

Discussion

The present study is the first characterization of trace metal concentrations in tissues of Kemp's ridleys stranded along the Texas coast. A summary of trace metal concentrations in internal tissues and carapace tissue of Kemp's ridley, loggerhead and green turtles reported by other studies is given in Appendix Table 38. Information in this table suggests metal concentrations have been detected in a variety of tissue types in stranded sea turtles collected around the world. Lack of consistent results may be due to differences in food habits, ambient contaminant loads, species monitored, and analytical techniques plus limited access to these endangered species and, thus, small sample sizes. The information inconsistencies and voids render it difficult to assess cumulative effects of trace metals as well as associated toxicity risks in Kemp's ridleys as well as all other sea turtle species.

The one study to monitor trace metal levels in muscle tissue (d.w., [Cd] = 450, [Cu] = 4,900, [Zn] = 82,000 ppb) of Kemp's ridleys (n = 6, SCL = 25.8 $\pm$ 5.2 cm, range = 21.3 - 34.5 cm) analyzed conspecifics stranded along the French Atlantic coast (Caurant *et al.*, 1999). Metal levels in the aforementioned study were higher than those of stranded ridleys (range = 33.9 - 64.0 cm SCL) in the present study.

Stranded ridleys in the present study yielded higher Ag, Cu, and Hg concentrations in carapace tissue that did those of green and loggerhead counterparts as shown in Appendix Table 38, while the latter exhibited higher Ag,

Cd, Cr, Cu, Pb levels in their liver and kidney tissues. Similar Zn levels in internal organs and tissues were found among these three sea turtle species. Variability observed in trace metal levels among the aforementioned species may be due to the relatively small number of samples analyzed as well as differences in their foraging habits. In addition, the current study utilized class-100 clean laboratory techniques that typically result in lower metal detection levels and more consistent results than those (i.e. non class-100 clean lab techniques) used in other studies.

Overall, significantly higher Ag, Cr, Hg and Zn concentrations were found in carapace tissue of stranded ridleys than in their liver, kidney and muscle tissues, while liver exhibited higher Cd and Cu concentrations. Although non-significant, carapace tissue of stranded ridleys also exhibited higher Pb levels than did those of their internal organs and muscle tissue. Mercury was the only metal whose concentration in kidney tissues increased with increasing size of ridleys. In addition, Hg was the only trace metal to exhibit a significantly positive correlation between liver and carapace tissue and, in doing so, justified accepting the hypothesis that trace metal concentrations in the organs and/or muscle tissue are correlated with those in their carapace tissue, at least for Hg.

Silver

Carapace tissue (256 ppb) of dead stranded ridleys exhibited higher Ag levels than did their liver (1.9 times), kidney (3.8 times, $p = 0.016$) and muscle

tissues (3.2 times, $p = 0.022$). Thus, the hypothesis that Ag concentrations in the internal organs are higher than those in their carapace tissue is rejected. Regardless of tissue type and statistical significance, the relative Ag distribution in stranded ridleys was carapace > liver > muscle > kidney. Similar tissue distribution patterns of Ag have been reported for green turtles (Anan *et al*., 2001; Wang *et al*., 2003) and marine mammals (Saeki *et al*., 2001; Anan *et al*, 2002b; Ikemoto *et al*., 2004).

Silver concentrations in liver of 37.0 to 71.4 cm SCL green turtles (n = 26; Anan *et al*., 2001) were about 76 times higher than those of ridleys in the present study. The fact that higher Ag levels were found in marine plants and/or algae (5.9 to 14,100 ppb) than those in blue crab from other studies (100 - 2,800 ppb; Eisler, 1996; Park and Presley, 1997) implies that different contaminant loads in food sources may explain this concentration difference between green and Kemp's ridley sea turtles. Conversely, similar Ag levels were reported in kidney and muscle tissues between ridleys in this study and those of greens reported by Anan *et al*. (2001).

Stranded ridleys of the present study yielded higher Ag levels in carapace tissue than did loggerhead (n = 14, SCL = 67.8 ± 8.5 cm, range = 52.5 - 88.2 cm) and green (n = 13, SCL = 40.8 ± 11.3 cm, range = 27.0 - 61.8 cm) counterparts from Texas, regardless of size, while the latter exhibited higher Ag concentrations in liver (Wang *et al*., 2003). There are several probable factors facilitating the disparity of Ag in these stranded sea turtles from Texas. It could be

due to a relatively small sample size used in assessing trace metal levels during this study. Another factor that may play a role in metal concentration differences is the different contaminant loads in diets of sea turtle species.

No significant correlation was detected in Ag concentrations between carapace tissue and internal organs or muscle tissue of stranded ridleys. Consequently, the hypothesis that Ag concentrations in the internal organs and/or muscle tissue are correlated with their carapace tissue is rejected. Although this result casts doubt that carapace tissue may be suitable as a surrogate sample source for Ag in Kemp's ridleys, the carapace did exhibit the potential to accumulate Ag, given the aforementioned comparison with other tissues analyzed during this study. The lack of correlation between carapace and internal tissues may simply mean it does not exist or that a larger sample size is warranted in future studies to assess the utility of the former tissue as a bioindicator of Ag in Kemp's ridleys. Furthermore, carapace tissue (237 ppb) of stranded ridleys yielded significantly higher Ag levels than those of their live conspecifics from Texas and Louisiana (116 ppb; Fig. 22). It is likely these larger stranded ridleys had time to accumulate higher Ag in their carapace tissue than did their smaller, live cohorts; a similar size-related dependency was noted for Ag in Chapter V. A sample size disparity between numbers of stranded versus live ridleys used in this comparison also may have impacted these results.

A significant positive correlation in Ag concentrations was found between liver and kidney of stranded ridleys. A similar relationship has been reported for

marine mammals (Ikemoto *et al*., 2004). Significant positive correlations were found between Ag and Cd in liver, and Ag and Pb in both liver and muscle tissue of stranded ridleys. Silver concentration was positively correlated with Hg level in liver of stranded ridleys. A similar result was also reported for marine mammals (Becker *et al*., 1995; Saeki *et al*., 2001).

Silver toxicity tests of marine vertebrates have been exclusively conducted with fish. Acute 96-h LC_{50} values in marine fish ranged from 108 to 1,170 ppb (Ratte, 1999). Silver levels in stranded ridley tissues analyzed in the present study were within or below the range of 0.8 to 1,444 ppb reported for marine mammals (Law *et al*., 2001; Saeki *et al*., 2001; Anan *et al*., 2002b; Ikemoto *et al*., 2004) and marine fish (Ratte, 1999). However, toxic effects of these concentrations in ridleys remain unknown. Further investigation on Ag toxicity is needed to evaluate adverse effects of this metal in live Kemp's ridleys.

Overall, both hypotheses that Ag concentrations in the internal organs are higher than those in carapace tissue and Ag levels in the internal organs and muscle tissue are correlated with carapace tissue of stranded ridleys are rejected. However, Ag appears to accumulate at higher concentrations in the carapace tissue of stranded ridleys rather than in their liver, kidney or muscle tissues. The fact that no correlation was detected between carapace tissue and other tissues analyzed during this study may be related to varying accumulation rates across tissue types. Furthermore, an unknown effect of post-mortem decomposition between soft (i.e. muscle) and hard tissues (carapace) also may

promote this disparity among tissues.

Cadmium

Kidney tissues of dead stranded ridleys (2,410 ppb) yielded higher Cd levels than did their muscle (44 times) and carapace tissue (15 times) and, thus, the hypothesis that Cd concentrations in the internal organs of stranded Kemp's ridleys are higher than in their carapace tissue is accepted, at least for kidney tissue. In addition, Cd levels in liver (2,130 ppb) were 13 and 39 times significantly higher in their carapace and muscle tissue, respectively. Regardless of tissue type and statistical significance, the hierarchy for Cd levels in these stranded ridleys was kidney > liver > carapace > muscle. Similar tissue distribution patterns of Cd have been reported for loggerheads from Texas (Wang *et al*., 2003). Caurant *et al*. (1999) reported the only published mean Cd concentration in muscle tissue (450 ppb) of stranded ridleys that was about 9 times higher than those of larger conspecifics in the present study. This disparity may have been due to the fact that Cd levels decrease with increasing size of ridleys as discussed in Chapter V. In addition, mean Cd level in carapace tissue (151 ppb) of stranded ridleys was slightly higher than that in loggerheads (129 ppb; Sakai *et al*., 2000a) but lower than those in greens and loggerheads investigated by Wang *et al*. (2003; Appendix Table 37).

The kidney has been documented as the primary body burden site for Cd in sea turtles (Gordon *et al*., 1998; Storelli *et al*., 1998a; Caurant *et al*., 1999; Sakai *et al*., 2000) and marine mammals (Meador *et al*., 1999; Becker, 2000;

Bustamante *et al*., 2003). However, stranded ridleys in the present study yielded higher Cd in liver than in their kidney. A similar result also was reported for seabirds (Lock *et al*., 1992; Zeisler *et al*., 1993; Muirhead and Furness, 1998). Normally, Cd concentrations in the kidneys of humans and marine mammals are 10 to 15 times higher than in their liver (Kostial, 1986; Thompson, 1990). In addition, Cd levels in the cortex of kidney are about twice as high as those in the medulla of marine mammals (Sonne-Hansen *et al*., 2002). Concentration disparities detected between liver and kidney tissues in stranded ridleys may be due to differences in type of kidney samples taken for analysis by the present study as well as the relatively small number of samples analyzed. A further investigation involving comprehensive research on trace metal concentrations in different tissue constituents from stranded ridleys is needed to more accurately assess this disparity.

Like that for Ag, no significant correlation was detected in Cd concentrations between carapace tissue and internal organs or muscle tissue of stranded ridleys. Therefore, the hypothesis that Cd concentrations in internal organs and/or muscle tissue are correlated with their carapace tissue must be rejected. The fact that liver exhibited higher Cd levels than did carapace tissue prevents this tissue from serving as a suitable surrogate sample source for Cd loading in Kemp's ridleys. Further investigation involving invasive biopsy techniques applied to internal organs of live ridleys needs to be conducted to assess whether the liver is a primary Cd accumulation site.

Additionally, stranded ridleys yielded higher but statistically insignificant Cd concentrations in their carapace tissue (151 ppb) compared to those of their smaller live constituents (132 ppb) from Texas and Louisiana. This variation may due to the expanded interval that larger stranded ridleys have to accumulate higher Cd in carapace tissue than did their smaller live constituents.

A significantly positive correlation between Cd and Cu was found in liver and carapace tissue of stranded ridleys. Sakai *et al*. (2000b) suggested this correlation might be related to specific metal binding proteins (metallothionein) that exist in the liver. For marine mammals, these proteins are important to metal homeostasis, especially for storage and detoxification. Metallothionein is normally induced by trace metal, such as Cd, Cu and Zn. As a result, the correlation between Cd and Cu could be due to the induction of metallothionein synthesis to prevent toxic effects of Cd and Cu.

Investigations on Cd-poisoned humans and laboratory mammals indicate that concentrations above 50,000 ppb wet weight in the kidney may induce histopathological changes (Sonne-Hansen *et al*., 2002). Fujise *et al*. (1988) suggested that renal dysfunction attributable to Cd can occur in marine mammals when liver concentrations exceeded 20,000 ppb. Eisler (1985) estimated Cd concentrations exceeding 100 ppb in feathers of seabirds may indicate signs of adverse effects, based on laboratory experiments. The 96h-LC_{50} value for marine fish larvae is 1,230 ppb (Hutchinson *et al*., 1994). Cadmium concentrations in stranded ridleys of the present study were at least

an order of magnitude lower than the aforementioned levels in marine mammals.

In summary, the hypothesis that Cd concentrations in internal organs of stranded ridleys are higher than in their carapace tissue is accepted for liver only. However, the hypothesis that Cd levels in internal organs and/or muscle tissue are correlated with their carapace tissue is rejected. Therefore, carapace tissue may not be suitable as a surrogate sample source for monitoring Cd accumulation in ridleys.

Chromium

Carapace tissue of dead stranded ridleys exhibited higher Cr concentrations (168 ppb) than did their liver (9.5 times, $p = 0.018$), kidney (8.2 times, $p = 0.020$) and muscle tissue (3 times). Thus, the hypothesis that Cr levels in the internal organs are higher than those in their carapace tissue is rejected. Chromium levels in stranded ridleys of the present study were about two orders of magnitude lower than those within a similar size range of green turtles (Anan *et al.*, 2001). Regardless of tissue type and statistical significance, the hierarchy for Cr concentrations was carapace > muscle > kidney > liver. A similar result also has been reported for stranded loggerhead and green turtles from Texas (Wang *et al.*, 2003). Anan *et al.* (2001) reported Cr accumulation levels in liver and kidney of green turtles that were in direct contrast to the aforementioned findings. This disparity was probably due to a relatively small sample size used in assessing metal levels during this study and uptake variations within the

constituent tissues.

No significant correlation was detected in Cr concentrations between carapace tissue and internal organs or muscle tissue and, thus, the hypothesis that Cr levels in internal organs and muscle tissue are correlated with their carapace tissue is rejected. Although this result casts doubt that carapace tissue may be suitable as a surrogate sample source for Cr in Kemp's ridleys, the carapace did exhibit the potential to accumulate Cr, given the aforementioned comparison with other tissues analyzed during this study. Conversely, larger stranded ridleys in the present study yielded lower but statistically insignificant Cr levels in their carapace tissue (158 ppb) compared to those of their smaller live cohorts (278 ppb) from Texas and Louisiana. This finding suggests that live/smaller ridleys may require higher concentrations of this essential element than do their older conspecifics. However, future studies employing a larger sample size are needed to assess the potential for utilizing carapace tissue as a indicator of Cr in Kemp's ridleys.

A significant positive correlation was detected in Cr concentration between liver and kidney tissues of stranded ridleys that has not been reported for sea turtles. No other correlation was detected between Cr and other trace metals in different tissues analyzed during this study.

Although toxicity of Cr is not well understood, it is known that acute 96-h LC_{50} values in fish range from 11,000 to 100,000 ppb (Anestis and Neufeld, 1986). It has been suggested (Eisler, 1986) that Cr levels exceeding 4,000 ppb in

internal tissues may cause adverse effects in seabirds. Chromium levels in stranded ridleys of the present study were at least an order of magnitude lower than those in fish and seabirds. Like that for Cd, Cr concentrations detected by the present study may not be sufficient to cause toxic risks in ridleys.

Overall, both hypotheses that Cr concentrations in internal organs are higher than those in their carapace tissue; and Cr levels in internal organs and muscle tissue are correlated with their carapace tissue are rejected.

Copper

Copper levels in liver tissue (14,700 ppb) of dead stranded ridleys exceeded those in their kidney (1.8 times, $p = 0.040$), muscle (6 times, $p = 0.001$) and carapace (9 times, $p = 0.001$) tissues. In addition, kidney tissue (7,780 ppb) of the aforementioned stranded ridleys also yielded significantly higher Cu concentrations than those in their carapace (5 times, $p = 0.001$) and muscle (3.5 times, $p = 0.001$) tissues and, consequently, the hypothesis that Cu levels in the internal organs are higher than in their carapace tissue is accepted. Regardless of tissue type and statistical significance, the hierarchy for Cu concentrations was liver > kidney > muscle > carapace. Similar tissue distribution patterns of Cu have been reported for loggerhead and green turtles (Caurant *et al*., 1999; Sakai *et al*., 2000a and 2000b; Wang *et al*., 2003). Caurant *et al*. (1999) reported the only published Cu level in muscle tissue (4,900 ppb) of stranded ridleys, with this level being twice as high as that detected by the present study. There are several

probable factors facilitating the disparity of Cu concentrations in these stranded ridleys, including a relative small sample size as well as the size disparity between conspecifics in the present study and those sampled by Caurant *et al*. (1999). Another factor that may play a role in these metal concentration disparities is a possible difference in food habits or ambient contaminant loads in respective species' diets.

Geographic and species differences were observed in Cu concentrations of stranded sea turtles from various locations (Appendix Table 38). Copper levels in liver tissue of stranded ridleys in the present study were similar to those of loggerheads from Texas (Sis and Landry, 1992; Wang *et al*., 2003) but at least 20 times lower than those in greens from Japan and Hawaii (Appendix Table 38). Copper levels in kidney and muscle tissue of stranded ridleys in the present study were higher than those of loggerheads from Texas and Japan (Sakai *et al*., 2000a; Wang *et al*., 2003). Furthermore, Cu levels in carapace tissue (1,560 ppb) of stranded ridleys in the present study were 6 times higher than those in larger loggerheads (251 ppb; n = 6, SCL = 83.6 ± 6 cm) analyzed by Sakai *et al*. (2000a).

No significant correlation was detected in Cu concentrations between carapace tissue and internal organs or muscle tissue. Consequently, the hypothesis that Cu concentrations in internal organs and muscle tissue of stranded ridleys are correlated with their carapace tissue is rejected. Therefore, carapace tissue may not be a suitable surrogate sample source for measuring

Cu levels in Kemp's ridleys. Nonetheless, this tissue did exhibit potential accumulation for Cu, given the results of their comparison with muscle tissue analyzed during this study.

Larger stranded ridleys yielded significantly lower Cu concentrations in carapace tissue (1,560 ppb) compared to those of their smaller live constituents (2,410 ppb) from Texas and Louisiana. This disparity mirrors higher Cu concentrations that have been documented in smaller or younger green turtles (Sakai *et al*., 2000b) and marine mammals (Das *et al*., 2003). These results suggest that smaller/younger animals may require more Cu during development. The fact that adult ridley females discussed in Chapter V exhibited significantly lower Cu levels than did their smaller conspecifics seems to verify this suggestion. However, any speculation in this regard must be adequately tested.

A significantly positive correlation was detected in Cu concentrations between liver and kidney tissue of stranded ridleys. A similar result has been reported for marine mammals (Watanabe *et al*., 1996; Ikemoto *et al*., 2004). A positive relationship was observed between Cu and Pb, and Cu and Zn in muscle tissue as well as Cu and Zn in carapace tissue of stranded ridleys. Similar findings also were reported in green turtles (Sakai *et al*., 2000b) and marine mammals (Endo *et al*., 2002; Kunito *et al*., 2002). In addition, a positive correlation was detected between Cu and Pb in kidney and carapace tissues of stranded ridleys.

The 96-h LC50 toxicity of Cu in marine fish larvae is greater than 220 ppb

(Hutchinson *et al*., 1994). Acute toxicity in saltwater fish from Cu uptake ranged from 28 to 510 ppb (USEPA, 1980b). Mean Cu concentration reported for liver tissue of seabirds is around 6,000 ppb, with few values greater than 10,000 ppb (Thompson, 1990). Copper levels in stranded ridleys of the current study were higher than those reported in fish but near the range for seabirds. Nonetheless, it is difficult to interpret possible health impacts of these levels in ridleys of the present study without further investigation on Cu toxicity.

Overall, the hypothesis that Cu concentrations in the internal organs (liver and kidney only) are higher than those in their carapace tissue is accepted. However, the hypothesis that Cu levels in internal organs and/or muscle tissue are correlated with those of carapace tissue is rejected. These findings imply that carapace tissue may not be suitable as a surrogate sample source for monitoring Cu accumulation in stranded ridleys.

Mercury

Carapace tissue of dead stranded ridleys yielded higher Hg concentrations (1,250 ppb) than did their kidney (1.7 times) and muscle tissue (2.9 times, $p = 0.043$). Thus, the hypothesis that Hg concentrations in the internal organs are higher than in their carapace tissue is rejected. This concentration disparity is in contrast to that reported by Sakai *et al*. (2000a) for green turtles whose liver tissue yielded higher Hg levels. Mercury concentrations in carapace tissue of stranded ridleys in the present study were higher than those reported in

loggerheads (43.2 ppb; Sakai *et al*., 2000a). Regardless of tissue type and statistical significance, the hierarchy for Hg levels in stranded ridleys was carapace > liver > kidney > muscle tissue. This finding was similar to that reported for loggerhead and green turtles (Sakai *et al*., 2000a and 2000b; Wang *et al*., 2003) and cetaceans (Zhou *et al*., 2001; Méndez *et al*., 2002; Das *et al.,* 2003). The fact that loggerheads accumulated higher methylmercury, a toxic form of Hg, in the muscle (Storelli *et al*., 1998b) could have adverse effects on this species. Therefore, further characterization of methylmercury levels in various tissues is needed to accurately assess health impacts in Kemp's ridleys from Hg toxicity.

Mercury concentrations in kidney tissues rose significantly with increasing size of stranded ridleys. Similar increases also have been reported for Kemp's ridleys and loggerheads (Presti *et al*., 2000; Kenyon *et al*., 2001; Day, 2003), and marine mammals (Medvedev *et al*., 1997; Monaci *et al*., 1998; Endo *et al*., 2002; Ikemoto *et al*., 2004). A significant positive correlation was detected in Hg concentration between liver and carapace tissue of stranded ridleys and, thus, provided support for accepting the hypothesis that Hg levels in internal organs and/or muscle tissue are correlated with those in their carapace tissue. This suggests that carapace tissue could serve as a suitable surrogate sample source to characterize Hg loadings in Kemp's ridleys. The lack of correlation between carapace tissue, and kidney and muscle tissue may be due to varying chemical forms of Hg accumulating in different tissues. For example, Storelli *et al*.

(1998b) reported that higher total Hg levels were found in liver tissue of loggerheads than in their muscle tissue, while the latter exhibited higher percentage of methylmercury/total Hg. This finding indicates that a detoxification process exists in the liver of loggerheads. It is possible that Kemp's ridleys may exhibit a similar demethylation of Hg in their liver; if so, this might impact correlation between tissues analyzed during this study.

Mercury concentration was positively correlated with Pb in carapace tissue, a trend mirrored by live constituents in Chapter V. Additionally, larger stranded ridleys yielded similar Hg concentrations in the carapace tissue (1,280 ppb) compared to those of their smaller live constituents (1,170 ppb) from Texas and Louisiana. This variability may be related to a relatively small sample size analyzed by the current study or to differences in primary foraging grounds prior to capture and tissue sampling.

Wolfe *et al*. (1998) reported that the Hg toxicity in reptiles is almost unknown. Liver-borne Hg concentrations ranging from 100,000 to 400,000 ppb are known to cause hepatic damage in dolphins (Beck *et al*., 1997). Mercury concentrations such as these are at least three orders of magnitude higher than those reported for stranded ridleys in the present study. Bekvar *et al*. (1996) recommended the Hg threshold for reproductive effect in whole fish collected from various locations ranges from 500 to 1,000 ppb. Mercury concentrations within the range of 800 – 2,000 ppb were reported to impair reproduction in birds (Clarkson, 1987). Eisler (1987) concluded Hg levels of 5,000 ppb in feathers of seabirds collected from

around the world are known to cause adverse reproduction effects. Carapace tissue of stranded ridleys analyzed by the present study exhibited Hg concentrations similar to the aforementioned Hg levels in fish and birds. Consequently, there may reason to suspect possible adverse health effects from Hg accumulation in ridleys foraging in the northwest Gulf of Mexico.

Overall, the hypothesis that Hg concentrations in the internal organs are higher than those in their carapace tissue is rejected. Conversely, the hypothesis that Hg levels in the internal organs or muscle tissue are positively correlated with those in carapace tissues is accepted only for liver. As a result, carapace tissue of stranded ridleys may be considered a potential surrogate sample source upon which to assess Hg exposure.

Lead

Although not significant, carapace tissue of dead stranded ridleys yielded higher Pb concentrations (1,500 ppb) than did their liver (10 times), kidney (9.2 times) and muscle tissue (10.8 times). Thus, the hypothesis that Pb concentrations in their internal organs are higher than those in the carapace tissue is rejected. Stranded ridleys of the present study yielded lower Pb levels in carapace tissue than did their loggerhead counterparts (2,420 ppb; Sakai *et al.*, 2000a). It is likely these larger loggerheads had time to accumulate higher Pb in their carapace tissue than did the smaller, stranded ridleys analyzed by the present study. Regardless of tissue type and statistical significance, the

hierarchy for Pb levels in stranded ridleys was carapace > kidney > liver > muscle. Similar tissue distribution patterns of Pb have been reported for loggerhead turtles (Sakai *et al*., 2000a). Other studies on green turtles (Sakai *et al*., 2000a; Anan *et al*., 2001) identified higher Pb levels in kidney tissue rather than in their liver. Differences in food habits of sea turtle species and contaminant loads in their foraging environment may account for this disparity.

Sakai *et al*. (2000a) suggested that carapace tissue as a useful surrogate sample source for monitoring Pb exposure in green turtles because of its correlation with the whole body burden. However, no significant correlation was detected in Pb concentrations between carapace tissue and internal organs or muscle tissue in the current study. Consequently, the hypothesis that Pb concentrations in internal organs and/or muscle tissue are correlated with their carapace tissue is rejected. Although this result casts doubt that carapace tissue may be suitable as a surrogate sample source for Pb in Kemp's ridleys, the carapace did exhibit the potential to accumulate Pb, given the aforementioned comparison with other tissues analyzed during this study.

Furthermore, stranded ridleys yielded higher but statistically non-significant Pb concentrations in their carapace tissue (1,380 ppb) compared to those of their live constituents (960 ppb) from Texas and Louisiana. This difference may have been due to the expanded interval that these larger stranded ridleys had to accumulate higher Pb in carapace tissue. Disparity in number of stranded versus live ridleys used in this comparison also may have influenced these results,

especially given the small sample of stranded ridleys and the potential for highly variable results from constituent carcasses.

A significantly positive correlation was detected between Hg and Pb in carapace tissue, a trend mirrored by live constituents in Chapter V. The fact that both metals tend to accumulate higher Pb levels in carapace tissue may account for this correlation.

No toxicity tests of Pb have been documented in marine vertebrates. Burger and Gochfeld (1995) reported that Pb levels of 4,000 ppb in feathers of seabirds from New Jersey could indicate sub-lethal and reproductive effects. Lead concentrations in liver tissue of common freshwater turtle species were reported as high as 1,000 ppb (Eisler, 1988). Carapace tissue of stranded ridleys in the present study yielded Pb concentrations approximately 3 times lower than those in seabirds but 1.5 times higher than those in freshwater turtles. As such, they may be considered a potential source of adverse health effects in ridleys. Additional research is needed to assess health risks in sea turtles from Pb accumulation. Permit restrictions may initially limit this additional research to studies on blood chemistry and developing correlations between metal concentrations and status of immune functions.

Overall, both hypotheses that Pb concentrations in the internal organs are higher than in their carapace tissue; and Pb levels in the internal organs and muscle tissue are correlated with their carapace tissue are rejected.

Zinc

Carapace tissue of dead stranded ridleys exhibited significantly higher Zn concentrations (271,000 ppb) than did their liver (3.9 times), kidney (2 times) and muscle tissue (8.7 times). Therefore, the hypothesis that Zn levels in the internal organs of stranded Kemp's ridleys are higher than in their carapace tissue is rejected. Kidney tissue (134,000 ppb) of the aforementioned stranded ridleys also yielded significantly higher Zn levels than did their liver (1.9 times) and muscle (4.3 times) tissues. In addition, Zn concentrations in liver tissue were 2.2 times higher than in their muscle tissue. Regardless of tissue type and statistical significance, the hierarchy for Zn levels in stranded ridleys was carapace > kidney > liver > muscle. Various tissue distribution patterns of Zn have been reported for loggerhead and green turtles (Sakai *et al*., 2000a; Anan *et al*., 2001; Wang *et al*., 2003). Differences in sample size as well as variability of contaminant loads in their diets may account for the disparity between Zn levels in ridleys versus those in other sea turtle species.

The only published mean Zn concentration in muscle (16,400 ppb; Caurant *et al*., 1999) of stranded ridleys was at least 2 times higher than that of larger conspecifics analyzed by the present study. This disparity may be explained by the fact that Zn levels in smaller ridleys were higher than in their larger conspecifics discussed in Chapter V. This finding also may reflect geographic differences as well as disparities in contaminant loading between stranded ridleys in the present study and those sampled by Caurant *et al*. (1999). Similar

Zn levels were detected in liver and kidney tissues between the present study and other investigations of loggerheads (Caurant *et al*., 1999; Sakai *et al*., 2000a) but lower than that for green turtles (Aguirre *et al*., 1994; Gordon *et al*., 1998; Anan *et al*., 2001). However, Zn levels in the present study were an order of magnitude lower than those in cetaceans (Méndez *et al*., 2002; Bustamante *et al*., 2003).

No significant correlation was detected in Zn concentrations between carapace tissue and internal organs or muscle tissue of stranded ridleys. Consequently, the hypothesis that Zn concentrations in internal organs or muscle tissue are correlated with their carapace tissue is rejected. Although this result casts doubt that carapace may be suitable as a surrogate sample source for Zn in Kemp's ridleys, carapace tissue did exhibit the potential to accumulate Zn, given the aforementioned comparison with other tissues analyzed during this study.

Additionally, larger stranded ridleys yielded higher but statistically non-significant Zn concentrations in the carapace tissue (270,000 ppb) compared to those of their smaller live constituents (262,000 ppb) from Texas and Louisiana. The fact that Zn levels did not increase with increasing size of ridleys in Chapter V may explain this finding. Differences in numbers of stranded versus live ridleys used in this comparison also may have influenced these results.

Very few toxicity tests involving Zn have been conducted on marine

vertebrates. It is difficult to interpret Zn concentrations in sea turtles because of the paucity of toxicity data. Eisler (1993) documented the acute 96h LC_{50} for marine teleosts and found it ranged from 191 to 38,000 ppb. Zinc levels in stranded ridleys exceeded aforementioned levels for marine fish but were within or below the range of 20,000 to 481,600 ppb reported for marine mammals (Monaci *et al*., 1998; Das *et al*., 2000; Law *et al*., 2001; Anan *et al*., 2002b; Ikemoto *et al*., 2004). This finding may suggest a cause for concern that Zn levels in the present study could have adverse health impacts in Kemp's ridleys.

Overall, both hypotheses that Zn concentrations in internal organs are higher than in carapace tissue and Zn levels in internal organs and muscle tissue are correlated with carapace tissue of stranded ridleys are rejected.

Summary

The current study characterized trace metal concentrations in liver, kidney, muscle, and carapace tissue of 11 Kemp's ridleys stranded along the Texas coast. A relative small sample size as well as the potential for a varying accumulation rate in different tissues or portions of respective tissues could have affected the distribution of metal loading reported herein. The integrity of trace metal concentrations between soft and hard tissues also may be adversely impacted by post-mortem decomposition in stranded carcasses. Sakai *et al*. (2000a) reported that certain trace metal concentrations (Hg and Zn) in carapace could better represent the whole body burden instead of soft tissues (i.e. kidney

and liver).

Generally, significantly higher Ag, Cr, Hg, Pb and Zn levels were found in the carapace tissue to suggest the potential for accumulation of these trace metals. Conversely, liver appeared to accumulate Cd and Cu in the present study. These concentration differences may be due to differences in diet or feeding grounds used by different life stages of ridleys over time. This specific tissue distribution also was observed by other studies (Caurant *et al*., 1999; Godley *et al*., 1999; Anan *et al*., 2001; Wang *et al*., 2003). These findings suggest that a further investigation characterizing trace metal concentrations in internal organs and muscle tissue of live ridleys is needed to determine other possible accumulation sites of these metals, especially for Cd and Cu, and to verify the aforementioned results from stranded counterparts. These findings could then be compared with the current study to evaluate carapace tissue as a non-invasive indicator of metal loading in Kemp's ridleys.

Stranded ridleys in the present study also exhibited higher Ag, Cd, Hg, Pb and Zn concentrations than did their live constituents from Texas and Louisiana. This finding implies that larger stranded ridleys had more time to eat more prey and, in doing so, to accumulate higher trace metal concentrations in their carapace tissue than did their smaller, live cohorts. The fact that higher Ag, Hg, Pb and Zn levels were found in larger ridleys (discussed in Chapter V) may help to elucidate this statement. Higher Cr and Cu levels were found in carapace tissue of live ridleys rather than in their stranded conspecifics. These results

mandate a thorough investigation of metabolism and essential element requirements of sea turtles in an effort to explain the aforementioned disparity between live and stranded ridleys.

Trace metal concentrations in stranded ridleys were normally within or below the range of those reported for marine mammals and seabirds. The fact that Cu, Hg, Pb and Zn levels in ridleys of the present study were higher than those in marine fish may suggest the potential for adverse impact to this species' health. However, the actual toxic effects of these concentrations and the differences in accumulation of trace elements in sea turtles remain unknown (Anan *et al*., 2001). Data generated herein provide valuable information that could be used to evaluate potential risks to sea turtles from trace metals. Larger sample sizes produced by future studies will help to more accurately assess trace metal-tissue distribution patterns in Kemp's ridleys. In addition, future research needs to include the characterization of different speciation (i.e. Cr (III) vs. Cr (VI); Hg vs. methylHg) of trace metals in order to adequately evaluate metal toxicity and possible adverse effects in this endangered species.

CHAPTER VII

SUMMARY AND CONCLUSIONS

Information on trace metal concentrations in tissues of the critically endangered Kemp's ridley sea turtle is extremely limited, especially as it relates to levels in live, wild conspecifics. This species' depleted population status and limited number of in-water studies enabling access to life history constituents represent barriers to obtaining tissue samples from which trace metals can be analyzed. Endangered species permit restrictions on sampling live conspecifics are, in large part, also responsible for this information void. Equipped with permits that restricted sample sources to blood and carapace tissue in live ridleys and internal organs from dead, stranded counterparts, the current study attempted to: 1) characterize baseline trace metal levels in captive ridleys on which to conduct strategic comparisons involving live, wild conspecifics; 2) determine the role of food as a potential pathway for trace metal uptake; 3) assess the potential for trace metal accumulation, especially in relation to life history stage and size; and 4) identify an external tissue type that could be used as an analytical sample-source surrogate for internal organs and tissues.

Baseline Trace Metal Concentrations in Captive Ridleys

Baseline trace metal levels were established in the current study from a limited size range (28 to 35 cm SCL) of captive Kemp's ridleys headstarted in relatively contaminant-free conditions and fed the same diet throughout their captivity. All trace metal concentrations in carapace tissue of captive ridleys were significantly higher than those in their blood. Mean trace metal concentrations in these captive ridleys, regardless of constituent year-class, are given in Table 4. Concentration differences ranged from 1.5 times for Cr and 88 times for Cu higher in carapace tissue. Additionally, carapace tissue of captive ridleys yielded higher Ag (28 times), Cu (1.8 times), Hg (4 times), and Zn (2.5 times) concentrations than did those in their pelleted food, while the latter exhibited higher Cr (275 times) and Pb (2 times) levels.

Captive vs. Wild Ridleys

Trace metal concentrations from 28 to 35 cm SCL, wild Kemp's ridleys captured from Texas and Louisiana were compared to those of similar-sized captive conspecifics. Mean trace metal levels in these captive and wild ridleys are shown in Tables 4 and 9. Significantly higher Cd, Cr, Hg, Pb and Zn concentrations were found in carapace tissue of wild Kemp's ridleys. It is likely that food and the foraging environment accounted for differences in metal concentrations between captive and wild conspecifics.

Blood vs. Carapace Tissue

Live Kemp's ridleys, regardless of captive or wild status, exhibited higher trace metal concentrations in carapace tissue than in their blood. This trend suggests two possible conclusions regarding trace metal uptake and accumulation in ridleys: 1) blood-borne concentrations are probably indicative of a recent exposure to respective trace metals, with this medium exhibiting little or no likelihood for continued accumulation; and 2) depending upon metal, carapace tissue displayed the possibility to accumulate trace metals.

Kemp's Ridleys vs. Blue Crab Prey

Potential trace metal relationships between Kemp's ridleys and their prey were examined in the present study. Carapace tissue of ridleys captured from Texas and Louisiana yielded higher Cr, Hg, Pb and Zn concentrations than did their blue crab prey while the latter exhibited higher Ag and Cu levels. Significantly higher Ag, Cd, Cr, Hg, Pb and Zn concentrations in carapace tissue of adult ridley females than in their blue crab prey was probably a result of much greater volumes of food eaten by these females over prolonged periods as well as the fact that they likely used the highly industrialized Texas and Louisiana coasts as prime foraging grounds as juveniles and subadults. Different contaminant loads in food sources and the accumulation possibility vested in specific tissues may explain this concentration disparity between Kemp's ridleys and their blue crab prey.

Trace Metal Concentrations in Kemp's Ridleys vs. Size

Trace metal concentrations were determined for different size classes of Kemp's ridleys. Various size-dependent relationships existed in blood (Hg, Pb, Zn) and carapace tissue (Ag, Cr, Hg) of Kemp's ridleys across all post-pelagic life stages. These results, especially in regard to carapace tissue, tend to suggest the potential for accumulation of particular trace metals in ridleys. Conversely, Cd was the only metal that decreased in concentration with increasing size of ridleys, thus suggesting lack of a size-dependent relationship.

Geographic Differences

Except for Cd and Cu, higher metal concentrations were detected in adult female ridleys nesting on the Rancho Nuevo, Mexico beach. It is likely that these larger ridleys cumulatively consumed more prey over time than did their smaller conspecifics, thus the greater potential for uptake and possible accumulation of larger metal quantities. In addition, this disparity could be due to differences between life history stages as they relate to: 1) size of prey items; 2) foraging regions; and 3) metal kinetics (e.g. uptake, excretion, accumulation rate). Another factor likely playing a role in this disparity is the strong possibility these adults, like their younger conspecifics captured in this study, used the Texas and Louisiana coast as a foraging ground. As such, these adults have probably been exposed to habitats with elevated trace metal contents and thus had increased potential to accumulate trace metals since their juvenile life history stage.

Blue crabs collected from Mexico exhibited lower trace metal levels (Ag, Cr, Cd, Cu, Pb) than did their Texas and Louisiana counterparts. This concentration difference is expected given the less industrialized coastal waters along the Rancho Nuevo, Mexico nesting beach where blue crabs were collected.

Trace Metal Concentrations in Stranded Ridleys

Trace metal concentrations were determined for various internal organs and tissues of Kemp's ridleys stranded dead along the Texas coast. Although a relatively small number (n = 11) was analyzed in the present study, data generated herein provided valuable information on metal distribution in these stranded turtles.

Overall, carapace tissue yielded higher Ag, Cr, Hg, Pb and Zn levels than did internal organs and muscle tissue of stranded ridleys and, thus the potential for accumulation of certain trace metals. The fact that liver tissue exhibited higher Cd and Cu concentrations than did other tissues lessens the possibility that carapace tissue can be used as a generic surrogate for internal tissues in Kemp's ridleys. However, positive correlation detected in Hg levels between liver and carapace tissue of stranded ridleys suggests the latter may be considered a potential surrogate sample upon which to assess Hg uptake.

Trace metal concentrations in stranded ridleys were normally within or below the range of those reported for marine mammals and seabirds. However, Hg, Pb and Zn levels in stranded ridleys were higher than those reported for

marine fish, a finding suggests further investigation of their being a potential risk to ridley health.

Comparison of Trace Metal Concentrations in Sea Turtles

Comparison of trace metal concentrations between Kemp's ridleys and other marine vertebrates, such fish and mammals, is very problematic given vast differences in size, diet and foraging habitats. As such, trace metal concentrations in Kemp's ridleys of the present study, regardless of tissue type, were compared to those of other studies on sea turtles listed in Appendix Table 37. The maximum Ag (680 ppb), Cd (2,380 ppb), Cr (1,860 ppb) and Cu (36,600 ppb) concentrations in Kemp's ridleys of the present study were within the range of those reported in loggerhead (Cd: 4 - 64,000 ppb, Cr: 200 - 6,800 ppb, Cu: 340 - 20,900 ppb) and green turtles (Ag: 1 - 9,300 ppb, Cd: 10 - 285,000 ppb, Cr: non-detected - 3,800 ppb, Cu: 76 - 340,000 ppb). However, the maximum Pb (11,000 ppb) and Zn (1,870,000 ppb) concentrations in ridleys were at least an order of magnitude higher than those for their loggerhead (Pb: non-detected – 5,530 ppb, Zn: 12,200 - 1,700,000 ppb) and green (Pb: below detection limit – 7,270 ppb, Zn: 3,300 - 439,000 ppb) counterparts. In addition, Hg level (5,400 ppb) in ridleys was about 2 times higher than that in greens (below detection limit - 2,760 ppb) but within the range of 0 - 7,500 ppb reported for loggerheads. These findings suggest that Hg, Pb and Zn concentrations in the present study may have the potential to cause adverse effects to Kemp's ridley health.

Recommendations

Knowledge of absorption, feeding and efflux rate of trace metal is critically important to understanding the transfer of these trace metals through the food chain and into Kemp's ridleys. Stable-isotope analyses may prove useful in providing knowledge on this transfer. More research is needed on trace metal metabolism and regulation in live ridleys to explain concentration differences between captive and wild ridleys.

Biopsy sampling of internal organs and muscle tissue is needed to provide additional information on trace metals (i.e. Cd and Cu) and their accumulation potential in live Kemp's ridleys. It also may provide evidence to assess the efficacy of using carapace tissue as a surrogate sample source for internal organs and tissue in monitoring certain trace metal concentrations in Kemp's ridleys. Also important is Day's (2003) suggestion that a laser ablation inductively coupled plasma mass spectrometer (ICPMS) be used to profile trace metal concentrations at different depth layers and locations on the carapace tissue.

Toxicity and bioavailability of trace metals in marine vertebrates are dependent on various factors including species, environmental parameters (i.e. salinity and temperature), season, speciation and interaction between metals. Speciation of trace metals in tissues of Kemp's ridleys, regardless of live or dead must be investigated. Different chemical forms (i.e. Hg and MeHg) may result in differences in absorption and metabolism across targeted tissues and, thus,

different toxicity risks to ridleys.

Other highly toxic trace metals, such as arsenic (As), must be characterized by the future studies on Kemp's ridleys. This is particularly true given higher As concentrations reported in loggerhead and green turtles (Storelli *et al.*, 1998a; Sakai *et al.*, 2000a). In addition, the essential element, selenium (Se), also must be a prime candidate for future trace metal studies in ridleys, since it could increase Ag deposition rate in body tissues and decrease the toxicity of Hg.

Future research must be aided by long-term, in-water monitoring that enables larger sample sizes, sex and a wider array of tissue types to be analyzed so as to provide a more complete assessment of trace metal loading in Kemp's ridleys. Long-term assessments would provide information prerequisite to strategic management of foraging areas and life history stages and, in doing so, could facilitate quicker population recovery.

Health Assessment

Very little is known about the immune system of sea turtles, especially in relation to marine pollution. It can be assumed that, when stressed, sea turtles decrease their ability to fight disease. Adrenal and hematological responses to stress have been studied in both captive and wild Kemp's ridley, olive ridleys, loggerhead and green sea turtles (Gregory *et al.*, 1996; Valverde *et al.,* 1999; Hoopes *et al.*, 2000; Gregory and Schmind, 2001). Data on immune function is only limited to studies of fibropapillomatosis in sea turtles (Aguirre *et al.*, 1995).

Keller (2003) reported contaminants (i.e. organochlorines) have been shown to suppress the immune system in loggerhead sea turtles. Although, no research has been conducted on effects of trace metals in sea turtles, it can be assumed that their immunological responses are similar to those of marine mammals and fish (Robert, 1997). The only corroborated research of trace metal and immune function in live Kemp's ridleys (Peden-Adams *et al*., 2003) targeted the relationship between hematology, blood chemistry and trace metals. A negative correlation detected between several metals in blood and carapace tissue and lymphocyte abundance suggested that immunosuppression occurs in the wild ridley population. This finding implies that certain trace metals have the potential to impair and reduce reproduction in sea turtles. However, the health impact of this suppression is yet not understood. Nonetheless, data generated in the aforementioned investigation suggest that exposure to low levels of environmental contaminants may alter sea turtle health and, in doing so, affect Kemp's ridley's recovery rate.

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APPENDIX

Appendix Table 1. Trace metal concentrations (ppb) in pelleted food (d.w.) fed to captive Kemp's ridleys in 2002.

Sample No.	Weight	Ag	Cd	Cr	Cu	Hg	Pb	Zn
1	0.111	42.0	42.6	287	4120	18.7	520.2	299000
2	0.111	18.1	34.0	200	4890	15.3	769.9	122000
3	0.179	26.1	40.0	196	5750	8.0	645.5	172000
4	0.164	32.7	41.5	130	2740	15.2	580.9	123000
5	0.225	15.2	43.3	226	4930	19.3	738.3	116000
6	0.206	20.6	40.2	256	5630	14.7	632.7	75600
7	0.285	15.5	39.4	194	4050	13.9	639.7	151000
8	0.299	14.9	38.0	159	3880	11.2	596.3	89800
9	0.348	68.9	42.6	307	4050	14.9	601.7	172000
10	0.325	12.0	41.0	245	4120	15.0	419.6	7640
Mean	0.225	26.6	40.3	220	4420	14.6	614	133000
S.D.	0.086	17.6	2.7	55.1	899	3.3	99.9	76200
Min.	0.111	12.0	34.0	130	2740	8.0	420	7640
Max.	0.348	68.9	43.3	307	5750	19.3	770	299000

Appendix Table 2. Size and trace metal concentrations (ppb) in captive Kemp's ridleys from 2001 and 2002.

Turtle no.	SCL	Weight	**Ag**		**Cd**		**Cr**		**Cu**	
2001	(cm)	(kg)	Blood	Carapace	Blood	Carapace	Blood	Carapace	Blood	Carapace
XXK 201	33.5	5.64	2.0	196	0.9	299	0.3	1.0	298	7270
XXK 202	31.2	5.16	5.4	161	1.0	92.5	0.5	1.0	276	6780
XXK 203	33.1	5.86	1.4	34.8	0.5	19.7	0.4	0.8	251	1050
XXK 204	32.1	5.44	2.9	58.8	0.7	28.1	1.4	0.6	323	1390
XXK 205	33.7	5.92	2.9	39.1	0.4	40.8	0.1	1.4	204	5150
XXK 206	33.2	6.1	4.0	236	1.2	110	1.3	0.8	394	7830
2002										
4-1	32.6	5.2	4.5	117	0.5	35.2	0.5	0.8	379	6430
4-2	32.2	5.2	1.7	159	0.4	44.4	0.1	1.0	338	16300
4-3	33.7	5.2	1.3	54.4	0.3	30.1	0.2	1.1	387	9170
4-5	31.0	4.6	6.0	43.4	0.8	41.8	0.3	0.7	390	7630
4-6	32.0	4.6	0.6	96.9	0.3	28.2	0.1	0.7	395	4880
4-7	31.2	4.6	1.8	58.8	0.4	35.3	9.3	0.7	406	5140
4-8	33.5	5.3	4.1	41.1	0.3	35.2	0.1	0.6	341	8760
4-9	31.9	5.3	1.2	86.0	0.1	33.5	0.1	1.0	311	6360
4-10	32.6	4.8	1.2	67.6	0.9	22.0	0.1	0.7	367	5560
4-11	32.8	5.2	1.7	71.7	0.4	33.5	0.1	0.9	357	6890
4-12	32.0	4.9	2.4	86.7	0.5	33.2	0.1	0.7	466	5690
4-13	32.0	4.9	2.4	36.2	0.6	30.9	0.3	1.0	400	13400
4-14	34.5	5.5	2.2	118	0.5	31.9	0.1	1.0	380	7310
5-1	32.5	4.9	1.8	32.4	0.9	36.4	0.8	0.8	436	6190
5-2	32.6	5.1	2.6	122	0.1	55.5	0.1	0.9	311	10400
5-3	32.4	5.0	2.2	64.4	0.4	41.0	0.5	0.5	456	5930
5-4	32.0	4.8	1.7	112	0.3	47.0	0.2	0.7	271	4360
5-5	31.6	4.4	1.0	107	1.5	63.3	0.1	0.9	423	4620
5-6	33.5	5.5	0.1	57.3	0.5	29.8	0.2	0.6	413	5190
5-7	32.8	5.0	0.6	28.7	0.3	36.1	0.3	0.8	429	7230
5-8	32.3	5.0	1.1	54.8	0.3	58.7	1.0	1.2	302	16200
5-9	32.8	5.1	2.9	83.6	0.1	66.3	0.4	1.0	282	7870
5-10	33.4	5.2	8.4	50.9	0.3	44.6	0.2	0.9	270	6110
5-11	32.8	4.9	2.9	76.7	0.4	48.1	0.1	0.9	327	4420
5-12	32.0	5.0	2.1	66.0	0.3	101	0.2	1.0	306	12300
5-13	28.1	3.3	9.8	39.4	1.7	33.2	0.5	0.6	388	7610
5-14	33.0	5.4	0.6	44.3	0.6	50.2	1.0	1.0	367	8650
Mean	32.4	5.1	2.7	81.8	0.6	52.6	0.6	0.9	353	7270
S.D.	1.2	0.5	2.1	49.2	0.4	49.1	1.6	0.2	63.5	3400
Min.	28.1	3.3	0.1	28.7	0.1	19.7	0.1	0.5	204	1050
Max.	34.5	6.1	9.8	236	1.7	299	9.3	1.4	466	16300

Appendix Table 2. Cont.

Turtle no.	SCL	Weight	**Hg**		**Pb**		**Zn**	
2001	(cm)	(kg)	Blood	Carapace	Blood	Carapace	Blood	Carapace
XXK 201	33.5	5.64	9.5	59.2	7.0	93.6	16700	280000
XXK 202	31.2	5.16	1.3	34.9	17.7	53.9	8220	119000
XXK 203	33.1	5.86	3.9	73.8	9.8	41.1	10700	233000
XXK 204	32.1	5.44	6.2	48.8	2.4	81.0	12690	94800
XXK 205	33.7	5.92	7.5	52.8	12.7	79.3	2940	59700
XXK 206	33.2	6.1	12.0	69.7	20.3	104	4810	682000
2002								
4-1	32.6	5.2	3.0	69.1	3.4	492	9550	202000
4-2	32.2	5.2	1.8	53.8	7.8	671	11900	820000
4-3	33.7	5.2	1.6	47.9	7.1	326	14400	169000
4-5	31.0	4.6	1.7	55.2	20.5	336	13900	296000
4-6	32.0	4.6	0.9	97.1	5.8	295	4100	341000
4-7	31.2	4.6	2.1	55.7	28.1	138	26500	260000
4-8	33.5	5.3	1.1	57.9	5.4	655	36700	136000
4-9	31.9	5.3	0.9	60.7	21.0	288	9100	174000
4-10	32.6	4.8	0.8	55.8	2.3	304	36000	371000
4-11	32.8	5.2	1.9	53.1	6.8	258	5340	580000
4-12	32.0	4.9	2.4	51.0	24.3	138	1110	479000
4-13	32.0	4.9	2.4	61.7	6.8	514	18400	408000
4-14	34.5	5.5	1.7	62.2	2.7	416	5630	424000
5-1	32.5	4.9	2.4	61.3	34.5	226	16900	1070000
5-2	32.6	5.1	1.6	55.0	13.7	468	9310	155000
5-3	32.4	5.0	1.4	54.7	7.5	386	7580	204000
5-4	32.0	4.8	1.0	51.2	2.0	184	10000	364000
5-5	31.6	4.4	0.9	61.1	8.4	180	9850	212000
5-6	33.5	5.5	1.3	88.0	27.7	291	5550	205000
5-7	32.8	5.0	3.5	71.2	7.0	274	13500	317000
5-8	32.3	5.0	2.4	55.4	6.5	807	14500	200000
5-9	32.8	5.1	1.2	55.2	15.7	293	11200	330000
5-10	33.4	5.2	2.0	62.3	11.3	121	11000	136000
5-11	32.8	4.9	2.5	68.1	12.4	116	33700	452000
5-12	32.0	5.0	1.9	39.6	23.8	671	14500	233000
5-13	28.1	3.3	2.9	77.2	20.5	281	8710	297000
5-14	33.0	5.4	2.3	70.2	7.9	425	16200	300000
Mean	32.4	5.1	2.7	60.3	12.4	303	13100	321000
S.D.	1.1	0.5	2.5	12.4	8.7	199	8800	214000
Min.	28.1	3.3	0.8	34.9	2.0	41.1	1110	59700
Max.	34.5	6.1	12.0	97.1	34.5	807	36700	1070000

Appendix Table 3. Range and mean trace metal concentrations (ppb) in blood (w.w.) and carapace tissue (d.w.) of captive Kemp's ridleys and their pelleted food (d.w.).

	2001-2002	2001	2002	Food
	n = 33	n = 6	n = 27	n = 10
Ag				
Blood	0.1 - 9.8	1.4 - 5.4	0.1 - 9.8	12.0 - 68.9
	2.7 ± 2.1	3.1 ± 1.4	2.6 ± 2.3	26.6 ± 17.6
Carapace	28.7 - 236	34.8 - 236	28.7 - 159	
	81.8 ± 49.2	121 ± 87.7	73.2 ± 32.8	
Cd				
Blood	0.1 - 1.7	0.4 - 1.2	0.1 - 1.7	34.0 - 43.3
	0.6 ± 0.4	0.8 ± 0.3	0.5 ± 0.4	40.3 ± 2.7
Carapace	19.7 - 299	19.7 - 299	22.0 - 101	
	52.6 ± 49.1	98.4 ± 105	42.5 ± 16.1	
Cr				
Blood	0.1 - 9.3	0.1 - 1.4	0.1 - 9.3	130 - 307
	0.6 ± 1.6	0.7 ± 0.5	0.6 ± 1.8	220 ± 55.1
Carapace	0.5 - 1.4	0.6 - 1.4	0.5 - 1.2	
	0.9 ± 0.2	0.9 ± 0.3	0.8 ± 0.2	
Cu				
Blood	204 - 466	204 - 394	270 - 466	2740 - 5750
	353 ± 63.5	291 ± 64.9	367 ± 55.5	4420 ± 900
Carapace	1050 - 16300	1050 - 7830	4360 - 16300	
	7270 ± 3400	4910 ± 3000	7800 ± 3300	
Hg				
Blood	6.8 – 12.0	1.3 - 12.0	0.8 - 3.5	8.0 - 19.3
	2.7 ± 2.5	6.7 ± 3.8	1.8 ± 0.7	14.6 ± 3.3
Carapace	34.9 – 97.1	34.9 - 73.8	39.6 - 97.1	
	60.3 ± 12.4	56.5 ± 14.3	61.2 ± 12.1	
Pb				
Blood	2.0 - 34.5	2.4 - 20.3	2.0 - 34.5	420 - 770
	12.4 ± 8.7	11.7 ± 6.7	12.6 ± 9.1	615 ± 99.9
Carapace	41.1 - 807	41.1 - 104	116 - 807	
	303 ± 199	75.4 ± 23.7	354 ± 184	
Zn				
Blood	1110 - 36700	2940 - 16700	1110 - 36700	7640 - 299000
	13100 ± 8810	9350 ± 5100	13900 ± 9300	133000 ± 76200
Carapace	59700 - 1070000	59700 - 682000	136000 - 1070000	
	321000 ± 214000	245000 ± 230000	338000 ± 210000	

Appendix Table 4. Correlation coefficients of trace metal concentrations in blood and carapace tissue of captive Kemp's ridleys from 2001 and 2002.

Blood	Pearson	Ag	Cd	Cr	Cu	Hg	Pb
	Cd	0.382*					
	Cr	-0.049	-0.005				
	Cu	-0.160	0.286	0.161			
	Hg	0.110	0.310	0.067	-0.174		
	Pb	0.148	0.158	0.329	0.217	0.015	
	Zn	-0.044	-0.032	0.250	-0.010	-0.172	-0.121
	Spearman	Ag	Cd	Cr	Cu	Hg	Pb
	Cd	0.181					
	Cr	0.162	0.327				
	Cu	-0.224	0.316	0.091			
	Hg	0.291	0.312	0.460**	-0.053		
	Pb	0.153	0.093	0.152	0.177	0.097	
	Zn	-0.064	-0.013	0.207	-0.068	0.034	-0.148
Carapace	Pearson	Ag	Cd	Cr	Cu	Hg	Pb
	Cd	0.607**					
	Cr	0.109	0.239				
	Cu	0.116	0.135	0.437*			
	Hg	-0.099	-0.165	-0.315	-0.239		
	Pb	-0.137	-0.145	0.140	0.810**	-0.075	
	Zn	0.215	-0.013	-0.068	0.199	0.104	0.032
	Spearman	Ag	Cd	Cr	Cu	Hg	Pb
	Cd	0.388*					
	Cr	0.136	0.399*				
	Cu	0.018	0.337	0.465*			
	Hg	-0.218	-0.227	-0.233	-0.144		
	Pb	-0.038	-0.018	0.123	0.695**	-0.014	
	Zn	0.147	-0.031	0.080	0.070	-0.006	0.070

* Correlation coefficient is significant at the 0.05 level

** Correlation coefficient is significant at the 0.01 level

Appendix Table 5. Mean trace metal concentrations (ppb) in blue crabs captured from Louisiana and Texas coastal waters.

Location	Metal			Reference
Calcasieu River	Ag	100		Ramelow *et al*., 1989
Neches River	Hg	411	d.w.	Barrera *et al*., 1995
	Zn	86000		
Galveston Bay	Ag	2800	d.w.	Park and Presley, 1997
	Cd	170		
	Cu	83500		
	Pb	12200		
	Zn	103000		
Lavaca Bay	Hg	1210	w.w.	Locarnini and Presley, 1996
		200	w.w.	Sager, 2002
Corpus Christi Bay	Cd	1360	d.w.	Barrera *et al*., 1995
	Cu	72500		
	Hg	145		
	Pb	2520		
	Zn	107000		
San Antonio Bay	Cd	100	d.w.	Sims and Presley, 1976
	Cu	54000		
	Pb	< 200		
	Zn	14000		

d.w.: dry weight, w.w.: wet weight

Appendix Table 6. Trace metal concentrations (ppb) in blood (w.w.) and carapace tissue (d.w.) of wild Kemp's ridleys captured from Texas during 2000-2002.

	SCL	Weight	**Ag**		**Cd**		**Cr**		**Cu**	
Turtle no.	(cm)	(kg)	Blood	Carapace	Blood	Carapace	Blood	Carapace	Blood	Carapace
SP01-5-1W	31.0	3.26	1.2	49.6	18.6	11.9	30.7	102	594	671
SP01-5-3W	29.0	3.54	0.8	2.7	9.5	17.0	23.4	18.4	466	255
SP01-7-1W	33.3	7.84	1.5	10.6	8.5	132	5.2	387	237	1910
PL01-7-1W	41.7	10.26	1.1	41.1	17.1	138	19.0	298	262	3050
PL01-7-2W	43.4	12.0	0.3	70.1	8.2	188	2.1	353	176	2200
SP01-8-1W	49.8	16.6	0.2	101	7.2	106	4.6	93.1	573	1670
PL01-8-1W	33.5	4.93	1.4	1.2	9.9	114	28.9	227	542	28500
PL02-5-1W	43.0	6.70	4.0	14.2	22.0	116	0.5	138	434	1720
PL02-5-2W	27.9	1.39	2.0	87.9	3.5	117	3.7	281	351	1920
PL02-5-3W	29.1	1.77	4.9	62.5	17.9	20.5	0.6	128	369	854
PL02-5-4W	32.8	3.04	5.1	30.0	17.8	149	0.3	167	278	2560
PL02-5-5W	32.8	3.17	2.6	19.4	9.3	62.0	196	141	272	1100
SP02-6-1W	33.3	6.18	2.9	38.9	9.6	167	0.8	117	380	1610
SP02-6-2W	34.6	5.24	2.4	104	2.6	215	203	365	406	2580
SP02-6-3W	31.7	4.86	5.7	75.3	5.2	137	1.2	24.5	441	1070
SP02-6-4W	31.1	4.88	2.9	55.7	4.8	60.0	3.0	43.5	495	15500
SP02-6-5W	35.6	5.94	8.6	127.4	6.6	96.2	187	58.6	408	1150
SP02-6-6W	34.4	3.24	0.8	45.1	10.6	126	69.6	114	393	1210
PL02-6-1R	46.7	14.0	3.5	15.9	17.4	729	0.8	243	504	1690
PL02-6-2W	33.7	5.64	3.3	25.4	9.1	281	0.2	243	433	1500
PL02-6-3R	36.9	6.62	37.0	123	5.2	399	0.3	446	373	1810
PL02-6-4W	33.5	4.94	2.3	78.2	11.1	162	2.0	875	398	2240
PL02-7-1W	32.0	4.52	2.8	3.9	19.5	62.6	5.9	201	417	1120
PL02-7-2W	35.0	5.68	9.6	2.3	6.9	19.5	19.9	90.2	291	5620
SP02-7-1W	35.6	5.43	1.0	35.6	7.9	84.8	0.3	101	367	1350
SP02-7-2h	52.8	20.0	5.5	37.5	1.7	100	0.2	55.4	429	1710
SP02-7-3W	36.1	4.64	6.0	7.8	6.6	51.1	0.3	27.9	368	1420
SP02-7-4W	38.1	5.38	0.4	104	9.2	200	0.5	190	497	1350
PL02-8-1W	28.5	1.18	1.6	45.5	20.2	28.3	0.3	161	768	1500
PL02-8-4W	33.4	2.92	5.3	16.8	17.1	75.8	0.6	254	366	3250
Mean	35.7	6.2	4.2	47.7	10.7	139	27.0	198	410	3140
S.D.	6.1	4.3	6.6	37.8	5.8	139	58.9	172	118	5490
Min.	27.9	1.2	0.2	1.2	1.7	11.9	0.2	18.4	176	255
Max.	52.8	20.0	37.0	127	22.0	729	203	875	768	28500

Appendix Table 6. Cont.

	SCL	Weight	**Hg**		**Pb**		**Zn**	
Turtle no.	(cm)	(kg)	Blood	Carapace	Blood	Carapace	Blood	Carapace
SP01-5-1W	31.0	3.26	13.1	452	10.3	100	9830	274000
SP01-5-3W	29.0	3.54	21.8	751	53.6	524	9550	71500
SP01-7-1W	33.3	7.84	7.3	437	27.3	3670	11300	158000
PL01-7-1W	41.7	10.26	56.4	430	19.7	134	21000	245000
PL01-7-2W	43.4	11.95	20.0	566	18.3	574	10800	93300
SP01-8-1W	49.8	16.26	15.1	760	167	1260	16000	274000
PL01-8-1W	33.5	4.93	49.7	5340	27.3	1090	15500	356000
PL02-5-1W	43.0	6.70	9.0	1920	26.4	783	4790	226000
PL02-5-2W	27.9	1.39	27.2	2300	26.6	624	1330	633000
PL02-5-3W	29.1	1.77	6.7	839	75.1	672	8800	471000
PL02-5-4W	32.8	3.04	16.4	1920	42.2	2000	3890	1870000
PL02-5-5W	32.8	3.17	15.4	1960	15.4	375	3280	13700
SP02-6-1W	33.3	6.18	16.1	2290	3.7	1280	4900	348000
SP02-6-2W	34.6	5.24	10.2	1710	2.6	2900	4680	104000
SP02-6-3W	31.7	4.86	7.7	1310	24.3	344	3170	158000
SP02-6-4W	31.1	4.88	13.7	846	16.2	379	2760	429000
SP02-6-5W	35.6	5.94	11.6	1220	52.0	523	3330	463000
SP02-6-6W	34.4	3.24	9.1	933	12.9	973	2460	184000
PL02-6-1R	46.7	13.98	11.2	1970	33.8	899	5800	282000
PL02-6-2W	33.7	5.64	6.7	1150	11.2	1020	3090	147000
PL02-6-3R	36.9	6.62	61.7	3580	8.6	788	2520	140000
PL02-6-4W	33.5	4.94	36.2	2800	16.7	1800	3790	235000
PL02-7-1W	32.0	4.52	16.5	1550	8.6	210	4070	313000
PL02-7-2W	35.0	5.68	15.8	2760	3.4	701	7540	346000
SP02-7-1W	35.6	5.43	9.1	2380	53.6	1340	5970	91800
SP02-7-2h	52.8	20.04	13.9	1130	61.5	713	3720	291000
SP02-7-3W	36.1	4.64	9.9	1780	12.7	855	7350	178000
SP02-7-4W	38.1	5.38	6.4	867	47.6	1120	7140	342000
PL02-8-1W	28.5	1.18	21.5	1160	18.0	1370	5750	183000
PL02-8-4W	33.4	2.92	21.2	1110	11.8	545	7240	404000
Mean	35.7	6.2	18.6	1610	30.3	985	6710	311000
S.D.	6.1	4.3	14.4	1060	31.9	778	4560	325000
Min.	27.9	1.2	6.4	430	2.6	100	1330	13700
Max.	52.8	20.0	61.7	5340	167	3670	21000	1870000

Appendix Table 7. Range and mean trace metal concentrations (ppb) in blood (w.w.) and carapace tissue (d.w.) of wild Kemp's ridleys captured from Texas study sites.

Blood	Texas combined	Sabine Pass	Lavaca Bay
Ag	0.2 - 37.0 4.2 ± 6.6	0.2 - 8.6 2.9 ± 2.6	0.3 - 37.0 5.4 ± 8.7
Cd	1.7 - 22.0 10.7 ± 5.8	1.7 - 18.6 7.8 ± 4.1	3.5 - 22.0 13.3 ± 5.9
Cr	0.2 - 203 27.0 ± 58.9	0.2 - 203 37.8 ± 69.3	0.2 - 196 17.5 ± 48.3
Cu	176 - 768 410 ± 118	237 - 594 433 ± 90.9	176 - 768 390 ± 138
Hg	6.4 - 61.7 18.6 ± 14.4	6.4 - 21.8 11.8 ± 4.2	6.7 - 61.7 24.5 ± 17.4
Pb	2.6 - 167 30.3 ± 31.9	2.6 - 167 38.9 ± 42.2	3.4 - 75.1 22.7 ± 17.2
Zn	1330 - 21000 6710 ± 4560	2450 - 16000 6580 ± 3920	1330 - 21000 6830 ± 5190
Carapace	**Texas combined**	**Sabine Pass**	**Lavaca Bay**
Ag	1.2 - 127 47.7 ± 37.8	2.7 - 127 56.8 ± 39.7	1.2 - 123 39.8 ± 35.4
Cd	11.9 - 729 139 ± 139	11.9 - 215 107 ± 61.3	19.5 - 719 166 ± 180
Cr	18.4 - 875 198 ± 172	18.4 - 387 121 ± 118	90.2 - 875 265 ± 187
Cu	255 - 28500 3140 ± 5490	255 - 15500 2390 ± 3820	854 - 28500 3790 ± 6680
Hg	430 - 5340 1610 ± 1060	437 - 2380 1200 ± 622	430 - 5340 1960 ± 1240
Pb	100 - 3670 986 ± 779	100 - 3670 1140 ± 998	134 - 2000 850 ± 518
Zn	13700 - 1870000 311000 ± 326000	71500 - 463000 240000 ± 125000	13700 - 1870000 373000 ± 428000

Appendix Table 8. Correlation coefficients of trace metal concentrations in blood and carapace tissue of wild Kemp's ridleys captured from Texas study sites combined.

Blood	Spearman	Ag	Cd	Cr	Cu	Hg	Pb
	Cd	-0.188					
	Cr	-0.321	-0.033				
	Cu	-0.143	0.152	-0.033			
	Hg	-0.068	0.082	0.158	-0.138		
	Pb	-0.245	-0.005	-0.177	0.153	-0.178	
	Zn	-0.407*	0.307	0.148	-0.012	0.073	0.245
Carapace	Pearson	Ag	Cd	Cr	Cu	Hg	Pb
	Cd	0.153					
	Cr	0.240	0.341				
	Cu	-0.210	-0.075	-0.005			
	Hg	-0.096	0.200	0.264	0.569**		
	Pb	0.048	0.146	0.411*	-0.017	0.072	
	Zn	-0.002	-0.035	-0.073	0.091	0.086	0.119
	Spearman	Ag	Cd	Cr	Cu	Hg	Pb
	Cd	0.339					
	Cr	0.151	0.617**				
	Cu	-0.072	0.309	0.469**			
	Hg	-0.129	0.200	0.142	0.235		
	Pb	0.034	0.422*	0.313	0.277	0.296	
	Zn	0.068	-0.132	-0.079	0.281	0.083	-0.099

* Correlation coefficient is significant at the 0.05 level
** Correlation coefficient is significant at the 0.01 level

Appendix Table 9. Correlation coefficients of trace metal concentrations in blood and carapace tissue of wild Kemp's ridleys captured from Sabine Pass, Texas.

Blood	Pearson	Ag	Cd	Cr	Cu	Hg	Pb
	Cd	-0.450					
	Cr	0.311	-0.190				
	Cu	-0.233	0.314				
	Hg	-0.098	0.096	-0.073	0.376		
	Pb	-0.194	-0.142	-0.088	0.387	0.256	
	Zn	-0.510	0.359	-0.301	0.253	0.231	0.622*
Carapace	Pearson	Ag	Cd	Cr	Cu	Hg	Pb
	Cd	0.498					
	Cr	0.118	0.615*				
	Cu	0.032	-0.119	-0.091			
	Hg	-0.033	0.279	-0.094	-0.121		
	Pb	-0.056	0.559*	0.924**	-0.113	0.054	
	Zn	0.475	0.026	-0.251	0.431	-0.157	-0.373

* Correlation coefficient is significant at the 0.05 level
** Correlation coefficient is significant at the 0.01 level

Table 10. Correlation coefficients of trace metal concentrations in blood and carapace tissue of wild Kemp's ridleys captured from Lavaca Bay, Texas.

Blood	Pearson	Ag	Cd	Cr	Cu	Hg	Pb
	Cd	-0.310					
	Cr	-0.106	-0.166				
	Cu	-0.084	0.303	-0.242			
	Hg	0.441	-0.267	-0.049	-0.015		
	Pb	-0.189	0.414	-0.110	-0.009	-0.235	
	Zn	-0.264	0.215	-0.032	-0.104	0.435	0.163
Carapace	Pearson	Ag	Cd	Cr	Cu	Hg	Pb
	Cd	0.039					
	Cr	0.354	0.283				
	Cu	-0.337	-0.072	-0.019			
	Hg	-0.128	0.183	0.276	0.729**		
	Pb	-0.052	0.090	0.286	0.128	0.247	
	Zn	-0.053	-0.120	-0.212	0.003	-0.020	0.537*

* Correlation coefficient is significant at the 0.05 level

** Correlation coefficient is significant at the 0.01 level

Appendix Table 11. Trace metal concentrations (ppb) in digestive gland (DG, w.w.) and muscle tissue (M, w.w.) of blue crabs captured from Texas study sites in 2001.

Location	Sex	Length	Weight	**Ag**		**Cd**		**Cr**		**Cu**	
Sabine Pass		(mm)	(g)	DG	M	DG	M	DG	M	DG	M
1	F	158	158	447	220	65.2	8.2	1.9	1.7	4550	5810
2	F	150	143	256	147	172	3.7	8.8	67.4	18900	6780
3	F	120	73.5	413	58.3	102	5.8	6.0	9.4	22400	5100
4	F(S)	128	138	663	236	136	1.6	4.3	4.1	18400	4010
5	M	152	131	262	83.7	442	32.3	1.2	3.9	9240	3810
6	F	105	56.6	364	268	77.5	22.9	3.9	8.5	14500	14900
7	M	131	79.5	648	244	253	31.7	4.8	20.3	33600	4870
8	F	100	34.8	746	186	128	88.7	34.6	5.4	27000	12900
9	M	106	53.0	738	334	159	41.5	26.6	4.6	32100	17300
10	F	76.0	19.9	248	158	256	100	6.1	1.5	10300	5050
Lavaca Bay											
1	F	115	75.6	239	140	63.7	8.7	5.5	6.2	15500	6700
2	M	179	297	525	77.1	186	39.1	21.1	0.5	31600	8790
3	M	142	130	566	145	104	3.9	4.0	0.4	10500	5660
4	F(S)	122	90.6	1660	173	222	11.0	28.2	4.1	32500	4400
5	F(S)	136	115	1160	440	123	207	7.8	10.4	11200	12600
Mean		128	106	596	194	166	40.4	11.0	9.9	19500	7910
S.D.		26.2	67.5	387	101	98.7	55.0	10.9	16.7	9800	4360
Min.		76.0	19.9	239	58.3	63.7	1.6	1.2	0.4	4550	3810
Max.		179	297	1660	440	442	207	34.6	67.4	33600	17300

Location	Sex	Length	Weight	**Hg**		**Pb**		**Zn**	
Sabine Pass		(mm)	(g)	DG	M	DG	M	DG	M
1	F	158	158	7.6	17.3	114	10.8	31800	22600
2	F	150	143	7.9	7.5	67.0	19.9	23700	11500
3	F	120	73.5	5.7	49.9	41.4	9.1	42900	24000
4	F(S)	128	138	10.5	24.7	56.7	89.6	35800	16500
5	M	152	131	34.3	54.9	186	40.6	146000	30500
6	F	105	56.6	23.5	31.7	75.6	18.2	19500	36700
7	M	131	79.5	5.6	30.6	124	123	80000	61300
8	F	100	34.8	41.9	27.1	188	21.4	46100	196000
9	M	106	53.0	15.5	60.1	82.0	38.5	29600	50300
10	F	76.0	19.9	9.1	6.6	32.6	61.5	47600	45300
Lavaca Bay									
1	F	115	75.6	20.5	64.8	36.0	108	23400	82700
2	M	179	297	5.5	310	12.3	11.1	60600	31600
3	M	142	130	34.5	89.6	45.8	11.4	40200	10100
4	F(S)	122	90.6	58.0	83.0	124	65.8	38000	50700
5	F(S)	136	115	9.0	11.9	38.8	35.5	19000	95800
Mean		128	106	19.3	58.0	81.5	44.3	45600	51000
S.D.		26.2	67.5	16.1	74.6	54.5	37.4	32200	47200
Min.		76.0	19.9	5.5	6.6	12.3	9.1	19000	10100
Max.		179	297	58.0	310	188	123	146000	196000

F: female; M: male; S: sponge female

Table 12. Correlation coefficients of trace metal concentrations in digestive gland and muscle tissue of blue crabs captured from Texas study sites combined.

Digestive gland	Pearson	Ag	Cd	Cr	Cu	Hg	Pb
	Cd	-0.021					
	Cr	0.553*	-0.023				
	Cu	0.438	0.095	0.708**			
	Hg	0.489	0.196	0.465	0.127		
	Pb	0.171	0.455	0.276	0.109	0.559*	
	Zn	-0.204	0.870**	-0.151	0.007	0.162	0.538*
Muscle tissue	Pearson	Ag	Cd	Cr	Cu	Hg	Pb
	Cd	0.588*					
	Cr	-0.017	-0.126				
	Cu	0.599*	0.41	-0.048			
	Hg	-0.404	-0.16	-0.282	0.013		
	Pb	0.142	-0.06	-0.028	-0.329	-0.213	
	Zn	0.262	0.540*	-0.154	0.432	-0.154	0.111

* Correlation coefficient is significant at the 0.05 level
** Correlation coefficient is significant at the 0.01 level

Table 13. Correlation coefficients of trace metal concentrations in digestive gland and muscle tissue of blue crabs captured from Sabine Pass, Texas.

Digestive gland	Pearson	Ag	Cd	Cr	Cu	Hg	Pb
	Cd	-0.327					
	Cr	0.641*	-0.216				
	Cu	0.716*	-0.096	0.595			
	Hg	0.150	0.266	0.499	-0.013		
	Pb	0.252	0.400	0.320	0.063	0.763*	
	Zn	-0.200	0.900**	-0.248	-0.118	0.381	0.632
Muscle tissue	Pearson	Ag	Cd	Cr	Cu	Hg	Pb
	Cd	0.027					
	Cr	-0.158	-0.344				
	Cu	0.636*	0.245	-0.103			
	Hg	0.021	-0.156	-0.383	0.307		
	Pb	0.283	0.091	-0.071	-0.356	-0.087	
	Zn	0.096	0.682*	-0.220	0.412	0.008	-0.048

* Correlation coefficient is significant at the 0.05 level
** Correlation coefficient is significant at the 0.01 level

Table 14. Correlation coefficients of trace metal concentrations in digestive gland and muscle tissue blue crabs captured from Lavaca Bay, Texas.

Digestive gland	Pearson	Ag	Cd	Cr	Cu	Hg	Pb
	Cd	0.704					
	Cr	0.618	0.952*				
	Cu	0.363	0.860	0.956*			
	Hg	0.573	0.386	0.407	0.275		
	Pb	0.806	0.533	0.558	0.371	0.927*	
	Zn	-0.139	0.575	0.533	0.665	-0.033	-0.156
Muscle tissue	Pearson	Ag	Cd	Cr	Cu	Hg	Pb
	Cd	-0.670					
	Cr	0.922*	0.761				
	Cu	0.859	0.919*	0.602			
	Hg	0.716	-0.345	-0.704	-0.058		
	Pb	-0.024	-0.212	0.459	-0.276	-0.454	
	Zn	0.679	0.634	0.953*	0.556	-0.547	0.614

* Correlation coefficient is significant at the 0.05 level
** Correlation coefficient is significant at the 0.01 level

Table 15. Comparison of trace metal concentrations (ppb) in Kemp's ridleys and blue crabs from Sabine Pass, Texas.

	Ag	Cd	Cr	Cu	Hg	Pb	Zn
Kemp's ridleys							
Carapace tissue (w.w.)	56.8	107[b]	121[ab]	2390	1200[ab]	1140[ab]	240000[ab]
Blue crab (w.w.)							
Digestive gland	478.3[a]	179[a]	9.8	19100[a]	16.2	96.6	50300
Muscle tissue	194[b]	33.6	12.7	8050[b]	31.0	43.3	49500

[a] significant difference between digestive gland of blue crabs and carapace tissue of ridleys;
[b] significant difference between muscle tissue of blue crabs and carapace tissue of ridleys

Table 16. Comparison of trace metal concentrations (ppb) in Kemp's ridleys and blue crabs from Lavaca Bay, Texas.

	Ag	Cd	Cr	Cu	Hg	Pb	Zn
Kemp's ridleys							
Carapace tissue (w.w.)	39.8	166	265[ab]	3790	1960[ab]	850[ab]	373000
Blue crab (w.w.)							
Digestive gland	831[a]	140	13.3	20300[a]	25.5	51.3	36200
Muscle tissue	195[b]	53.9	4.3	7630[b]	112	46.4	54200

[a] significant difference between digestive gland of blue crabs and carapace tissue of ridleys;
[b] significant difference between muscle tissue of blue crabs and carapace tissue of ridleys

Appendix Table 17. Trace metal concentrations (ppb) in blood (w.w.) and carapace tissue (d.w.) of wild Kemp's ridleys captured from Louisiana during 2000-2002.

	SCL	Weight	**Ag**		**Cd**		**Cr**		**Cu**	
Turtle no.	(cm)	(kg)	Blood	Carapace	Blood	Carapace	Blood	Carapace	Blood	Carapace
C00-5-2W	48.7	14.46	-	13.5	-	130	-	1180	-	4520
C00-5-3W	36.1	6.73	-	303	-	186	-	610	-	4360
C00-6-1W	37.0	6.7	24.0	55.3	9.8	243	3.0	500	407	3550
C00-6-2W	33.3	5.38	19.4	124	16.3	96.9	4.0	156	75.0	1880
C00-6-3W	25.4	2.23	24.0	524	13.8	69.1	2.6	393	782	1550
C00-6-4R	35.2	6.27	20.2	203	3.4	30.3	3.6	186	221	2950
C00-6-5W	41.9	9.52	23.4	26.5	4.1	68.1	2.7	140	1080	1450
C00-6-6W	38.5	7.11	15.0	253	5.7	116	3.0	194	223	2060
C00-6-7W	55.6	21.45	13.8	215	2.9	108	4.5	240	149	1470
C00-6-8W	36.6	7.07	21.7	240	9.0	105	3.6	539	164	2170
C006-9W	36.3	6.78	18.4	231	3.6	82.7	14.4	249	820	2700
C00-7-1W	37.0	7.73	23.3	677	3.0	266	6.8	1870	172	8380
C00-7-2R	36.1	7.03	24.3	242	6.0	52.0	3.1	471	918	2370
C00-7-3W	64.2	31.45	9.9	578	2.8	121	4.6	1240	219	36600
C00-7-4W	35.4	6.57	24.7	224	5.2	64.6	3.0	253	93.6	1500
M00-7-1W	46.1	12.67	16.9	582	7.1	65.6	2.6	305	209	2170
C00-8-1W	45.0	11.49	2.5	183	1.4	308	3.1	920	176	4710
C00-8-2W	37.7	7.21	21.4	112	7.1	115	2.6	11.3	351	4960
C00-8-3W	40.3	8.09	14.9	125	5.3	129	2.4	43.4	105	1630
C00-8-4W	30.3	3.82	7.9	87.5	9.9	71.7	3.0	159	162	1290
C01-5-1W	41.3	9.92	1.1	7.2	7.7	76.3	5.6	7.0	163	815
C01-5-2W	30.2	3.61	0.7	18.9	15.8	28.3	24.2	55.3	455	385
C01-5-3W	33.8	4.88	0.1	20.4	4.3	20.2	49.8	34.7	247	378
C01-6-1W	31.2	4.29	1.0	105	23.9	167	60.5	327	203	3690
C01-6-2W	30.1	3.85	0.5	313	26.1	295	27.4	213	438	4540
C01-6-3W	27.8	3.28	-	301	-	234	-	456	-	4220
C01-6-4W	31.6	4.65	3.5	574	8.3	390	27.9	334	363	9120
C01-6-5W	42.1	11.27	0.5	79.9	17.6	85.4	24.7	42.7	477	2340
C01-6-6W	42.8	11.24	14.3	56.8	7.3	56.5	21.3	25.4	168	9040
C01-7-1W	32.2	4.74	0.1	115	33.3	72.6	6.3	175	145	1110
C01-7-2W	66.3	37.06	1.0	13.7	30.9	126	18.0	114	232	2020
C01-7-3W	29.9	3.97	0.1	99.7	27.1	157	54.8	449	470	2410
C01-7-5W	28.8	3.85	4.0	25.2	14.2	138	23.8	265	213	1970
C01-7-6W	31.9	5.13	15.4	53.7	15.3	114	31.6	133	431	752
C01-7-7W	22.5	1.99	0.5	42.7	10.6	82.6	23.2	262	325	1040
C01-8-1W	34.8	5.33	2.2	70.2	17.8	169	21.7	112	173	2750
C01-8-2W	35.2	5.84	3.3	97.2	16.4	99.8	11.0	332	162	1530
C01-8-3W	37.1	6.62	1.2	47.3	12.5	94.3	1.5	91.1	158	1440
C02-5-1W	32.1	NT	4.7	211	7.0	877	1.3	1030	381	7770
C02-5-2W	37.4	NT	4.0	33.3	2.7	171	201	143	425	2450
C02-5-3W	31.5	NT	2.8	11.8	9.1	61.8	1.6	79.2	354	3360
C02-5-4W	31.7	NT	3.4	10.4	4.2	59.0	0.4	23.8	354	396
C02-6-1W	42.9	10.25	0.4	53.9	14.1	97.4	5.3	204	557	1700
C02-6-2W	25.7	3.04	4.1	61.7	14.5	66.7	0.2	120	377	1300
C02-6-3W	32.5	4.56	6.4	303	6.1	143	0.2	190	424	2020
C02-6-4W	36.4	6.46	4.7	69.1	10.4	179	2.0	301	341	1990

NT: not measured; -: no sample

Appendix Table 17. Cont.

	SCL	Weight	**Ag**		**Cd**		**Cr**		**Cu**	
Turtle no.	(cm)	(kg)	Blood	Carapace	Blood	Carapace	Blood	Carapace	Blood	Carapace
C02-7-1W	35.4	4.52	0.7	56.7	6.2	192	0.3	805	583	3280
C02-7-2W	32.5	2.47	3.7	49.8	7.4	222	0.4	1110	451	3770
C02-7-3W	23.2	1.52	2.6	183	6.9	187	0.5	672	437	3230
C02-7-4W	39.9	6.67	3.9	37.1	7.6	46.7	0.8	236	609	1510
C02-7-5W	32.0	2.31	6.5	75.8	7.8	97.1	0.1	474	451	3900
C02-7-6W	32.4	3.11	4.7	92.4	16.7	32.5	1.7	83.1	477	301
C02-7-7W	24.3	1.82	3.0	41.5	7.9	96.5	4.6	148	342	1290
C02-7-8W	35.9	6.12	2.6	47.6	8.2	94.4	0.4	135	485	735
C02-7-9W	37.9	6.53	3.4	5.8	48.9	42.1	5.7	80.6	394	535
C02-7-10W	35.8	6.03	3.5	50.2	7.9	56.2	7.6	242	446	1680
C02-7-11W	38.7	7.30	3.9	203	8.3	65.7	0.2	5.5	332	1010
C02-7-12W	36.2	6.07	0.7	20.6	10.1	87.8	3.7	139	429	2140
C02-7-13W	32.1	4.75	3.2	38.9	7.2	171	1.0	189	405	1900
C02-7-14W	39.0	7.11	2.0	38.9	9.8	55.5	0.6	129	512	452
C02-7-15W	39.7	8.34	0.3	71.2	5.4	24.1	1.7	14.8	221	592
Mean	36.3	7.4	7.8	144	11.0	130	12.9	322	361	3100
S.D.	8.0	6.2	8.3	160	8.6	123	28.6	359	203	4800
Min.	22.5	1.5	0.1	5.8	1.4	20.2	0.1	5.5	75.0	301
Max.	66.3	37.1	24.7	677	48.9	877	201	1870	1080	36600

Appendix Table 17. Cont.

	SCL	Weight	Hg		Pb		Zn	
Turtle no.	(cm)	(kg)	Blood	Carapace	Blood	Carapace	Blood	Carapace
C00-5-2W	48.7	14.5	-	236	-	1640	-	290000
C00-5-3W	36.1	6.73	-	1200	-	2890	-	205000
C00-6-1W	37.0	6.7	6.1	2150	39.3	1080	2480	29500
C00-6-2W	33.3	5.38	4.5	220	20.4	406	2120	8070
C00-6-3W	25.4	2.23	3.6	115	19.0	550	1060	163000
C00-6-4R	35.2	6.27	3.2	432	12.9	210	454	147000
C00-6-5W	41.9	9.52	14.4	373	16.8	475	1090	36400
C00-6-6W	38.5	7.11	9.3	228	20.5	401	401	37300
C00-6-7W	55.6	21.5	30.6	785	15.8	546	350	121000
C00-6-8W	36.6	7.07	21.8	316	15.1	1430	1580	208000
C006-9W	36.3	6.78	15.1	294	14.5	1520	2180	58600
C00-7-1W	37.0	7.73	5.7	291	12.7	1460	2010	42600
C00-7-2R	36.1	7.03	13.3	486	16.2	549	2830	51300
C00-7-3W	64.2	31.5	10.6	1270	22.5	804	1950	154000
C00-7-4W	35.4	6.57	14.5	342	17.1	309	1880	36100
M00-7-1W	46.1	12.7	5.4	448	9.2	489	1590	98700
C00-8-1W	45.0	11.5	18.9	540	12.8	2620	3290	181000
C00-8-2W	37.7	7.21	9.7	421	10.4	1990	1550	49900
C00-8-3W	40.3	8.09	11.3	524	12.3	104	1220	252000
C00-8-4W	30.3	3.82	11.5	487	16.3	147	1270	420000
C01-5-1W	41.3	9.92	20.5	368	18.9	247	12600	126000
C01-5-2W	30.2	3.61	6.6	835	29.8	77.3	9120	64300
C01-5-3W	33.8	4.88	6.0	1100	32.0	278	2560	102000
C01-6-1W	31.2	4.29	12.5	1100	33.0	349	12400	206000
C01-6-2W	30.1	3.85	6.3	857	30.5	1150	9450	279000
C01-6-3W	27.8	3.28	-	488	-	521	-	238000
C01-6-4W	31.6	4.65	18.9	2000	14.9	901	8650	950000
C01-6-5W	42.1	11.3	10.0	1160	30.6	229	18000	311000
C01-6-6W	42.8	11.2	10.4	976	16.9	114	7540	241000
C01-7-1W	32.2	4.74	5.8	1760	27.1	1220	7550	564000
C01-7-2W	66.3	37.1	179	3180	23.3	2320	18500	237000
C01-7-3W	29.9	3.97	9.0	1340	11.6	532	10500	117000
C01-7-5W	28.8	3.85	5.1	1220	1.2	764	7220	516000
C01-7-6W	31.9	5.13	4.3	1140	47.1	269	5880	277000
C01-7-7W	22.5	1.99	4.2	950	13.4	503	5950	628000
C01-8-1W	34.8	5.33	8.6	1520	21.2	622	13800	141000
C01-8-2W	35.2	5.84	10.4	1630	8.9	270	10900	851000
C01-8-3W	37.1	6.62	10.1	1140	8.8	376	9660	248000
C02-5-1W	32.1	NT	11.8	2000	14.1	3190	3190	476000
C02-5-2W	37.4	NT	44.9	2490	14.5	826	6510	1340000
C02-5-3W	31.5	NT	15.5	1360	33.3	219	3390	558000
C02-5-4W	31.7	NT	5.8	336	39.2	50.6	3390	71300
C02-6-1W	42.9	10.3	17.2	972	11.7	1630	4060	361000
C02-6-2W	25.7	3.04	16.8	1710	48.7	1490	3590	134000
C02-6-3W	32.5	4.56	6.5	616	15.3	11000	3300	1450000
C02-6-4W	36.4	6.46	4.3	504	6.4	1670	5770	327000

Appendix Table 17. Cont.

	SCL	Weight	**Hg**		**Pb**		**Zn**	
Turtle no.	(cm)	(kg)	Blood	Carapace	Blood	Carapace	Blood	Carapace
C02-7-1W	35.4	4.52	25.8	1260	13.3	3190	9370	480000
C02-7-2W	32.5	2.47	5.8	991	145	4000	3370	584000
C02-7-3W	23.2	1.52	6.9	874	0.9	1810	6460	315000
C02-7-4W	39.9	6.67	6.7	1140	31.3	651	5210	352000
C02-7-5W	32.0	2.31	12.0	2050	49.0	6830	6080	516000
C02-7-6W	32.4	3.11	6.9	1350	73.0	727	5250	531000
C02-7-7W	24.3	1.82	3.6	1360	73.6	554	3410	884000
C02-7-8W	35.9	6.12	21.1	1340	37.6	1270	5380	370000
C02-7-9W	37.9	6.53	9.0	1110	126	38.7	4980	528000
C02-7-10W	35.8	6.03	13.8	2210	42.9	1970	6450	374000
C02-7-11W	38.7	7.30	3.7	1690	3.8	652	3070	327000
C02-7-12W	36.2	6.07	11.4	1920	33.5	682	4360	572000
C02-7-13W	32.1	4.75	22.7	3570	35.9	800	10200	318000
C02-7-14W	39.0	7.11	0.6	2040	55.7	394	5290	243000
C02-7-15W	39.7	8.34	11.8	1460	57.0	190	3170	262000
Mean	36.3	7.4	14.2	1120	28.2	1220	5430	329000
S.D.	8.0	6.2	23.3	734	26.2	1720	4170	294000
Min.	22.5	1.5	0.6	115	0.9	38.7	350	8070
Max.	66.3	37.1	179	3570	145	11000	18500	1450000

Appendix Table 18. Correlation coefficients of trace metal concentrations in blood and carapace tissue of wild Kemp's ridleys captured from Louisiana.

Blood	Spearman	Ag	Cd	Cr	Cu	Hg	Pb
	Cd	-0.404**					
	Cr	-0.221	0.279*				
	Cu	-0.087	0.048	-0.172			
	Hg	-0.073	-0.212	0.009	0.016		
	Pb	-0.155	0.205	-0.078	0.271*	-0.009	
	Zn	-0.683**	0.570**	0.322**	0.127	0.139	0.097
Carapace	Pearson	Ag	Cd	Cr	Cu	Hg	Pb
	Cd	0.277*					
	Cr	0.488**	0.505**				
	Cu	0.505**	0.261*	0.530**			
	Hg	-0.287*	0.184	-0.134	0.025		
	Pb	0.105	0.266*	0.275*	0.060	0.084	
	Zn	-0.088	0.155	-0.066	-0.050	0.361**	0.450**
	Spearman	Ag	Cd	Cr	Cu	Hg	Pb
	Cd	0.320*					
	Cr	0.467**	0.583**				
	Cu	0.452**	0.660**	0.596**			
	Hg	-0.299*	0.083	-0.110	-0.038		
	Pb	0.234	0.574**	0.556**	0.476**	0.209	
	Zn	-0.263	0.122	0.030	-0.048	0.523**	0.227

* Correlation coefficient is significant at the 0.05 level

** Correlation coefficient is significant at the 0.01 level

Appendix Table 19. Trace metal concentrations (ppb) in digestive gland (DG, w.w.) and muscle tissue (M, w.w.) of blue crab captured from Louisiana in 2001.

	Sex	Length	Weight	**Ag**		**Cd**		**Cr**		**Cu**	
No.		(mm)	(g)	DG	M	DG	M	DG	M	DG	M
1	M	154	173	364	85.3	439	30.5	11.5	2.4	8150	5130
2	M	144	140	472	121	1070	10.5	17.5	0.5	21300	5330
3	M	130	99.3	179	66.6	398	5.8	7.4	0.0	15800	9480
4	F	175	192	51.5	66.9	196	2.4	3.1	1.1	2750	7470
5	F	98.0	44.3	440	238	870	47.5	25.8	2.2	21400	3320
6	M	160	227	44.0	38.7	137	7.1	21.8	BDL	2550	4130
7	M	146	203	482	48.0	344	4.8	6.0	BDL	30600	6990
8	M	112	56.8	375	51.4	1760	3.4	7.8	BDL	24100	5190
9	F	110	91.6	119	105	493	41.9	26.6	0.1	11300	9410
10	F	105	64.5	91.7	68.4	157	28.1	11.2	1.7	18700	6330
Mean		133	129	262	88.9	586	18.2	13.9	1.1	15700	6280
S.D.		26.3	66.6	181	58.1	512	17.2	8.5	1.0	9320	2080
Min.		98.0	44.3	44.0	38.7	137	2.4	3.1	BDL	2550	3320
Max.		175	227	482	238	1760	47.5	26.6	2.4	30600	9480

	Sex	Length	Weight	**Hg**		**Pb**		**Zn**	
		(mm)	(g)	DG	M	DG	M	DG	M
1	M	154	173	22.2	32.7	78.0	20.9	95800	73600
2	M	144	140	33.8	9.9	142	5.3	89400	85400
3	M	130	99.3	14.4	16.4	81.5	11.7	18100	118000
4	F	175	192	4.3	22.2	38.6	0.2	2920	55600
5	F	98.0	44.3	2.5	14.0	105	45.5	13900	1840000
6	M	160	227	9.8	19.5	46.7	24.0	4430	50000
7	M	146	203	16.9	22.4	31.2	2.4	170000	39000
8	M	112	56.8	26.0	13.2	151	22.8	22700	116000
9	F	110	91.6	6.3	27.2	39.9	22.7	35800	80500
10	F	105	64.5	5.2	16.5	69.4	5.2	23800	68100
Mean		133	129	14.1	19.4	78.2	16.1	47700	253000
S.D.		26.3	66.6	10.5	6.9	42.5	13.9	54000	558000
Min.		98.0	44.3	2.5	9.9	31.2	0.2	2920	39000
Max.		175	227	33.8	32.7	151	45.5	170000	1840000

BDL: below detection limit

Appendix Table 20. Correlation coefficients of trace metal concentrations in digestive gland and muscle tissue of blue crabs captured from Louisiana.

Digestive gland	Pearson	Ag	Cd	Cr	Cu	Hg	Pb
	Cd	0.597					
	Cr	-0.005	0.054				
	Cu	0.745*	0.507	-0.129			
	Hg	0.611	0.591	-0.232	0.359		
	Pb	0.544	0.873**	0.052	0.417	0.639*	
	Zn	0.668*	-0.007	-0.227	0.533	0.503	-0.104
Muscle tissue	Pearson	Ag	Cd	Cr	Cu	Hg	Pb
	Cd	0.727*					
	Cr	0.360	0.446				
	Cu	-0.364	-0.146	-0.805*			
	Hg	-0.238	0.260	0.227	0.282		
	Pb	0.647*	0.632	0.402	-0.499	-0.026	
	Zn	0.903**	0.598	0.458	-0.491	-0.292	0.754*
	Spearman	Ag	Cd	Cr	Cu	Hg	Pb
	Cd	0.721*					
	Cr	0.357	0.429				
	Cu	-0.127	-0.345	-0.857*			
	Hg	-0.152	0.127	0.250	0.224		
	Pb	0.139	0.503	0.143	-0.624	-0.212	
	Zn	0.564	0.358	-0.214	-0.091	-0.588	0.503

* Correlation coefficient is significant at the 0.05 level

** Correlation coefficient is significant at the 0.01 level

Appendix Table 21. Trace metal concentrations (ppb) in blood (w.w.) and carapace tissue (d.w.) of wild Kemp's ridleys captured from the southeast Atlantic during 2001-2002.

	SCL	Wt	Ag		Cd		Cr		Cu	
Turtle no.	(cm)	(kg)	Blood	Carapace	Blood	Carapace	Blood	Carapace	Blood	Carapace
LK0003	56.3	25	3.0	127	10.5	44.9	2.5	115	485	646
LK0004	45.0	14.0	5.3	49.5	3.9	37.4	3.3	73.4	410	1860
LK0005	47.7	17.0	5.2	445	5.5	175	3.6	151	504	2710
LK4002	45.9	8.0	0.8	-	8.8	-	0.5	-	459	-
LK4003	51.8	22.7	3.7	-	3.7	-	0.4	-	420	-
LK4004	31.8	4.3	5.5	-	5.7	-	1.5	-	500	-
LK4005	50.1	16.0	4.1	-	0.4	-	211	-	419	-
LK4006	54.2	27.2	2.5	-	0.5	-	1.3	-	442	-
LK4007	57.0	31.8	2.2	51.7	19.5	22.9	0.3	47.3	379	1730
LK4008	50.2	20.4	3.7	21.3	5.3	28.9	0.6	27.7	463	463
LK4009	47.3	20.4	2.6	99.1	7.1	30.6	209	104	471	443
LK4010	46.3	20.0	2.3	-	6.5	-	0.4	-	394	-
LK4011	50.0	22.7	2.7	58.4	4.9	22.0	3.8	97.9	602	444
LK2018	42.8	8.0	2.4	39.0	17.0	20.7	0.7	713	600	600
LK2019	43.5	12.0	1.4	106	5.9	33.5	3.3	97.6	556	943
LK2020	42.0	11.5	1.9	65.4	10.2	96.6	161	409	461	834
LK2021	41.2	12.5	2.6	-	6.2	-	137	-	500	-
LK2022	30.9	22.7	1.5	-	21.0	-	1.2	-	415	-
Mean	46.3	17.6	3.0	106	7.9	51.3	41.1	184	471	1070
S.D.	7.1	7.4	1.4	123	5.9	48.9	77.5	214	65.0	773
Min.	30.9	4.3	0.8	21.3	0.4	20.7	0.3	27.7	379	443
Max.	57.0	31.8	5.5	445	21.0	175	211	713	602	2710

	SCL	Wt	Hg		Pb		Zn	
Turtle no.	(cm)	(kg)	Blood	Carapace	Blood	Carapace	Blood	Carapace
LK0003	56.3	25	30.5	801	14.8	184	4590	356000
LK0004	45.0	14.0	15.5	521	3.5	1480	3510	731000
LK0005	47.7	17.0	11.0	603	20.2	3300	2770	858000
LK4002	45.9	8.0	24.1	-	4.9	-	2730	-
LK4003	51.8	22.7	20.5	-	48.0	-	6460	-
LK4004	31.8	4.3	20.5	-	8.1	-	2810	-
LK4005	50.1	16.0	6.6	-	21.2	-	3660	-
LK4006	54.2	27.2	1.8	-	35.0	-	1440	-
LK4007	57.0	31.8	43.3	1180	21.8	110	4710	355000
LK4008	50.2	20.4	15.3	666	49.4	463	3990	90400
LK4009	47.3	20.4	17.8	406	0.8	34.9	7120	102000
LK4010	46.3	20.0	20.5	-	1.8	-	3590	-
LK4011	50.0	22.7	28.2	605	1.1	140	3790	103000
LK2018	42.8	8.0	21.3	498	15.6	240	2910	102000
LK2019	43.5	12.0	23.5	1047	5.4	872	3540	168000
LK2020	42.0	11.5	23.3	885	153	457	6220	192000
LK2021	41.2	12.5	16.7	-	6.1	-	2430	-
LK2022	30.9	22.7	14.9	-	9.3	-	3860	-
Mean	46.3	17.6	19.7	721	23.3	728	3900	306000
S.D.	7.1	7.4	9.2	252	35.6	1010	1470	278000
Min.	30.9	4.3	1.8	406	0.8	34.9	1440	90400
Max.	57.0	31.8	43.3	1180	153	3300	7120	858000

-: no sample

Appendix Table 22. Correlation coefficients of trace metal concentrations in blood and carapace tissue of wild Kemp's ridleys captured from the southeast Atlantic.

Blood	Pearson	Ag	Cd	Cr	Cu	Hg	Pb
	Cd	-0.458					
	Cr	-0.028	-0.214				
	Cu	-0.064	-0.028	-0.094			
	Hg	-0.321	0.561*	-0.248	0.084		
	Pb	-0.087	0.013	0.278	-0.142	-0.008	
	Zn	-0.092	0.158	0.406	-0.218	0.363	0.400
Carapace	Pearson	Ag	Cd	Cr	Cu	Hg	Pb
	Cd	0.882**					
	Cr	-0.108	0.053				
	Cu	0.692*	0.670*	-0.197			
	Hg	-0.112	-0.052	-0.222	0.139		
	Pb	0.851**	0.830**	-0.119	0.842**	-0.162	
	Zn	0.679*	0.650*	-0.243	0.921**	-0.067	0.841**

* Correlation coefficient is significant at the 0.05 level

** Correlation coefficient is significant at the 0.01 level

Appendix Table 23. Trace metal concentrations (ppb) in coastal waters and surface sediment of Louisiana and Texas.

Location	Metal	Water	Surface sediment
Calcasieu River	Ag	< 100[h]	67[e]
	Cd	1 – 8[h]	980[e]
	Cr	1 – 3[h]	30000 – 15000[g, i]
	Cu	< 8[h]	15000 – 50000[i]
	Pb	< 1[h]	13000[e]
	Zn	20 – 100[h]	36000[e]
Sabine-Neches estuary	Ag	0.06[f]	
	Cr		41000[j]
	Cu		12000[j]
	Pb		52000[j]
Galveston Bay	Ag	16.1[a]	150[b]
	Cd		160[b]
	Cr		37000 – 68000[b, c]
	Cu	0.5[c]	14000[b]
	Hg		80[c]
	Pb	0.14[c]	267000[e]
	Zn	1.7 – 136[c]	55000[d]
Lavaca Bay	Hg	0.04l[k]	200[b]

References: [a]Ward and Armstrong, 1992; [b]Santschi *et al*., 2001; [c]Morse *et al*., 1993; [d]Benoit *et al*., 1994; [e]Wen *et al*., 1999; [f]Wen *et al*., 1997; [g]Simon *et al*., 1994; [h]Ramelow *et al*., 1987; [i]Gauthreaux *et al*., 1998; [j]Ravichandran *et al*., 1995a; [k]Mason *et al*., 1998

Appendix Table 24. Trace metal concentrations (ppb) in blood (w.w.) and carapace tissue (d.w.) of wild, female Kemp's ridleys nesting at Rancho Nuevo, Mexico in 2002.

	SCL	Ag		Cd		Cr		Cu	
Turtle no.	(cm)	Blood	Carapace	Blood	Carapace	Blood	Carapace	Blood	Carapace
1	61.9	7.2	211	10.1	124	3.8	1320	482	2340
2	64.8	6.2	634	21.3	72.0	1.2	303	386	512
3	61.9	9.1	93.9	7.0	29.0	4.0	735	453	230
4	62.4	3.0	557	3.2	63.3	0.8	321	376	589
5	63.9	3.7	300	7.9	25.1	0.7	641	256	558
6	67.7	18.0	-	6.8	-	2.9	-	360	-
7	65.3	6.5	215	12.3	44.5	2.4	292	450	615
8	63.9	6.0	117	13.6	23.8	2.9	732	467	528
9	61.9	1.1	246	15.0	25.2	1.0	289	494	610
10	63.4	2.3	147	9.0	28.1	2.2	965	324	836
11	64.3	15.1	80.7	25.8	22.9	4.3	599	377	489
12	61.4	4.1	99.1	4.8	14.7	25.0	349	450	600
13	68.0	0.4	368	45.5	44.8	1.9	774	485	625
14	65.8	70.0	114	6.1	29.8	29.6	333	245	568
15	63.9	65.9	69.5	17.2	58.6	16.7	656	504	581
16	65.1	7.8	99.3	51.4	24.7	1.4	1770	557	904
17*	68.8	3.4	244	3.3	23.0	0.9	463	241	1120
18*	75.2	1.8	76.4	7.6	34.6	0.9	347	362	840
Mean	65.0	12.9	216	14.9	40.5	5.7	641	404	738
S.D.	3.3	20.6	168	13.7	26.9	8.7	406	94.0	457
Min.	61.4	0.4	69.5	3.2	14.7	0.7	289	241	230
Max.	75.2	70.0	634	51.4	124	29.6	1770	557	2340

	SCL	Hg		Pb		Zn	
Turtle no.	(cm)	Blood	Carapace	Blood	Carapace	Blood	Carapace
1	61.9	60.0	2470	72.7	2390	18300	396000
2	64.8	22.2	518	12.9	2630	15500	817000
3	61.9	75.6	2180	87.7	1220	24200	105000
4	62.4	40.9	1960	32.1	1990	18600	285000
5	63.9	117	2020	33.6	272	16300	264000
6	67.7	69.2	-	58.3	-	16900	-
7	65.3	94.0	1860	75.6	1200	17600	273000
8	63.9	102	3370	68.1	193	19400	105000
9	61.9	94.9	4020	28.4	1100	14700	412000
10	63.4	125	4060	39.6	1220	15700	127000
11	64.3	92.5	5400	40.9	499	43300	152000
12	61.4	53.0	1010	61.2	447	14000	465000
13	68.0	21.9	539	23.8	988	18800	260000
14	65.8	37.9	3930	16.0	1120	48600	121000
15	63.9	145	1850	24.7	903	55200	87200
16	65.1	12.8	3350	47.5	1280	11900	217000
17*	68.8	18.6	1090	91.9	1960	22000	124000
18*	75.2	52.5	1470	51.2	598	17600	207000
Mean	65.0	68.6	2420	48.1	1180	22700	260000
S.D.	3.3	39.5	1390	24.2	712	12600	184000
Min.	61.4	12.8	518	12.9	193	11900	87200
Max.	75.2	145	5400	91.9	2630	55200	817000

-: no sample; *: female did not nest

Appendix Table 25. Correlation coefficients of trace metal concentrations in blood and carapace tissue of female Kemp's ridleys nesting at Rancho Nuevo, Mexico in 2002.

Blood	Pearson	Ag	Cd	Cr	Cu	Hg	Pb
	Cd	-0.085					
	Cr	0.726**	-0.216				
	Cu	-0.123	0.559*	-0.098			
	Hg	0.207	-0.291	0.019	0.046		
	Pb	-0.361	-0.316	-0.210	0.059	-0.026	
	Zn	0.874**	-0.050	0.571*	-0.143	0.301	-0.287
	Spearman	Ag	Cd	Cr	Cu	Hg	Pb
	Cd	0.040					
	Cr	0.693**	0.044				
	Cu	0.041	0.666**	0.210			
	Hg	0.088	0.09	0.197	0.074		
	Pb	0.073	-0.337	0.085	-0.056	0.040	
	Zn	0.391	-0.119	0.390	-0.129	0.102	0.030
Carapace	Pearson	Ag	Cd	Cr	Cu	Hg	Pb
	Cd	0.408					
	Cr	-0.288	0.203				
	Cu	-0.038	0.673**	0.485*			
	Hg	-0.461	-0.261	0.241	-0.034		
	Pb	0.612**	0.683**	0.082	0.468	-0.287	
	Zn	0.663**	0.397	-0.215	0.111	-0.410	0.481

* Correlation coefficient is significant at the 0.05 level

** Correlation coefficient is significant at the 0.01 level

Appendix Table 26. Trace metal concentrations (ppb) in digestive gland (DG, w.w.) and muscle tissue (M, w.w.) of blue crabs captured from Rancho Nuevo, Mexico in 2002.

	Sex	Length	Weight	**Ag**		**Cd**		**Cr**		**Cu**	
No.		(mm)	(g)	DG	M	DG	M	DG	M	DG	M
1	F	157	167	417	38.5	50.8	1.8	5.3	0.1	11900	2020
2	F	131	129	245	161	49.6	1.1	9.3	7.4	7690	2860
3	M	128	116	131	71.8	56.2	2.9	5.8	0.9	7420	4710
4	M	115	78.7	127	69.3	31.5	2.2	4.0	0.7	8770	2970
5	M	118	105	126	60.2	34.9	3.7	1.1	0.8	11300	2240
6	M	142	153	176	37.3	31.1	28.2	3.7	1.6	9860	3460
7	M	120	124	144	88.5	39.3	1.5	6.3	0.8	10100	4070
8	F	120	76.8	139	81.5	56.0	4.8	0.7	3.2	13100	2730
9	F	150	139	345	107	145	2.8	7.6	0.6	26600	5130
10	F	132	98.0	109	40.2	27.2	2.3	2.9	0.2	6760	2850
Mean		131	119	196	75.5	52.1	5.1	4.7	1.6	11400	3300
SD		14.3	29.9	106	37.9	34.3	8.2	2.7	2.2	5740	1030
Min		115	76.8	109	37.3	27.2	1.1	0.7	0.1	6760	2020
Max		157	167	417	161	145	28.2	9.3	7.4	26600	5130

	Sex	Length	Weight	**Hg**		**Pb**		**Zn**	
No.		(mm)	(g)	DG	M	DG	M	DG	M
1	F	157	167	19.8	17.5	63.8	1.7	44600	13200
2	F	131	129	BDL	2.0	90.2	43.9	22300	65000
3	M	128	116	7.4	48.1	23.0	0.7	17300	93900
4	M	115	78.7	9.0	9.0	15.6	8.3	115000	120000
5	M	118	105	4.3	25.4	18.4	15.9	25400	21400
6	M	142	153	6.3	29.5	39.1	1.8	45400	65400
7	M	120	124	8.5	17.4	76.1	5.1	39900	9430
8	F	120	76.8	1.8	23.2	22.4	55.1	32500	49400
9	F	150	139	21.0	35.4	46.6	31.1	44400	51700
10	F	132	98.0	2.2	24.0	5.5	14.0	27500	94100
Mean		131	119	8.9	23.2	40.1	17.8	41400	58400
SD		14.3	29.9	7.0	13.0	28.5	19.2	27700	37100
Min		115	76.8	BDL	2.0	5.5	0.7	17300	9430
Max		157	167	21.0	48.1	90.2	55.1	115000	120000

BDL: below detection limit

Appendix Table 27. Correlation coefficients of trace metal concentrations in digestive gland and muscle tissue of blue crabs captured from Rancho Nuevo, Mexico in 2002.

Digestive gland	Pearson	Ag	Cd	Cr	Cu	Hg	Pb
	Cd	0.575					
	Cr	0.514	0.435				
	Cu	0.558	0.920**	0.184			
	Hg	0.917**	0.678*	0.759*	0.661		
	Pb	0.558	0.183	0.759*	0.099	0.609	
	Zn	- 0.014	-0.089	-0.064	0.048	0.211	-0.189
Muscle tissue	Pearson	Ag	Cd	Cr	Cu	Hg	Pb
	Cd	-0.387					
	Cr	0.784**	-0.027				
	Cu	0.277	0.046	-0.155			
	Hg	-0.361	0.232	-0.499	0.599		
	Pb	0.642*	-0.239	0.678*	-0.097	-0.277	
	Zn	- 0.011	0.063	0.050	0.187	0.072	-0.043

* Correlation coefficient is significant at the 0.05 level
** Correlation coefficient is significant at the 0.01 level

Appendix Table 28. Size and condition code of Kemp's ridleys stranded along the Texas coast during 2001-2002.

Turtle Number	SCL (cm)	Code	Stranding location
Lk-01-26	52.8	2	Mustang Island
Lk-01-38	36.9	2	Mustang Island
Lk-01-39	51.4	0	PINS*
Lk	54.2	2	Mustang Island
Lk-01-40	64.0	2	South Padre Island
Lk-02-01	62.9	2	South Padre Island
LK-02-02	63.4	2	South Padre Island
Lk-02-09	40.4	1	PINS north boundry
Lk-02-34	33.9	2	North Padre Island
Lk-02-36	34.5	1	North Padre Island
Lk-02-37	45.0	1	Mustang Island
Mean	49.0		
SD	11.6		
Min.	33.9		
Max.	64.0		

Code 0 - live turtles; code 1 - fresh dead carcasses; and code 2 - moderately decomposed carcasses
* PINS: Padre Island National Seashore

Appendix Table 29. Silver concentration (ppb) in tissues (d.w.) and blood (w.w.) of stranded Kemp's ridleys from Texas during 2001-2002.

Turtle no.	SCL (cm)	Carapace	Liver	Kidney	Muscle	Blood
Lk-01-26	52.8	116	-	-	117	-
Lk-01-38	36.9	103	135	85.5	61.5	-
Lk-01-39	51.4	45.2	-	-		19.2
Lk	54.2	124	79.2	40.0	29.0	-
Lk-01-40	64.0	187	572	181	31.0	-
Lk-02-01	62.9	528	65.7	97.0	29.0	-
LK-02-02	63.4	107	21.1	-	206	-
Lk-02-09	40.4	188	80.5	29.0	122	-
Lk-02-34	33.9	690	160	45.5	82.5	-
Lk-02-36	34.5	237	84.8	37.0	54.5	-
Lk-02-37	45.0	276	39.9	26.5	73.0	-
Mean	49.0	237	138	67.7	80.5	
S.D.	11.6	199	168	52.7	55.4	
Min.	33.9	45.2	21.1	26.5	29.0	
Max.	64.0	690	572	181	206	

-: no sample

Appendix Table 30. Cadmium concentration (ppb) in tissues (d.w.) and blood (w.w.) of stranded Kemp's ridleys from Texas during 2001-2002.

Turtle no.	SCL (cm)	Carapace	Liver	Kidney	Muscle	Blood
Lk-01-26	52.8	52.5	-	-	157	-
Lk-01-38	36.9	106	595	4340	23.5	-
Lk-01-39	51.4	54.0	-	-		38.2
Lk	54.2	84.9	1520	11900	5.5	-
Lk-01-40	64.0	62.7	5740	814	48.0	-
Lk-02-01	62.9	70.2	2440	628	34.0	-
LK-02-02	63.4	148	1930	-	62.5	-
Lk-02-09	40.4	140	767	132	38.5	-
Lk-02-34	33.9	177	2170	399	32.5	-
Lk-02-36	34.5	93.1	2650	534	100	-
Lk-02-37	45.0	671	1320	527	47.0	-
Mean	49.0	151	2130	2410	54.9	
S.D.	11.6	177	1530	4070	43.8	
Min.	33.9	52.5	595	132	5.5	
Max.	64.0	671	5740	11900	157	

-: no sample

Appendix Table 31. Chromium concentration (ppb) in tissues (d.w.) and blood (w.w.) of stranded Kemp's ridleys from Texas during 2001-2002.

Turtle no.	SCL (cm)	Carapace	Liver	Kidney	Muscle	Blood
Lk-01-26	52.8	230	-	-	15.0	-
Lk-01-38	36.9	587	6.6	8.5	9.0	-
Lk-01-39	51.4	52.5	-	-		6.6
Lk	54.2	147	8.9	14.5	37.5	-
Lk-01-40	64.0	119	18.2	22.0	0.5	-
Lk-02-01	62.9	55.2	5.9	18.0	7.5	-
LK-02-02	63.4	116	13.2	-	57.5	-
Lk-02-09	40.4	38.6	10.6	12.5	301.5	
Lk-02-34	33.9	98.8	28.1	36.5	15.0	
Lk-02-36	34.5	29.0	49.2	35.0	35.5	
Lk-02-37	45.0	264	18.8	17.0	50.5	-
Mean	49.0	158	17.7	20.5	53.0	
S.D.	11.6	161	13.7	10.2	89.4	
Min.	33.9	29.0	5.9	8.5	0.5	
Max.	64.0	587	49.2	36.5	301.5	

-: no sample

Appendix Table 32. Copper concentration (ppb) in tissues (d.w.) and blood (w.w.) of stranded Kemp's ridleys from Texas during 2001-2002.

Turtle no.	SCL (cm)	Carapace	Liver	Kidney	Muscle	Blood
Lk-01-26	52.8	704	-	-	1550	-
Lk-01-38	36.9	1580	2420	3190	1390	-
Lk-01-39	51.4	1170	-	-		4560
Lk	54.2	565	21700	13000	329	-
Lk-01-40	64.0	517	26700	8440	1290	-
Lk-02-01	62.9	1190	15100	7990	2060	-
LK-02-02	63.4	1470	7490	-	5890	-
Lk-02-09	40.4	817	12800	6100	1730	-
Lk-02-34	33.9	1580	20500	11200	2890	-
Lk-02-36	34.5	749	16300	10500	3150	-
Lk-02-37	45.0	6850	8980	4780	3180	-
Mean	49.0	1560	14700	7780	2350	
S.D.	11.6	1800	7650	3350	1540	
Min.	33.9	517	2420	3190	329	
Max.	64.0	6850	26700	13000	5890	

-: no sample

Appendix Table 33. Mercury concentration (ppb) in tissues (d.w.) and blood (w.w.) of stranded Kemp's ridleys from Texas during 2001-2002.

Turtle no.	SCL (cm)	Carapace	Liver	Kidney	Muscle	Blood
Lk-01-26	52.8	1880	-	-	55.0	-
Lk-01-38	36.9	375	18.8	211	254	-
Lk-01-39	51.4	488	-	-		40.0
Lk	54.2	284	1030	1310	6.5	-
Lk-01-40	64.0	516	171	1160	967	-
Lk-02-01	62.9	3730	6110	1120	949	-
LK-02-02	63.4	1510	184	-	302	-
Lk-02-09	40.4	154	189	425	487	-
Lk-02-34	33.9	1840	2300	880	502	-
Lk-02-36	34.5	840	496	280	191	-
Lk-02-37	45.0	1390	537	560	589	-
Mean	49.0	1180	1230	743	430	
S.D.	11.6	1060	1960	429	336	
Min.	33.9	154	18.8	211	6.5	
Max.	64.0	3730	6110	1310	967	

-: no sample

Table 34. Lead concentrations (ppb) in tissues (d.w.) and blood (w.w.) of stranded Kemp's ridleys from Texas during 2001-2002.

Turtle no.	SCL (cm)	Carapace	Liver	Kidney	Muscle	Blood
Lk-01-26	52.8	545	-	-	97.0	-
Lk-01-38	36.9	844	34.0	29.0	162	-
Lk-01-39	51.4	262	-	-		47.0
Lk	54.2	446	60.7	471	11.0	-
Lk-01-40	64.0	137	475	289	6.5	-
Lk-02-01	62.9	9170	71.6	82.0	26.5	-
LK-02-02	63.4	822	119	-	591	-
Lk-02-09	40.4	410	84.5	76.0	50.5	
Lk-02-34	33.9	1450	71.3	52.5	288	
Lk-02-36	34.5	143	310	156	121	
Lk-02-37	45.0	1000	118	145	29.5	-
Mean	49.0	1390	149	162	138	
S.D.	11.6	2610	146	149	181	
Min.	33.9	137	34.0	29.0	6.5	
Max.	64.0	9170	475	471	591	

-: no sample

Appendix Table 35. Zinc concentration (ppb) in tissues (d.w.) and blood (w.w.) of stranded Kemp's ridleys from Texas during 2001-2002.

Turtle no.	SCL (cm)	Carapace	Liver	Kidney	Muscle	Blood
Lk-01-26	52.8	218000	-	-	35200	-
Lk-01-38	36.9	146000	75400	142000	29100	-
Lk-01-39	51.4	256000	-	-		52700
Lk	54.2	262000	63000	132000	10300	-
Lk-01-40	64.0	51100	63200	172000	20600	-
Lk-02-01	62.9	537000	45200	123000	20600	-
LK-02-02	63.4	294000	53100	-	60800	-
Lk-02-09	40.4	254000	59700	98900	39100	-
Lk-02-34	33.9	249000	83900	121000	31700	-
Lk-02-36	34.5	88500	69800	155000	26400	-
Lk-02-37	45.0	614000	108000	127000	40000	-
Mean	49.0	270000	69000	134000	31400	
S.D.	11.6	170000	18600	22400	13800	
Min.	33.9	51100	45200	98900	10300	
Max.	64.0	614000	108000	172000	60800	

-: no sample

Appendix Table 36. Correlation coefficients of trace metal concentrations among tissues of stranded Kemp's ridleys from Texas during 2001-2002.

	Ag			Cd		
	Liver	Kidney	Muscle	Liver	Kidney	Muscle
Kidney	0.874**			-0.248		
Muscle	0.384	-0.552		0.296	-0.600	
Carapace	-0.042	-0.080	-0.264	0.286	-0.229	-0.141
	Cr			Cu		
	Liver	Kidney	Muscle	Liver	Kidney	Muscle
Kidney	0.855**			0.797*		
Muscle	-0.156	-0.305		-0.407	-0.146	
Carapace	-0.365	-0.536	-0.302	-0.408	-0.471	0.297
	Hg			Pb		
	Liver	Kidney	Muscle	Liver	Kidney	Muscle
Kidney	0.466			0.302		
Muscle	0.482	0.332		-0.199	-0.554	
Carapace	0.896**	0.316	0.392	-0.292	-0.286	-0.148
	Zn					
	Liver	Kidney	Muscle			
Kidney	-0.002					
Muscle	0.105	-0.448				
Carapace	0.242	-0.551	0.187			

* Correlation coefficient is significant at the 0.05 level

** Correlation coefficient is significant at the 0.01 level

Appendix Table 37. Correlation coefficients of trace metal concentrations within tissues of stranded Kemp's ridleys from Texas during 2001-2002.

Liver	Pearson	Ag	Cd	Cr	Cu	Hg	Pb
	Cd	0.844**					
	Cr	0.054	0.281				
	Cu	0.615	0.723*	0.268			
	Hg	-0.183	0.064	-0.202	0.178		
	Pb	0.774*	0.885**	0.494	0.556	-0.282	
	Zn	- 0.063	-0.216	0.305	-0.178	-0.351	-0.065
Kidney		Ag	Cd	Cr	Cu	Hg	Pb
	Cd	-0.129					
	Cr	-0.079	-0.387				
	Cu	-0.093	0.391	0.588			
	Hg	0.403	0.386	0.042	0.606		
	Pb	0.141	0.725*	-0.090	0.605	0.643	
	Zn	0.658	0.054	0.250	0.126	0.102	0.361
Muscle		Ag	Cd	Cr	Cu	Hg	Pb
	Cd	0.337					
	Cr	0.367	-0.124				
	Cu	0.722*	0.191	0.000			
	Hg	-0.300	-0.317	-0.032	0.020		
	Pb	0.794**	0.087	-0.071	0.825**	-0.235	
	Zn	0.933**	0.293	0.308	0.830**	-0.101	0.751*
Carapace		Ag	Cd	Cr	Cu	Hg	Pb
	Cd	0.164					
	Cr	-0.262	0.206				
	Cu	0.139	0.978**	0.288			
	Hg	0.643*	0.056	-0.184	0.129		
	Pb	0.570	-0.077	-0.157	0.005	0.847**	
	Zn	0.344	0.660*	-0.044	0.705*	0.585	0.580

* Correlation coefficient is significant at the 0.05 level

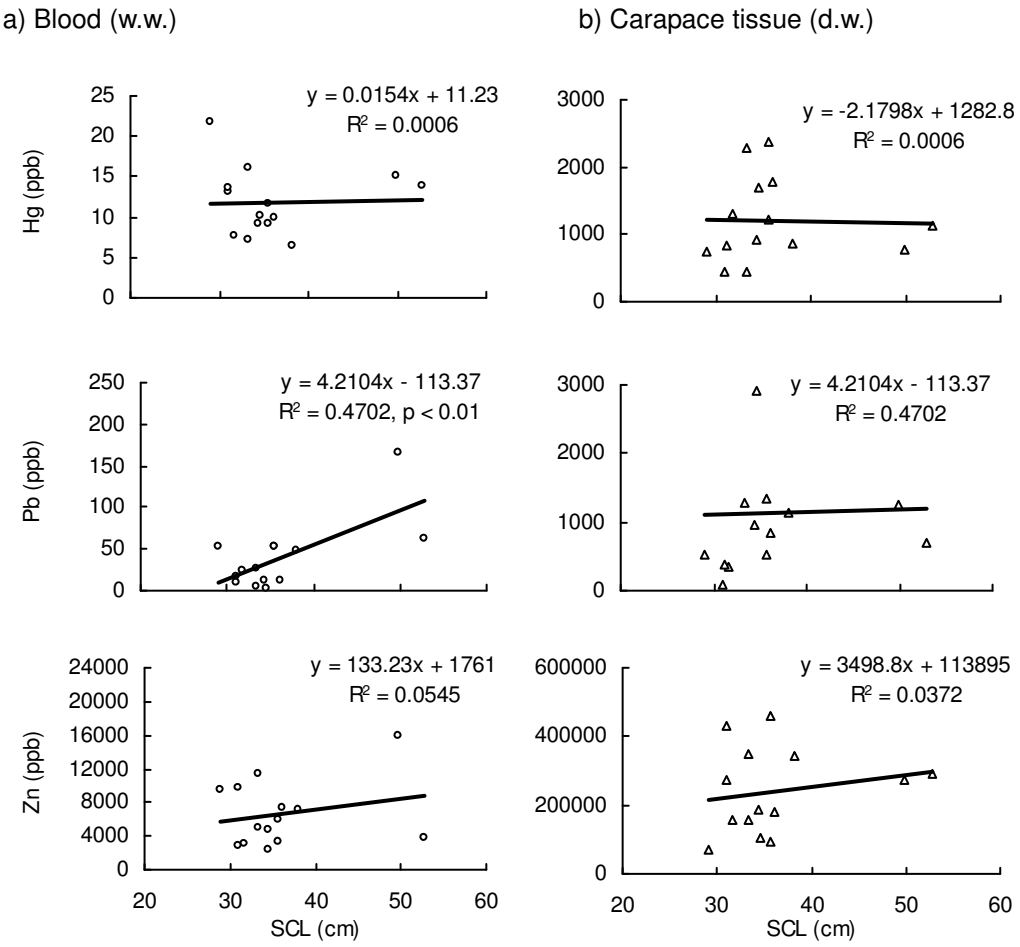
** Correlation coefficient is significant at the 0.01 level

Appendix Table 38. Trace metal concentrations (ppb) in tissues (d.w.) of stranded sea turtles from different locations.

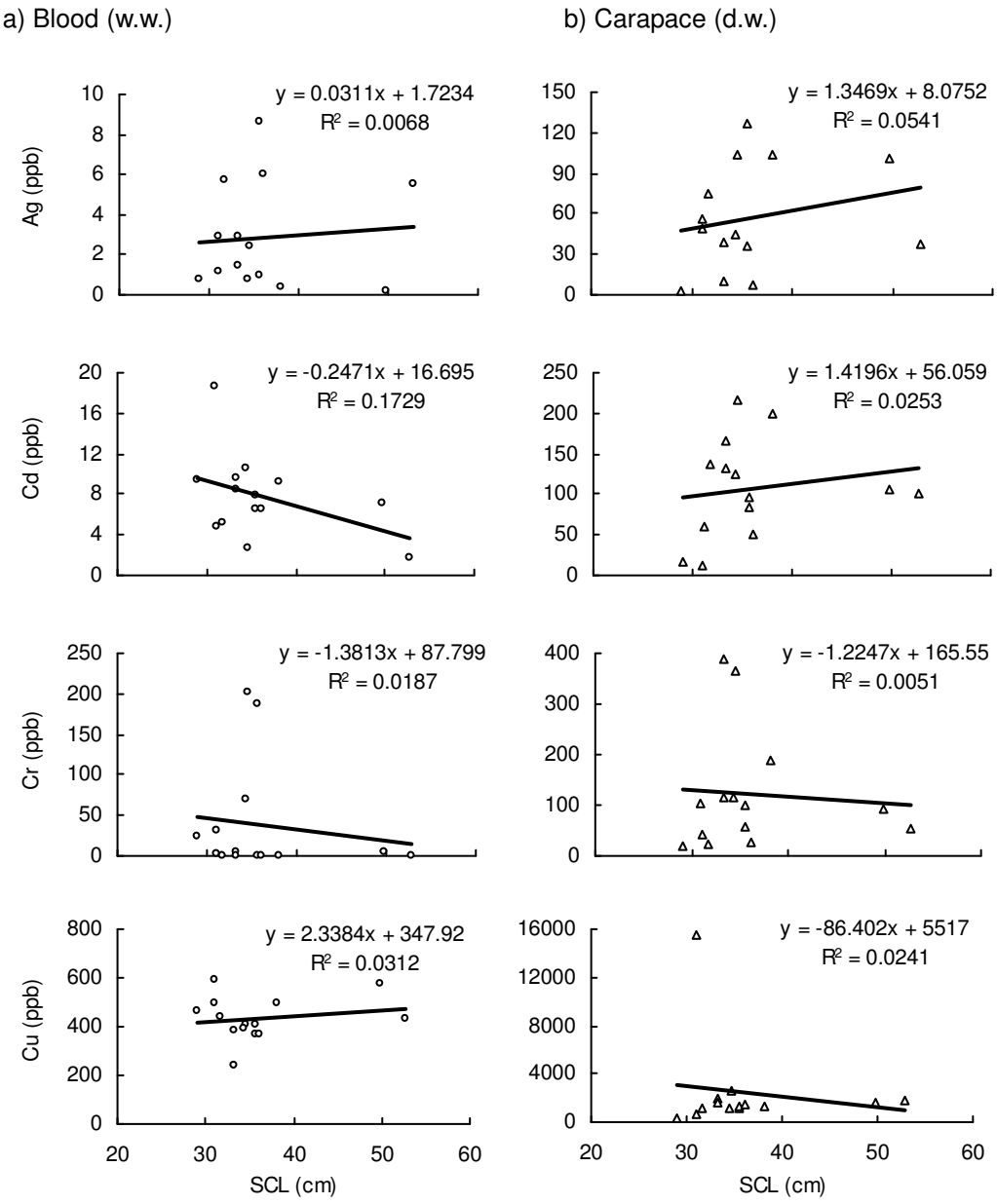
Species	Location	Metal	Liver	Kidney	Muscle	Carapace	Reference
Kemp's ridley	Texas	Ag	138	68	81	237	Present study (d.w.)
	n = 11	Cd	2130	2410	55	151	SCL = 49.0 ± 11.6 cm
		Cr	18	21	53	158	Range = 33.9 - 64.0 cm
		Cu	14700	8150	2350	1560	
		Hg	1230	743	430	1180	
		Pb	149	162	138	1380	
		Zn	690000	134000	31400	270000	
	France	Cd			450		Caurant *et al*., 1999 (d.w.)
	n = 6	Cu			4900		SCL = 25.8 ± 5.2 cm
		Zn			82000		Range = 21.3 - 34.5 cm
Loggerhead	Texas	Cd	3730	18900			Sis and Landry, 1992
	n = 10	Cu	4540	1520			SCL = 59.7 ± 7.6 cm
		Hg	378	178			Range = 51.2 - 76.5 cm
		Pb	109	160			
		Zn	26600	33800			
	Texas	Ag	637	186	71	87	Wang *et al*., 2003 (d.w.)
	n = 14	Cd	13400	5750	539	212	SCL = 67.8 ± 8.5 cm
		Cr	37	77	88	131	Range = 52.5 - 88.2 cm
		Cu	19600	3970	2980	953	
		Hg	147	96	96	240	
		Pb	299	191	748	532	
		Zn	63500	123000	30700	380000	
	Japan	Cd	32100	192000	320	129	Sakai *et al*., 2000a (d.w.)
	n = 6	Cu	58400	6500	4050	251	SCL = 83 ± 6 cm
		Hg	1320	1190	472	43	
		Pb	264	800	100	2420	
		Zn	92700	127000	125000	198000	
	France	Cd	8510	66500	400		Caurant *et al*., 1999 (d.w.)
	n = 21	Cu	27200	11100	3650		SCL = 29.4 ± 15.3 cm
		Zn	82500	118000	98000		Range = 21.3 - 34.5 cm
	Cyprus	Cd	8640	30500	570		Godley *et al*., 1999
	n = 7	Hg	2410	470	480		CCL = 63.5 ± 14.2cm
		Pb		2450	2460		Range = 56.0 - 79.0 cm
	Italy	Cd	7660	24230	550		Storelli *et al*., 1998a (d.w.)
	n = 12	Cr	1050	1570	1430		Unknown SCL
		Hg	1680	650	690		
		Pb	1230	700	540		
	Australia	Cd	54100	142000			Gordon *et al*., 1998 (d.w.)
	n = 8	Hg	50	225			Unknown SCL
		Zn	75200	92000			

Appendix Table 38. Cont.

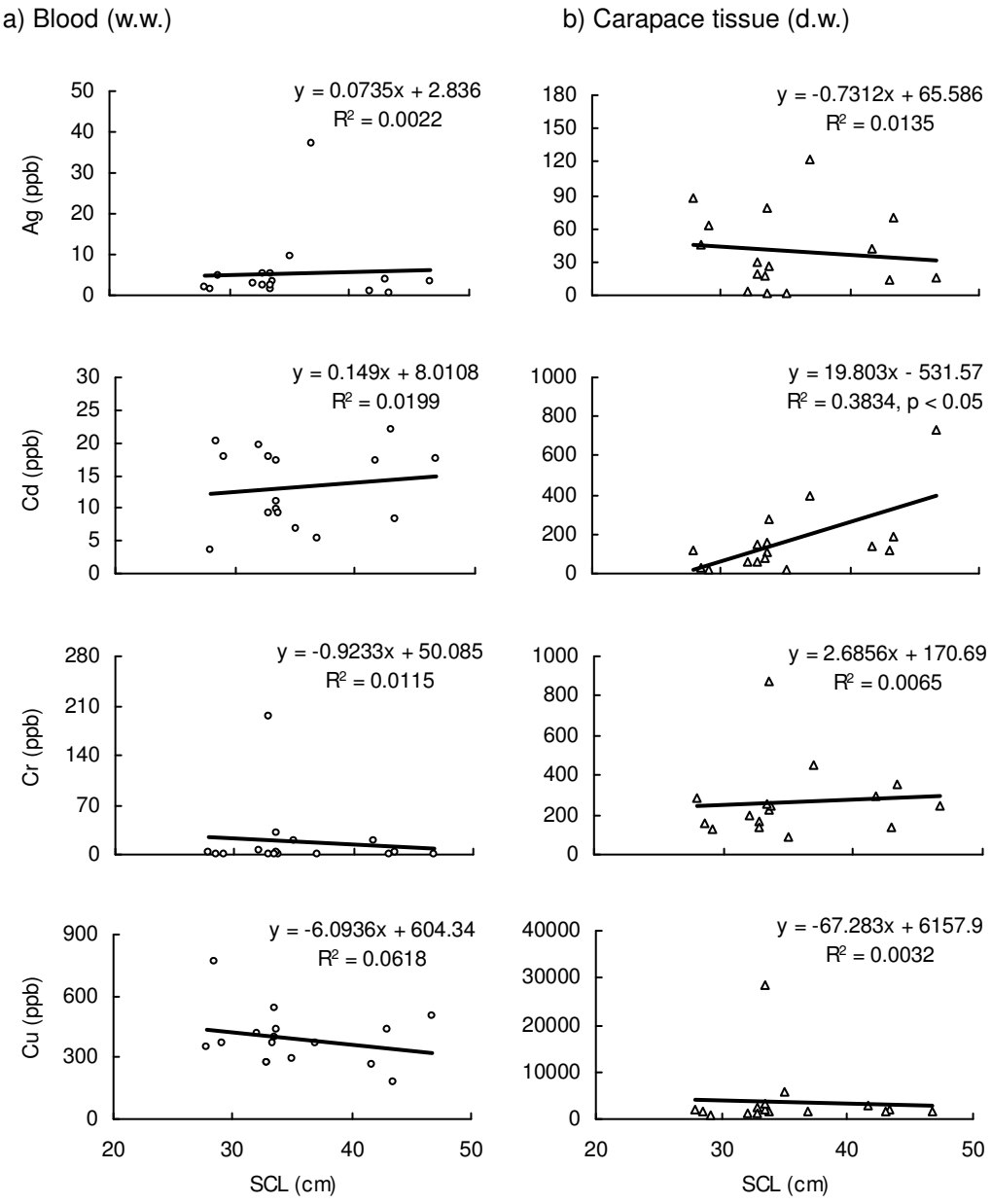
Species	Location	Metal	Liver	Kidney	Muscle	Carapace	Reference
Green	Texas	Ag	761	100	54	232	Wang *et al*., 2003 (d.w.)
	n = 13	Cd	3110	731	117	199	SCL = 40.8 ± 11.3 cm
		Cr	23	32	54	313	Range = 27.0 - 61.8 cm
		Cu	40300	7380	1320	1040	
		Hg	239	72	61	530	
		Pb	559	114	306	1620	
		Zn	62700	164000	23100	164000	
	Japan	Cd	18400	193000	250		Sakai *et al*., 2000b (d.w.)
	n = 50	Cu	166000	10800	1770		SCL = 51.0 cm
		Hg	957	650	145		
		Pb	< 100	900	< 100		
		Zn	100000	148000	44000		
	Japan	Ag	3200	29	13		Anan *et al*., 2001 (d.w.)
	n = 26	Cd	18200	142000	238		SCL = 51.2 ± 9.0 cm
		Cr	2200	2200	1400		Range = 37.0 - 71.4 cm
		Cu	139000	8270	879		
		Hg	420	300	40		
		Pb	507	813	94		
		Zn	87200	169000	47700		
	Japan	Ag	2937				Anan *et al*., 2002a (d.w.)
	n = 8	Cd	29600				Unknown SCL
		Cu	165000				
		Pb	462				
		Zn	110000				
	Cyprus	Cd	5890	3460	370		Godley *et al*., 1999 (d.w.)
	n = 6	Hg	550		90		CCL = 49.5 ± 16.6 cm
		Pb		1810			Range = 27.5 - 56.0 cm
	Hawaii	Cd	30700	130000			Aguirre *et al.*, 1994 (d.w.)
	n = 12	Cu	289000	18000			SCL = 54.5 ± 11.5 cm
		Zn	10100	112000			Range = 28.7 - 71.3 cm
	Australia	Cd	41300	76500			Gordon *et al*., 1998 (d.w.)
	n = 38	Hg	69	100			Unknown SCL
		Zn	131000	107000			



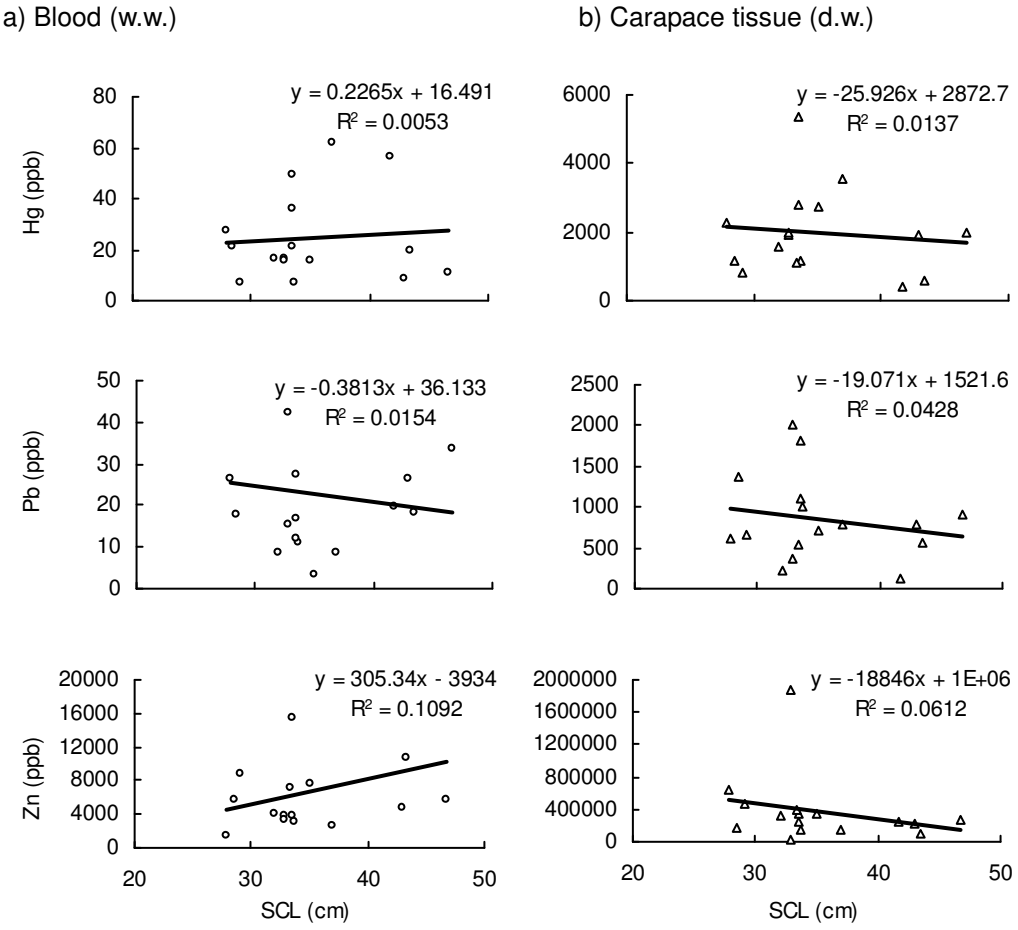
Appendix Fig. 1. Trace metal (Hg, Pb and Zn) concentrations (ppb) in blood (w.w.) and carapace tissue (d.w.) as a function of SCL of Kemp's ridleys (n = 14) captured from Sabine Pass, Texas during 2000-2002.



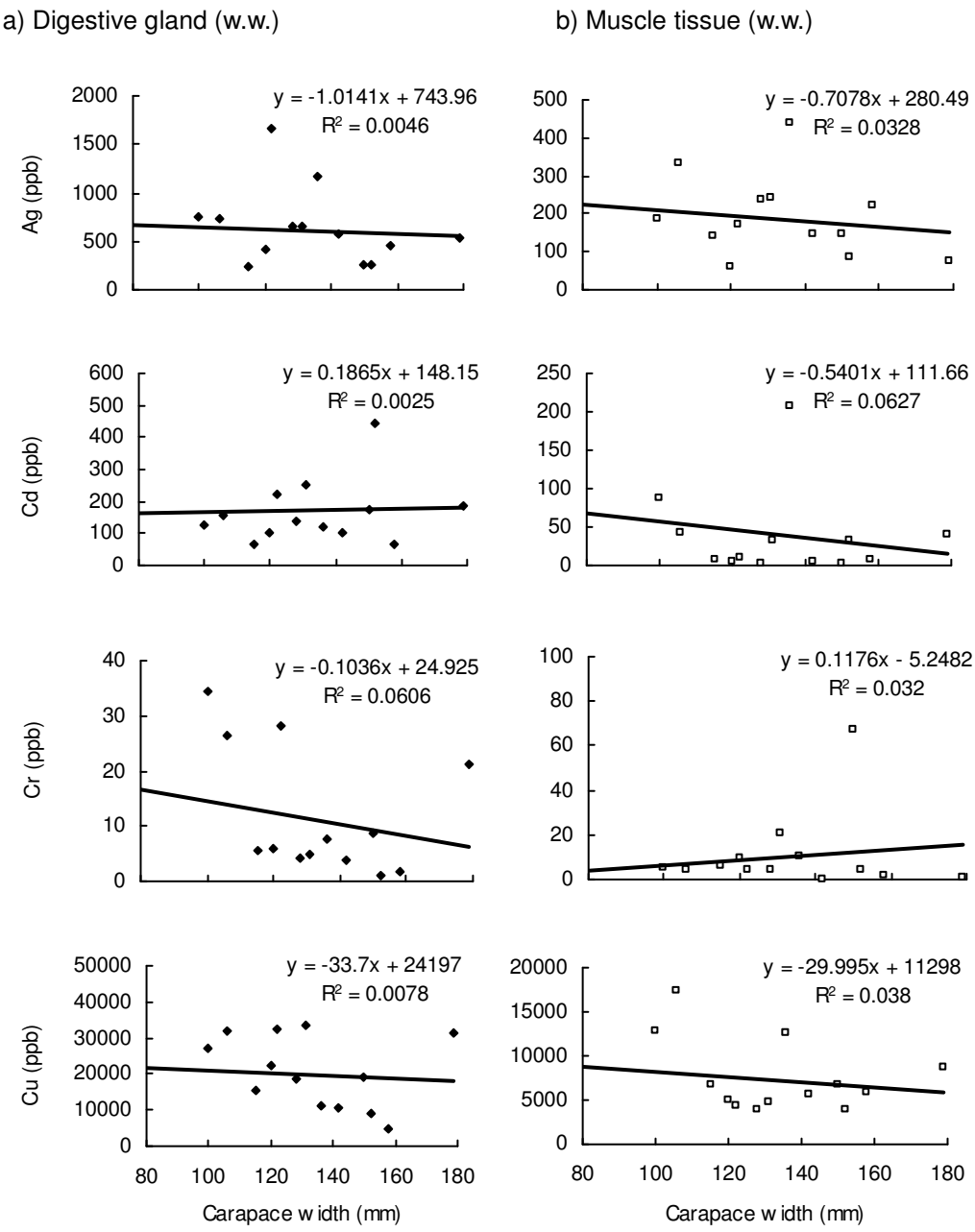
Appendix Fig. 2. Trace metal (Ag, Cd, Cr and Cu) concentrations (ppb) in blood (w.w.) and carapace tissue (d.w.) as a function of SCL of Kemp's ridleys (n = 14) captured from Sabine Pass, Texas during 2000-2002.



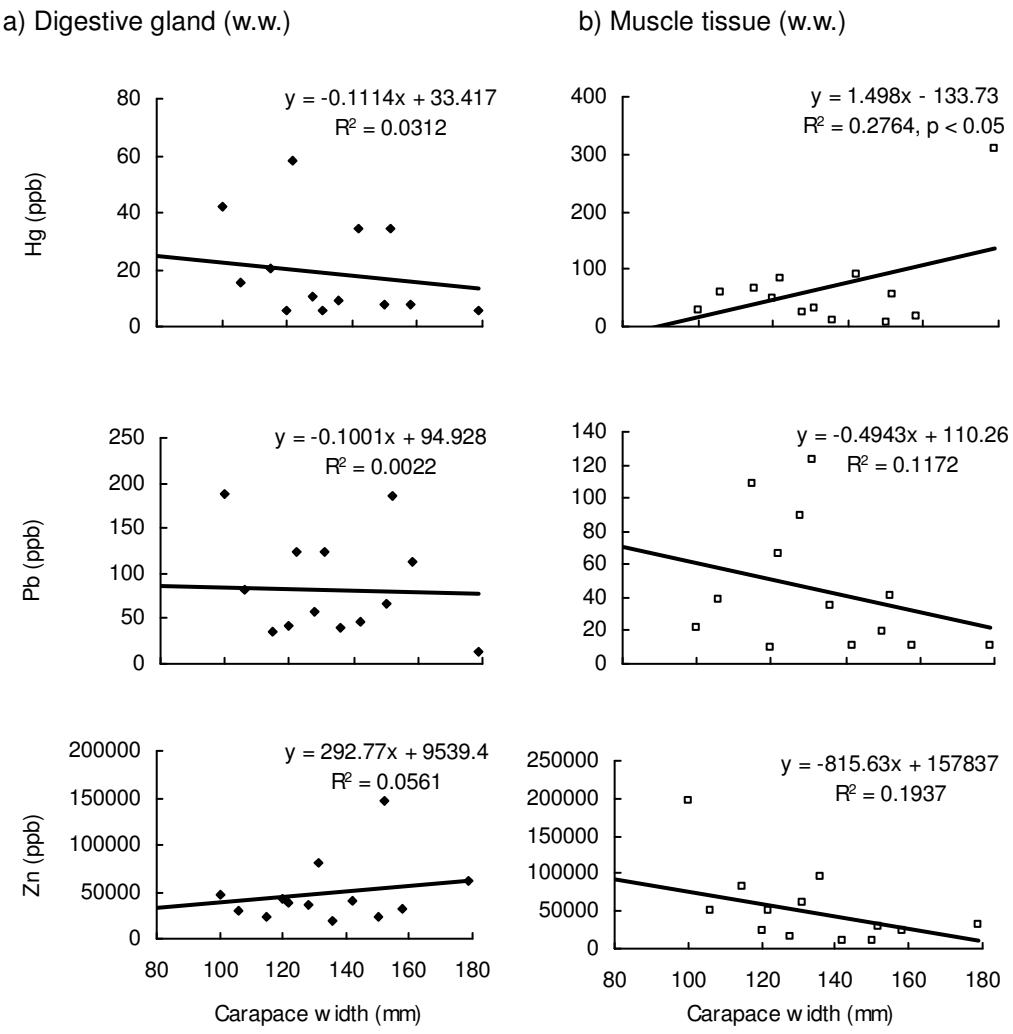
Appendix Fig. 3. Trace metal (Ag, Cd, Cr and Cu) concentrations (ppb) in blood (w.w.) and carapace tissue (d.w.) as a function of SCL of Kemp's ridleys (n = 16) captured from Lavaca Bay, Texas during 2001-2002.



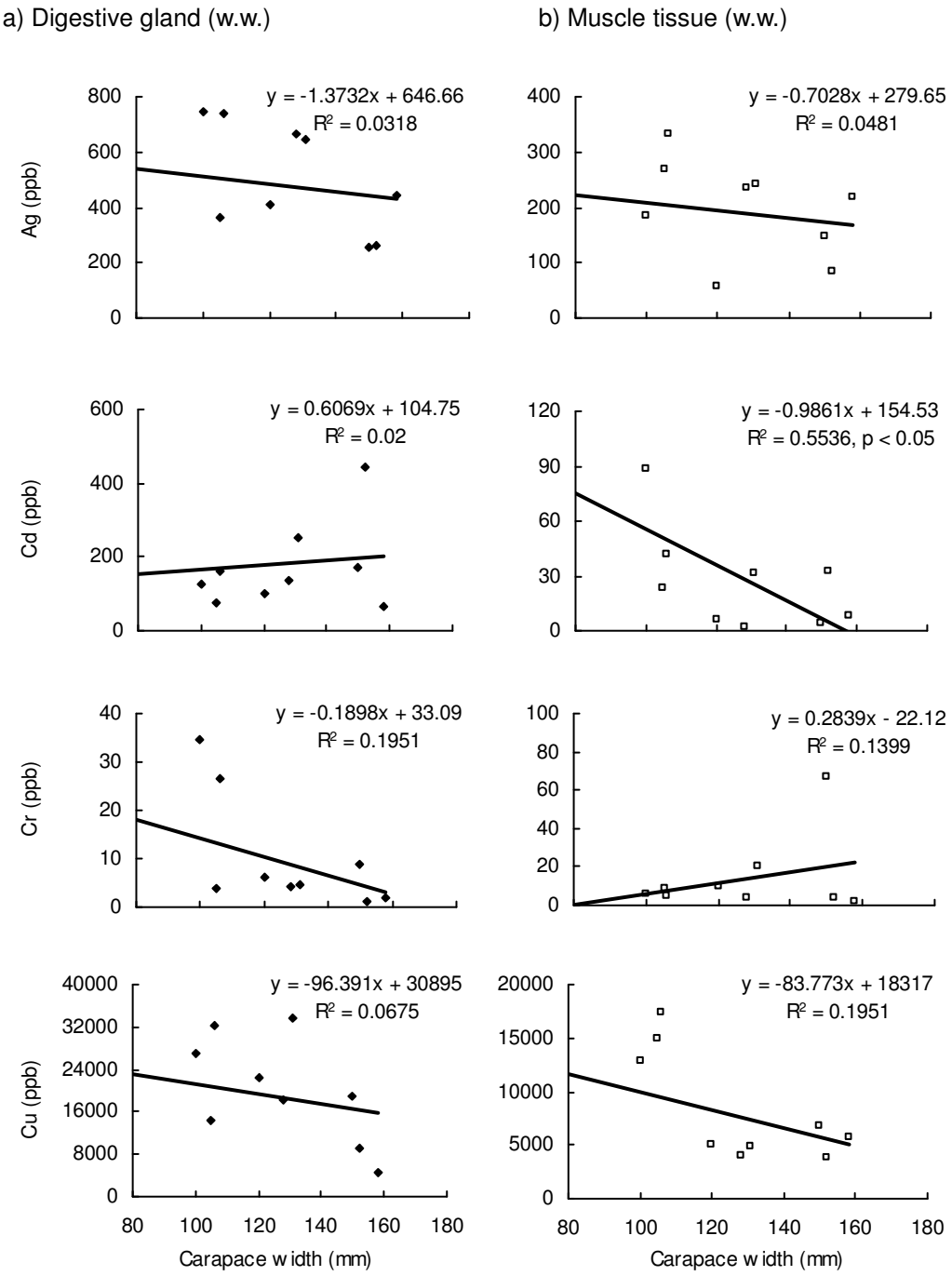
Appendix Fig. 4. Trace metal (Hg, Pb and Zn) concentrations (ppb) in blood (w.w.) and carapace tissue (d.w.) as a function of SCL of Kemp's ridleys (n = 16) captured from Lavaca Bay, Texas during 2001-2002.



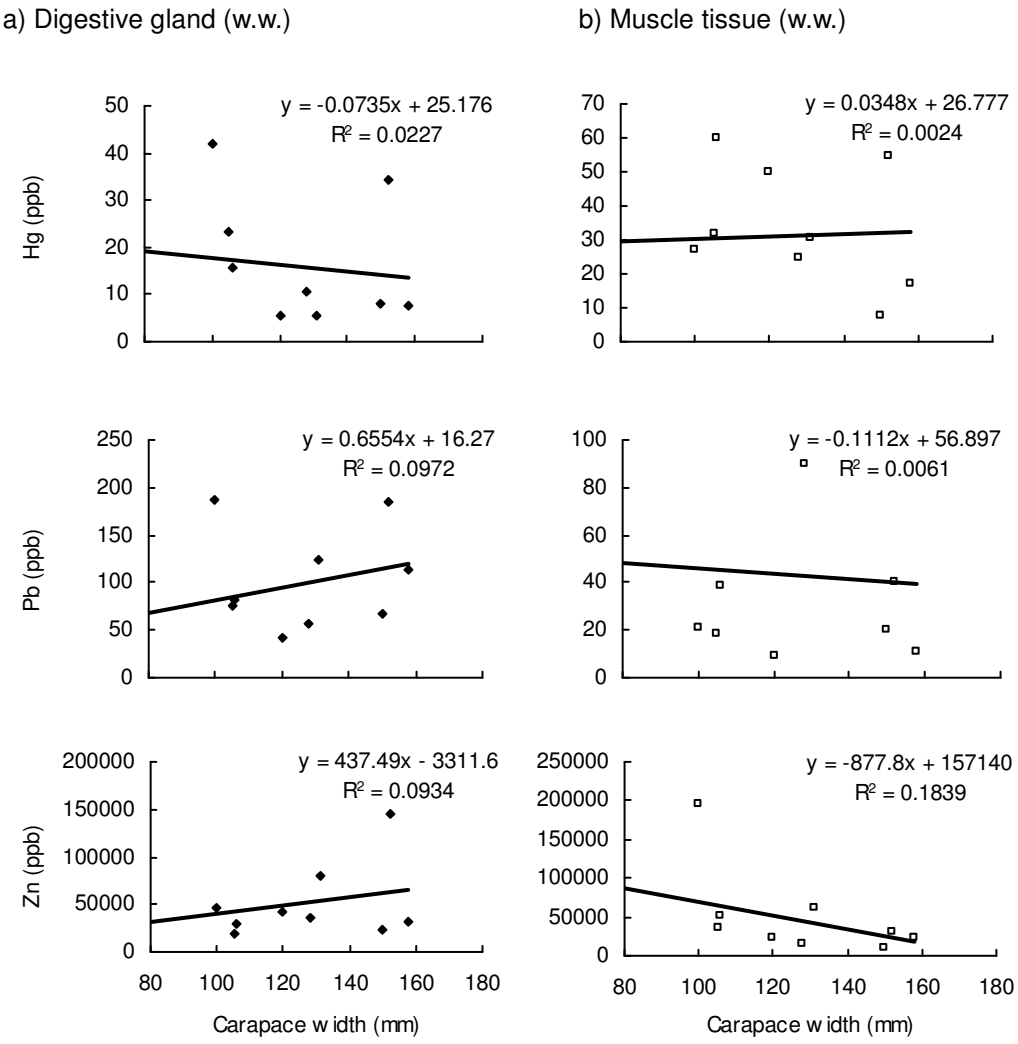
Appendix Fig. 5. Trace metal (Ag, Cd, Cr, Cu) concentrations (ppb) in digestive gland (w.w.) and muscle tissue (w.w.) as a function of carapace width of blue crabs (n = 15) captured from Sabine Pass and Lavaca Bay, Texas combined study sites in 2001.



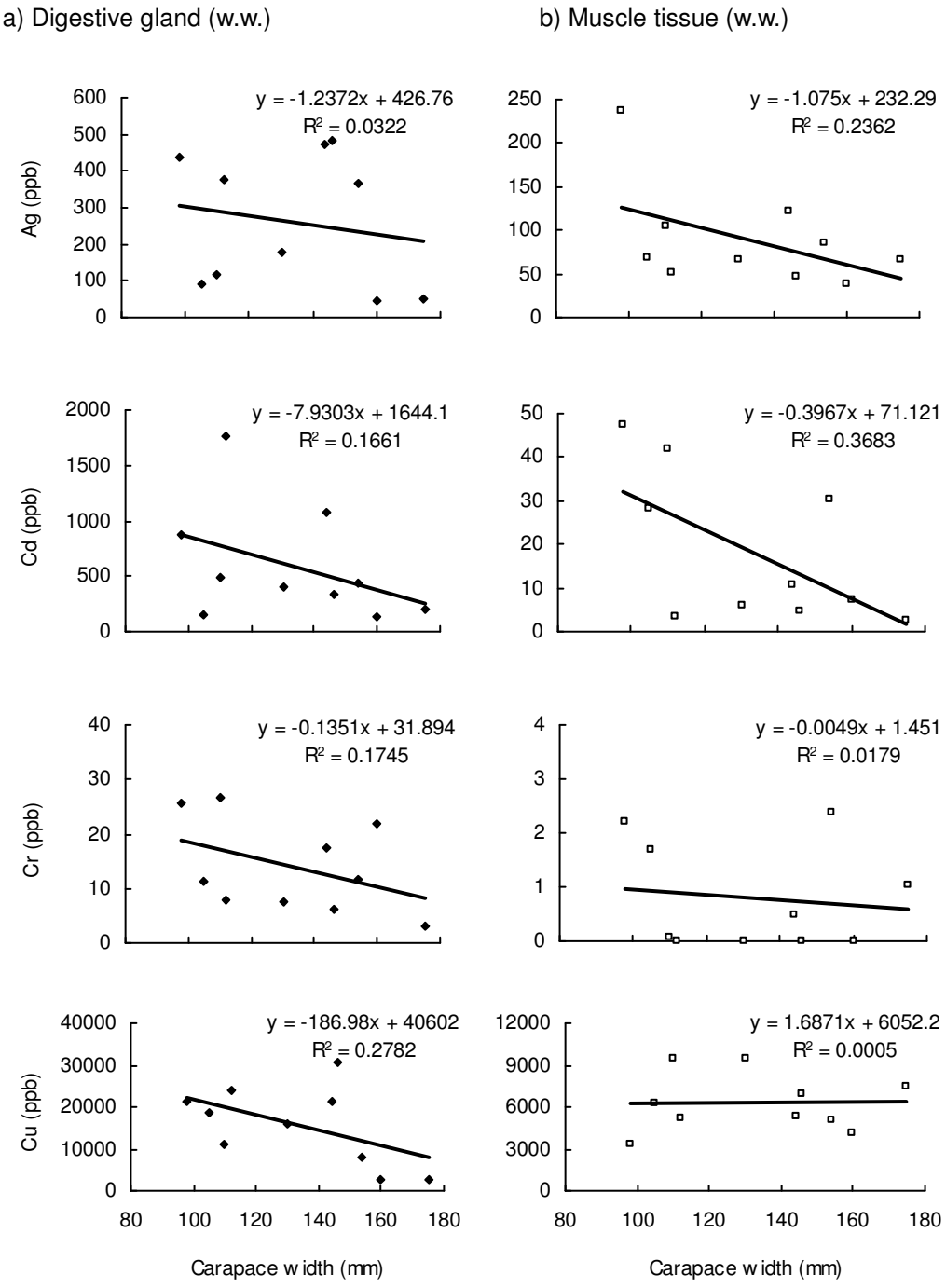
Appendix Fig. 6. Trace metal (Hg, Pb and Zn) concentrations (ppb) in digestive gland (w.w.) and muscle tissue (w.w.) as a function of carapace width of blue crabs (n = 15) captured from Sabine Pass and Lavaca Bay, Texas combined study sites in 2001.



Appendix Fig. 7. Trace metal (Ag, Cd, Cr and Cu) concentrations (ppb) in digestive gland (w.w.) and muscle tissue (w.w.) as a function of carapace width of blue crabs (n = 10) captured from Sabine Pass, Texas in 2001.



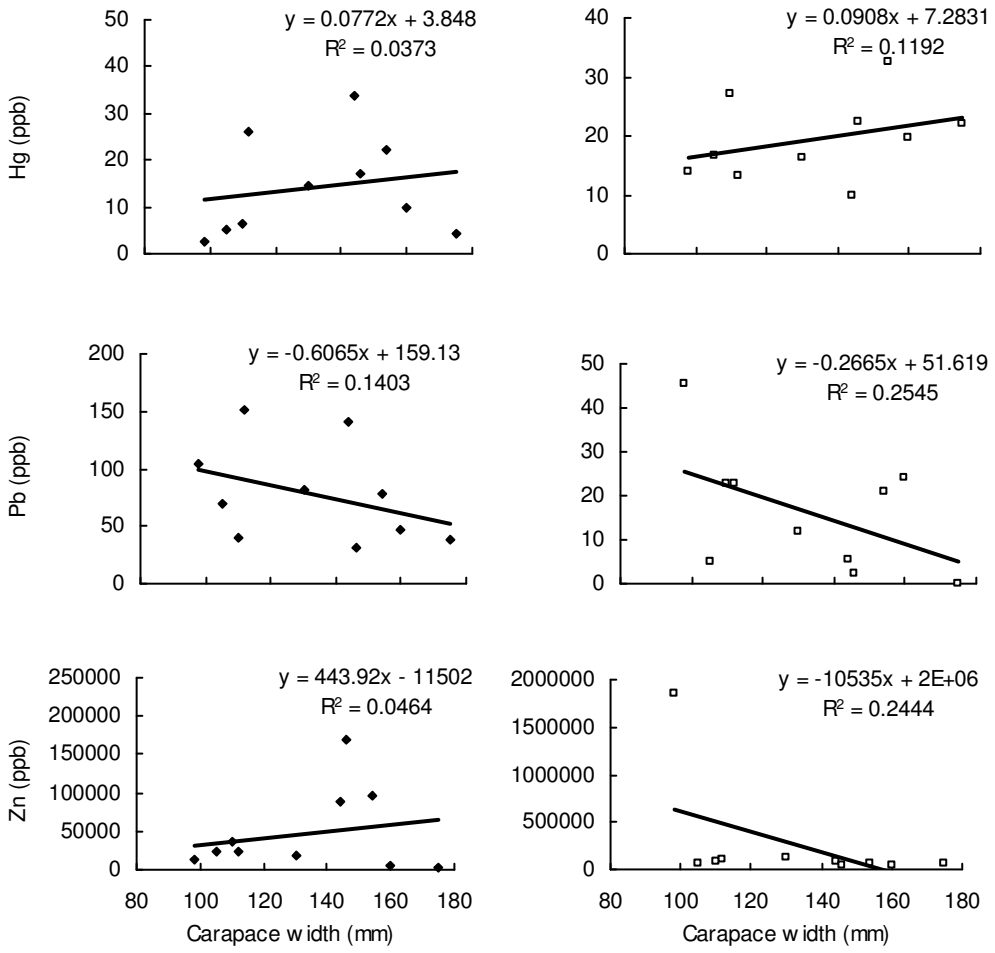
Appendix Fig. 8. Trace metal (Hg, Pb and Zn) concentrations (ppb) in digestive gland (w.w.) and muscle tissue (w.w.) as a function of carapace width of blue crabs (n = 10) captured from Sabine Pass, Texas in 2001.



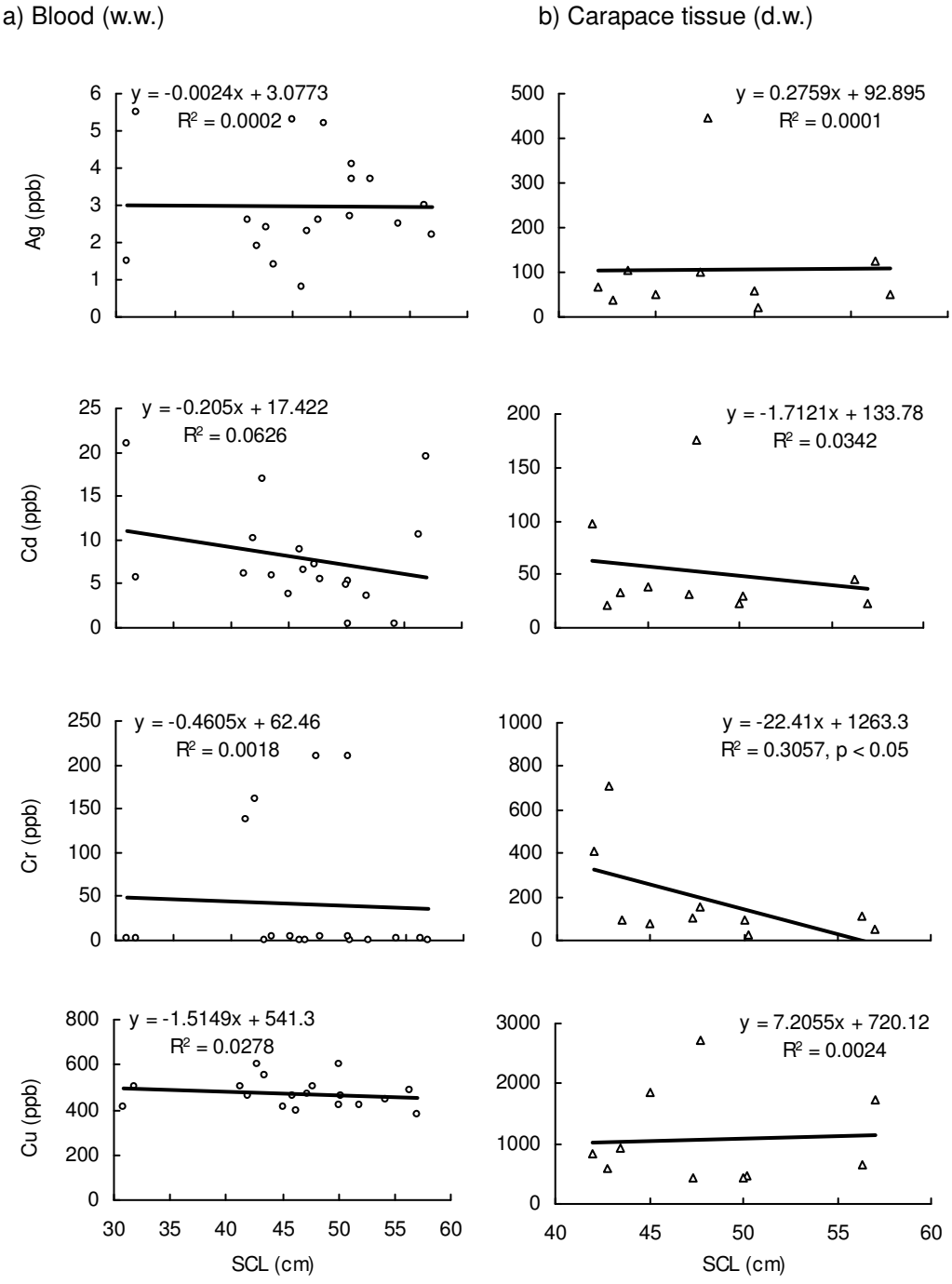
Appendix Fig. 9. Trace metal (Ag, Cd, Cr and Cu) concentrations (ppb) in digestive gland (w.w.) and muscle tissue (w.w.) as a function of carapace width of blue crabs (n = 10) captured from Calcasieu Pass, Louisiana in 2001.

a) Digestive gland (w.w.)

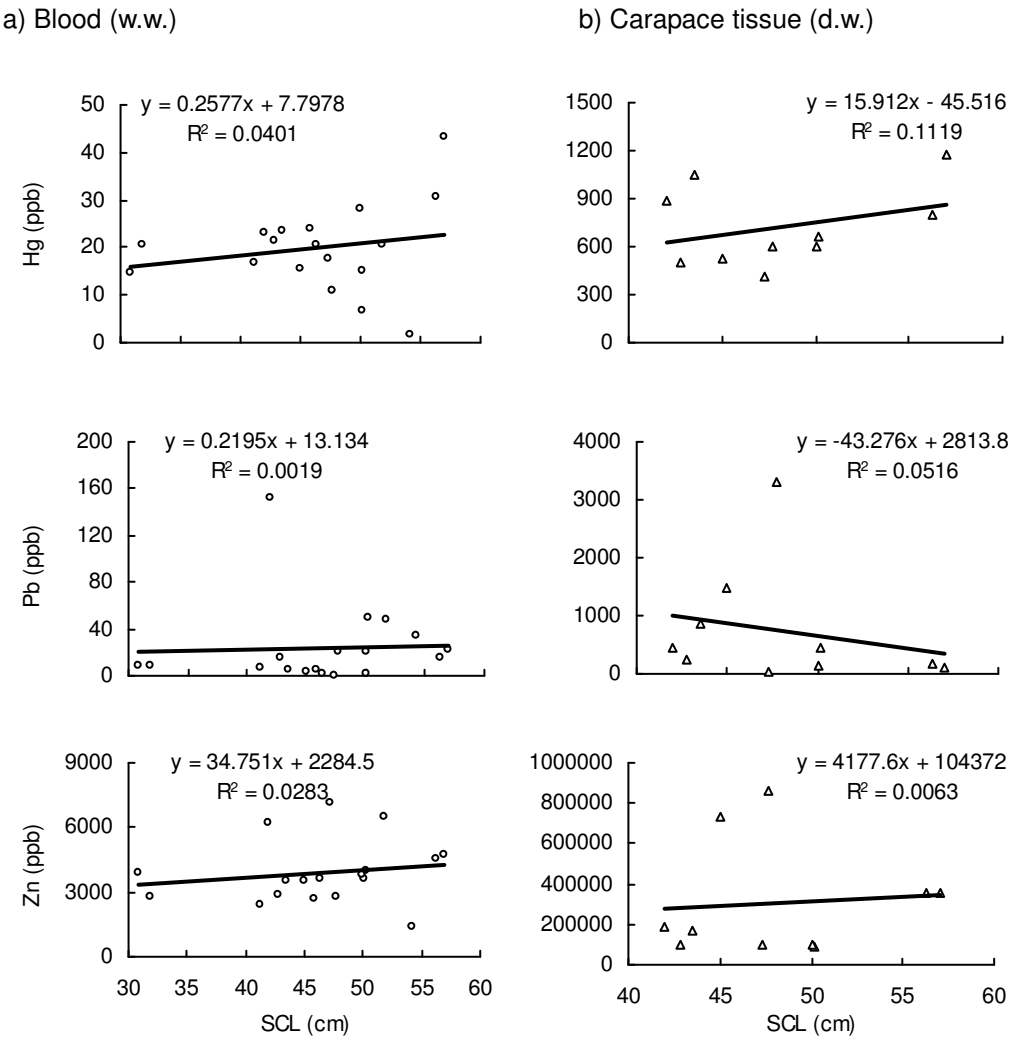
b) Muscle tissue (w.w.)



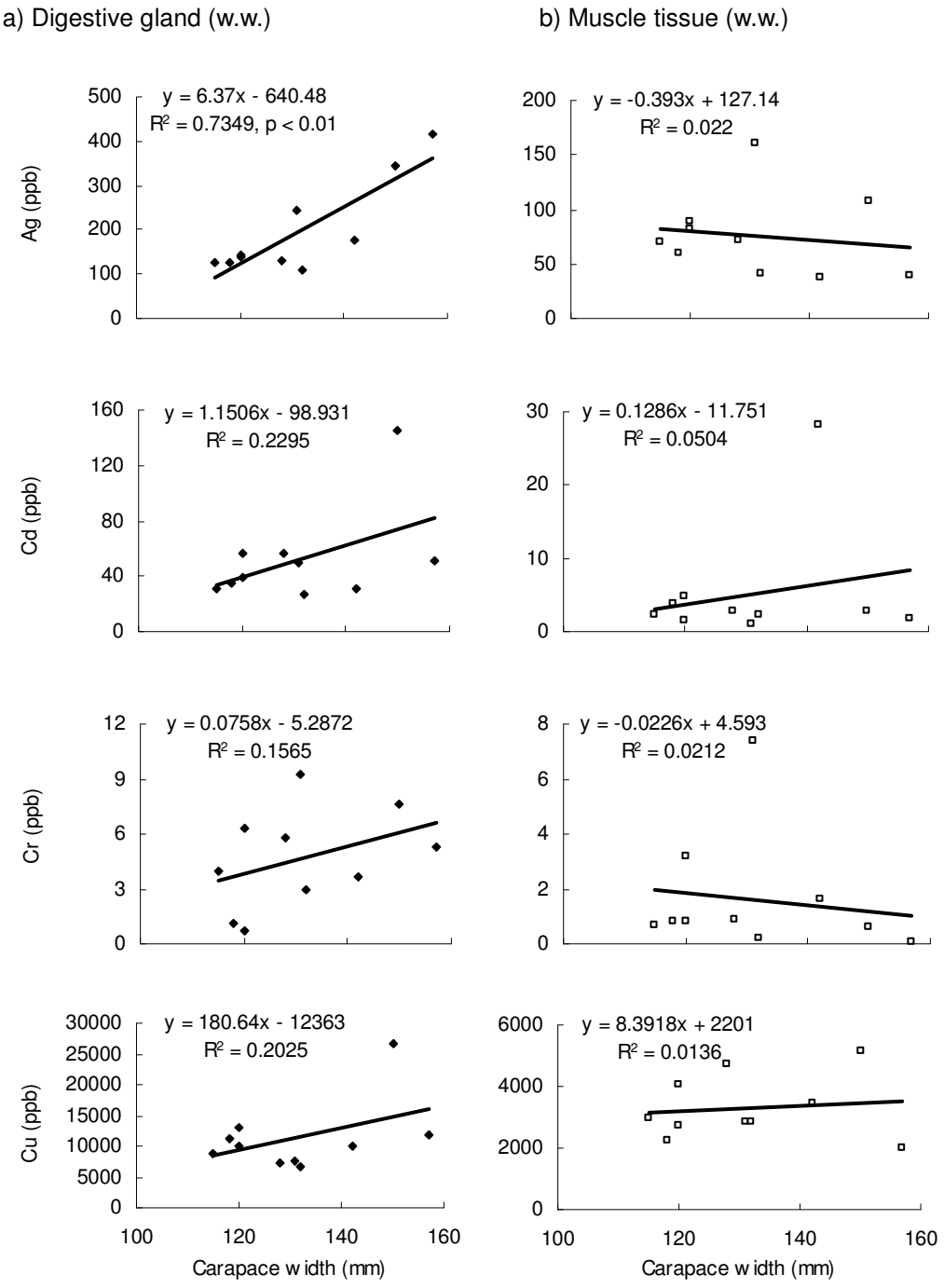
Appendix Fig. 10. Trace metal (Hg, Pb and Zn) concentrations (ppb) in digestive gland (w.w.) and muscle tissue (w.w.) as a function of carapace width of blue crabs (n = 10) captured from Calcasieu Pass, Louisiana in 2001.



Appendix Fig. 11. Trace metal (Ag, Cd, Cr and Cu) concentrations (ppb) in blood (w.w.) and carapace tissue (d.w.) of Kemp's ridleys (n = 18) captured from the southeast Atlantic during 2001-2002.



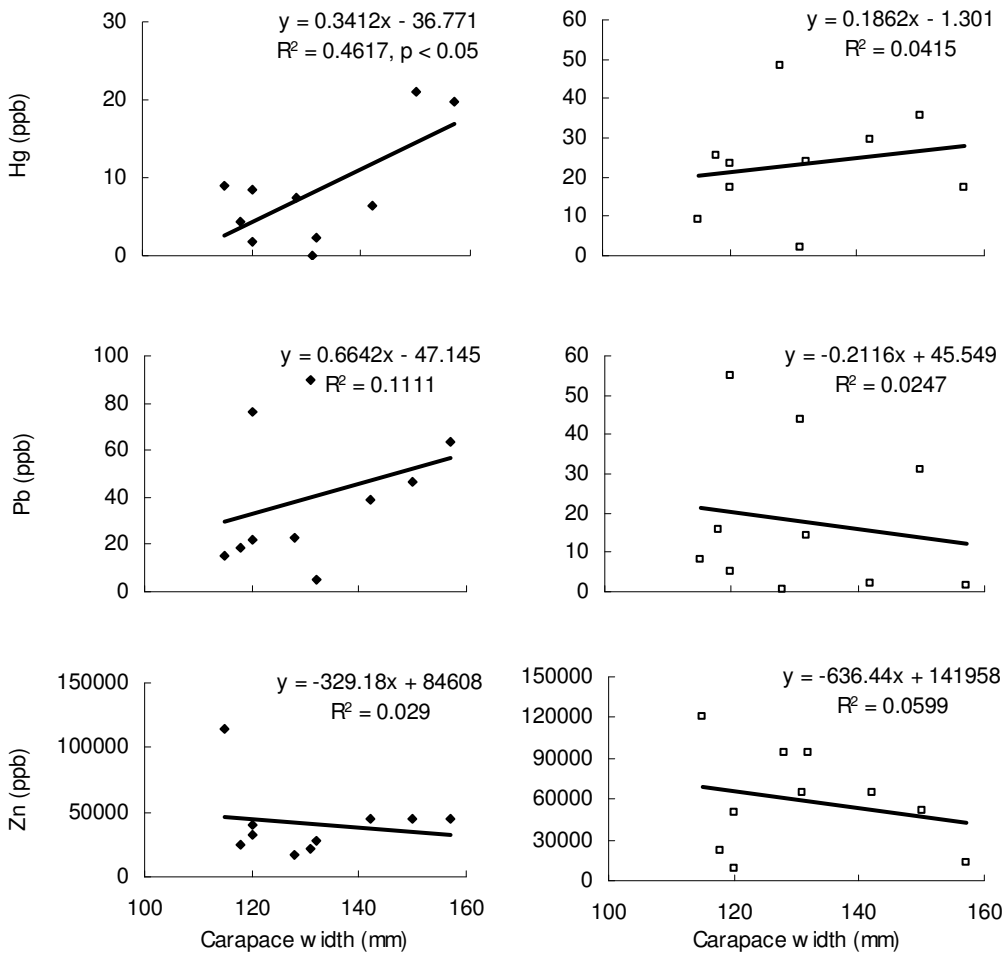
Appendix Fig. 12. Trace metal (Hg, Pb and Zn) concentrations (ppb) in blood (w.w.) and carapace tissue (d.w.) of Kemp's ridleys (n = 18) captured from the southeast Atlantic during 2001-2002.



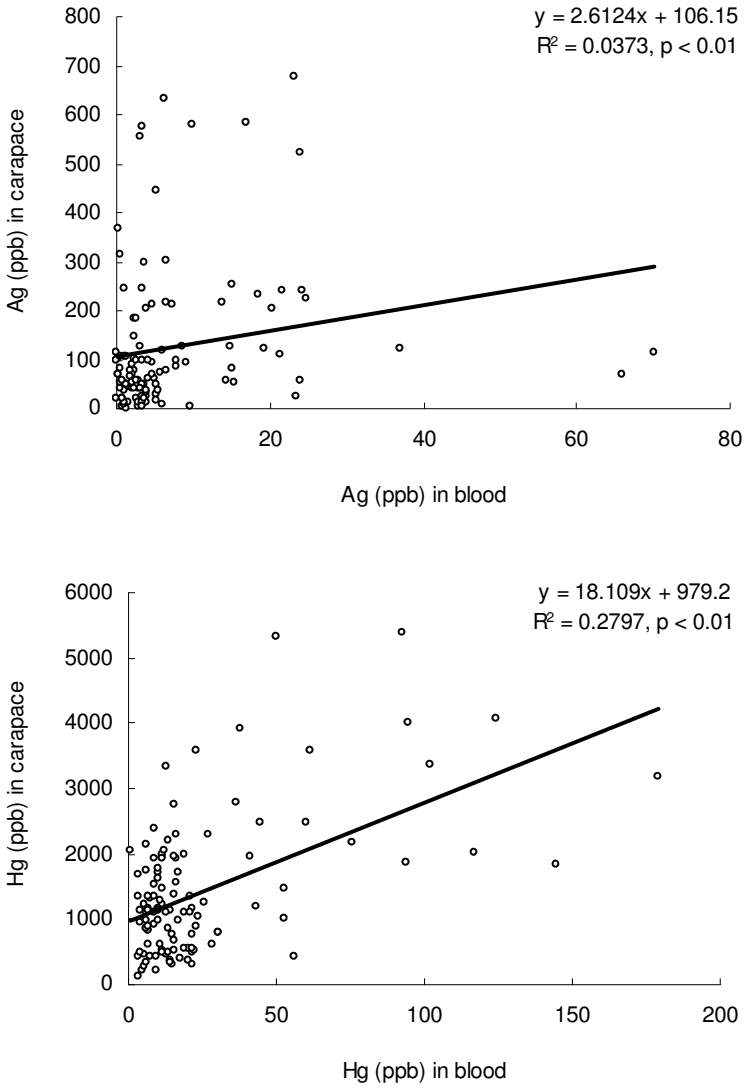
Appendix Fig. 13. Trace metal (Ag, Cd, Cr and Cu) concentrations (ppb) in digestive gland (w.w.) and muscle tissue (w.w.) as a function of carapace width of blue crabs (n = 10) captured from Rancho Nuevo, Mexico in 2002.

a) Digestive gland (w.w.)

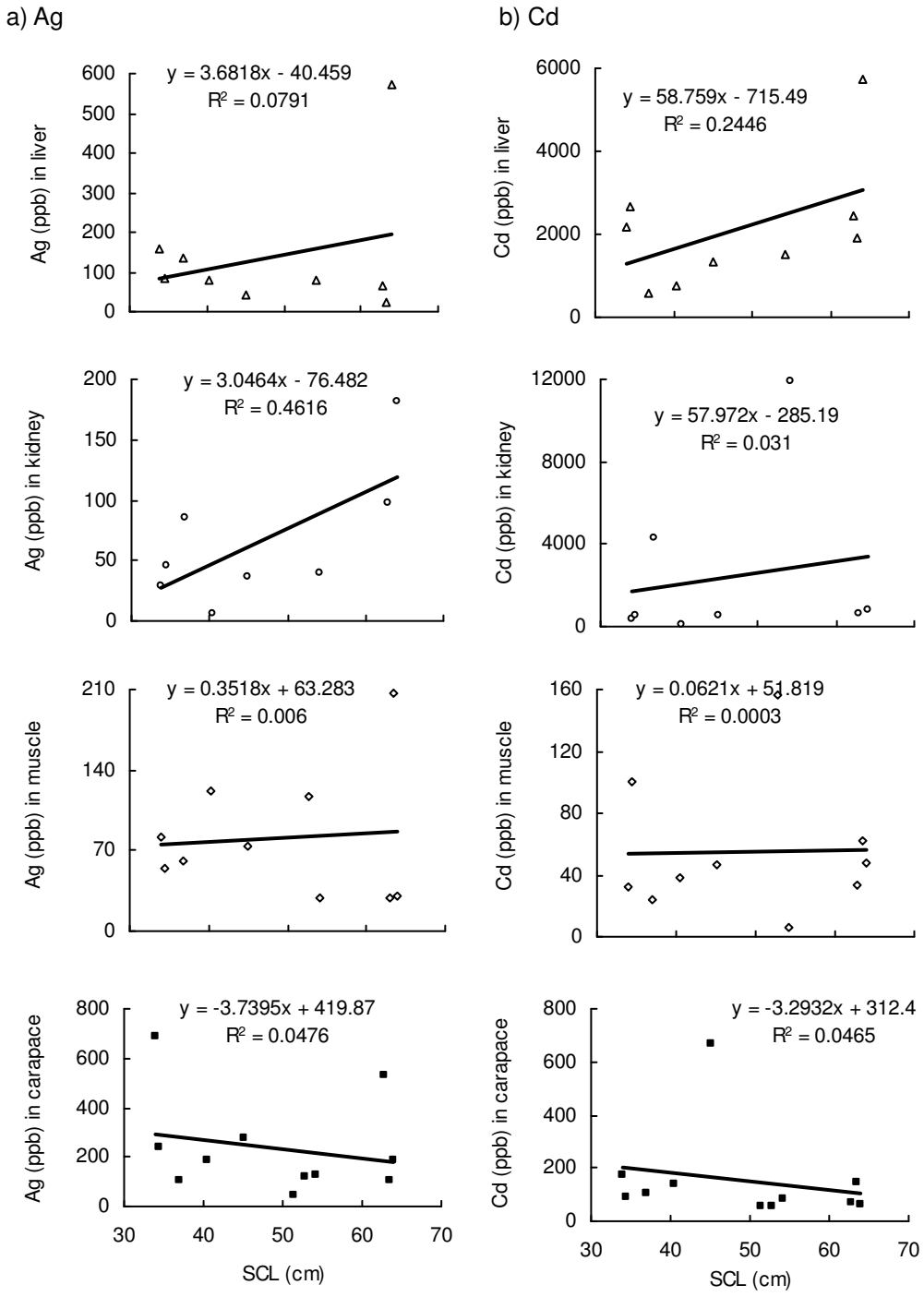
b) Muscle tissue (w.w.)



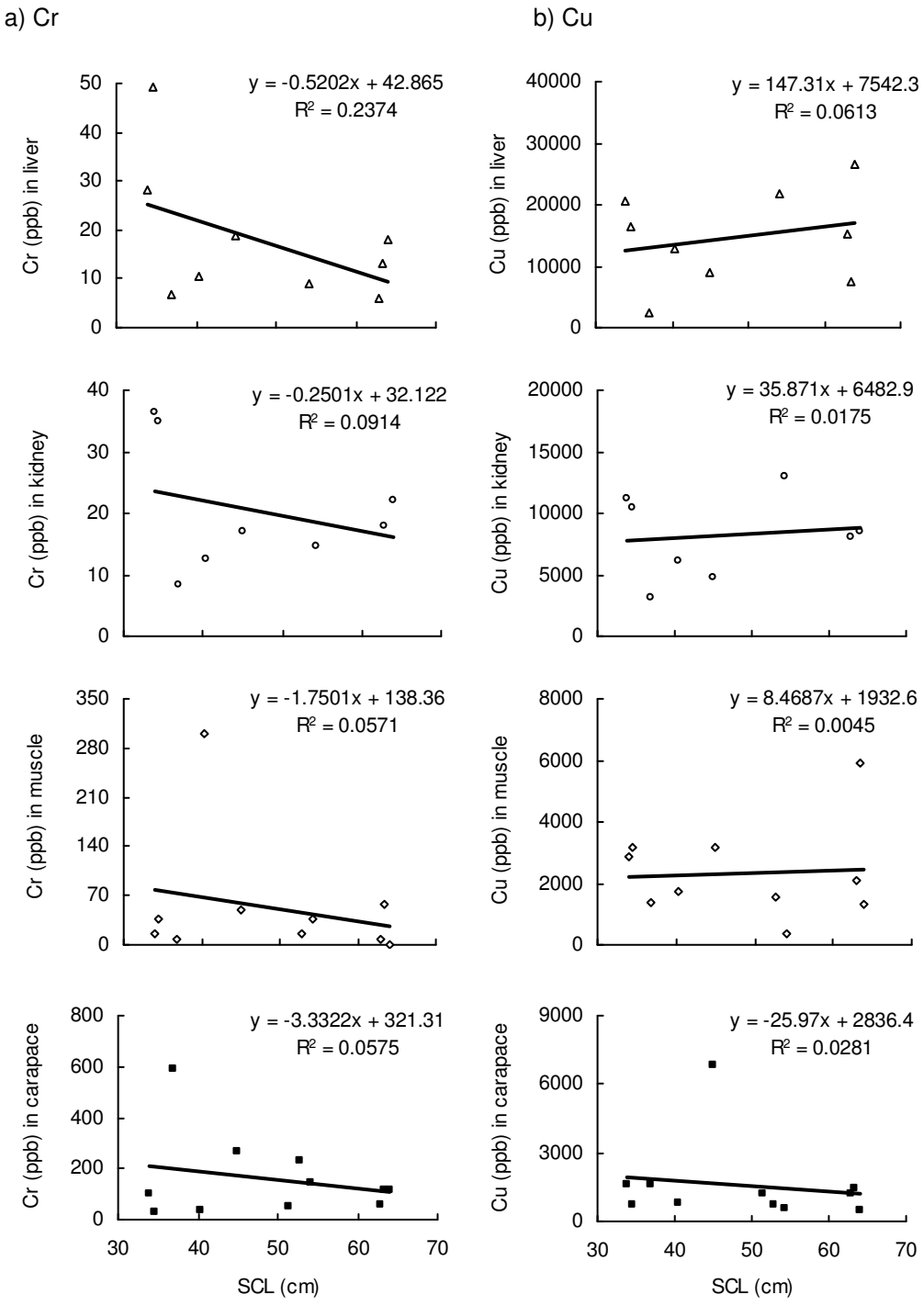
Appendix Fig. 14. Trace metal (Hg, Pb and Zn) concentrations (ppb) in digestive gland (w.w.) and muscle tissue (w.w.) as a function of carapace width of blue crabs (n = 10) captured from Rancho Nuevo, Mexico in 2002.



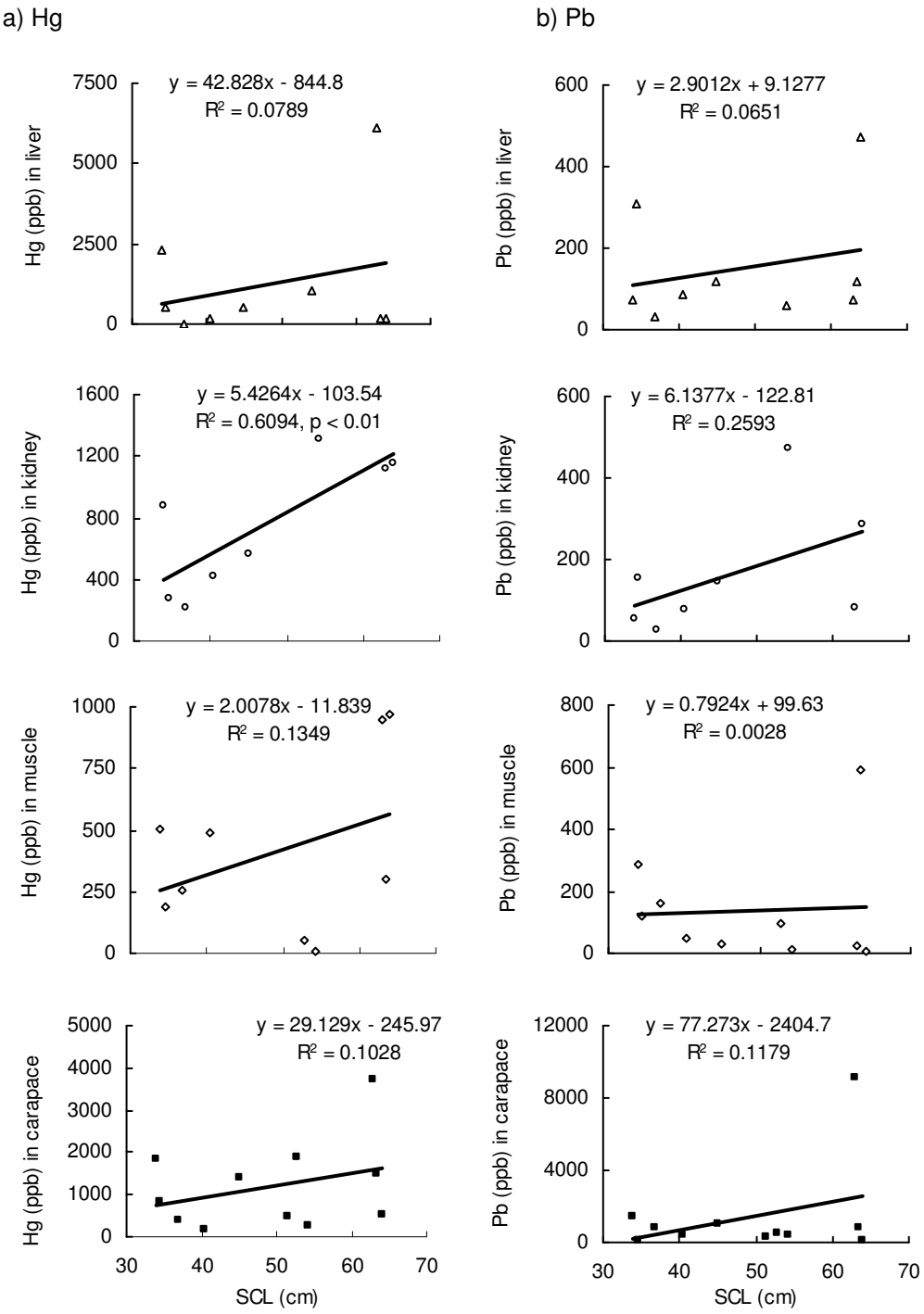
Appendix Fig. 15. Correlation of Ag and Hg concentrations (ppb) between blood and carapace tissue of wild Kemp's ridleys across all post-pelagic life stages.



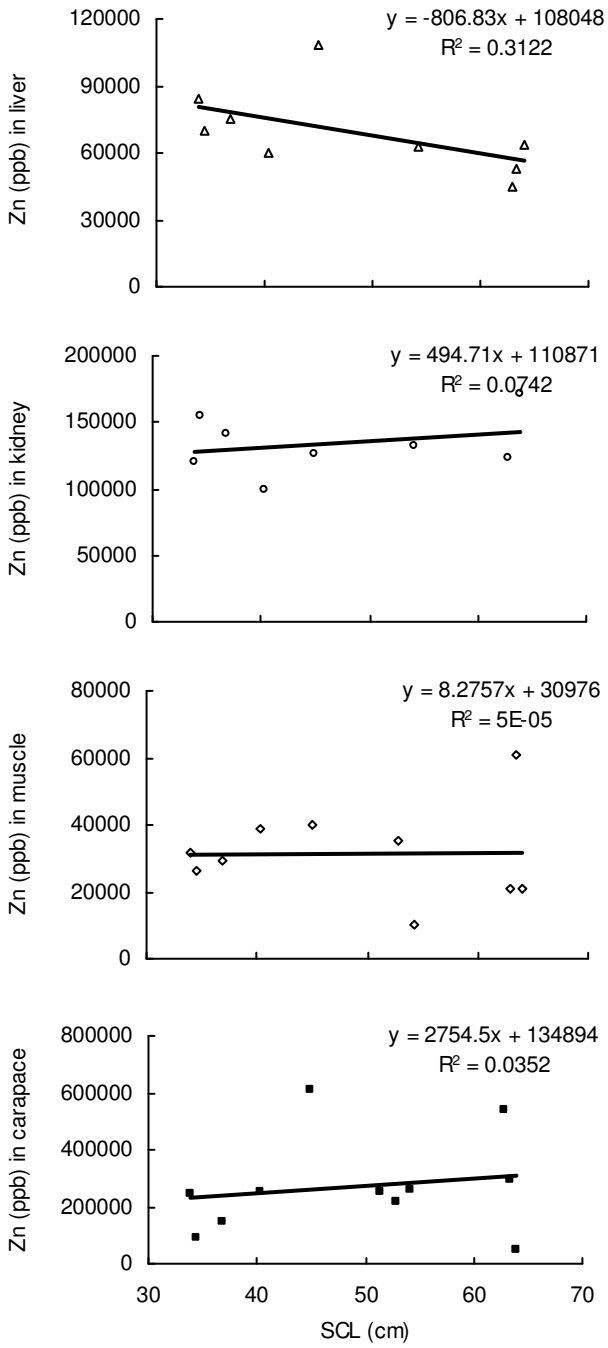
Appendix Fig. 16. Trace metal (Ag, Cd) concentrations (ppb) in liver, kidney, muscle, and carapace tissues (d.w.) as a function of SCL of stranded Kemp's ridleys from the Texas coast during 2001-2002.



Appendix Fig. 17. Trace metal (Cr, Cu) concentrations (ppb) in liver, kidney, muscle, and carapace tissues (d.w.) as a function of SCL of stranded Kemp's ridleys from the Texas coast during 2001-2002.



Appendix Fig. 18. Trace metal (Hg, Pb) concentrations (ppb) in liver, kidney, muscle, and carapace tissues (d.w.) as a function of SCL of stranded Kemp's ridleys from the Texas coast during 2001-2002.



Appendix Fig. 18. Zinc concentration (ppb) in liver, kidney, muscle, and carapace tissues (d.w.) as a function of SCL of stranded Kemp's ridleys from the Texas coast during 2001-2002.

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2003 Wang, H.-C., Landry, A.M. Jr., and Gill, G.A., 2003. Heavy metal concentrations in the Kemp's ridley (*Lepidochelys kempii*) and its blue crab (*Callinectes sapidus*) prey. In: Seminoff, J.A. complier, Proceedings of the Twenty-Second Annual Symposium on Sea Turtle Biology and Conservation. NOAA Technical Memo NMFS-SEFSC-503, p. 68. NOAA, Miami, Florida.

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