OCCURRENCE AND EFFECTS OF ORGANOCHLORINE CONTAMINANTS IN SEA TURTLES

by

Jennifer Michelle Keller

Nicholas School of the Environment and Earth Sciences
Division of Coastal System Science and Policy
and
Integrated Toxicology Program
Duke University

Date: March 10, 2003

Approved:

Patricia McClellan-Green, Supervisor

Celia Bonaventura

Kirk T. Fitzgerald

David Windsor

Dissertation submitted in partial fulfillment of
the requirements for the degree of Doctor
of Philosophy in the Nicholas School of
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ABSTRACT

Organochlorine (OC) contaminants, including polychlorinated biphenyls (PCBs) and pesticides, may have contributed to population declines by negatively affecting the immune and endocrine systems of many wildlife species. Little is known about the effects of contaminants on threatened or endangered sea turtles. This study investigated the effects of OC concentrations in tissues of juvenile loggerhead sea turtles (*Caretta caretta*) on their clinical health, immunity, and endocrine parameters. OC concentrations in blood, determined by gas chromatography with electron capture detection and mass spectrometry, significantly correlated with concentrations measured in adipose tissue. The correlations suggest that non-invasive blood sampling can be used to monitor accumulated concentrations of OCs in sea turtles. The OC concentrations significantly correlated to indicators of health, such as blood chemistries and hematology. Concentrations of some OC compounds correlated with an indicator of tissue damage (↑ aspartate aminotransferase), with alterations of protein, carbohydrate, and ion regulation (↑ blood urea nitrogen, ↑ total plasma protein, ↓ albumin:globulin ratio, ↓ glucose, ↑ osmolality, ↑ sodium, ↓ magnesium), with indicators of anemia and a modulated immune system (↓ red blood cells, ↓ hematocrit, ↓ hemoglobin, ↑ total white blood cells, ↑ heterophil:lymphocyte ratio). Moreover, turtles exhibiting signs of wasting (extremely emaciated and lethargic) had two orders of magnitude higher blood OC concentrations than apparently healthy turtles. OC concentrations also correlated positively with mitogen-induced lymphoproliferative responses, suggesting immunoenhancement. Vitellogenin (VTG),

an egg yolk precursor protein, was detected in the plasma of most female loggerhead turtles with straight carapace lengths of 77 cm or longer. Two to three percent of the females smaller than 77 cm were precociously expressing VTG and had higher blood OC concentrations compared to normal females of this size class that were not expressing VTG. Since it is not feasible to experimentally expose endangered sea turtles to toxicants, this study characterized a green sea turtle testes cell line for the activity of cytochrome P450 aromatase, an enzyme responsible for converting testosterone to estradiol. Aromatase activity in this *in vitro* model was induced by atrazine and inhibited by 4,4'-DDE. These findings suggest that the current concentrations of OCs in loggerhead sea turtles may be affecting their immune and endocrine systems.

DEDICATION

For Alex Girdley, who made learning fun by teaching earth science out of a van, tents, and well-worn hiking boats. His adventurous spirit will live forever in my memory and provide enduring inspiration, as I strive to follow his many footsteps.

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ALP	Alkaline phosphatase
AST	Aspartate aminotransferase
BUN	Blood urea nitrogen
cAMP	8Br- cyclic AMP
ConA	Concanavalin A
CPK	Creatine phosphokinase
DDD	Dichlorodiphenyldichloroethane
DDE	Dichlorodiphenyldichloroethylene
DDT	Dichlorodiphenyltrichloroethane
Dex	Dexamethasone
ECD	Electron capture detection
FBS	Fetal bovine serum
FP	Fibropapillomatosis
GC	Gas chromatography
GGT	Gamma glutamyl transferase
G:L ratio	Granulocyte to lymphocyte ratio
GST-TS	Green sea turtle testes cell line
HCH	Hexachlorohexane
HCB	Hexachlorobenzene
HCG	Human chorionic gonadotropin
HCT	Hematocrit or packed cell volume
HGB	Hemoglobin

H:L ratio	Heterophil to lymphocyte ratio
LDH	Lactate dehydrogenase
LOD	Limit of detection
LP	Lymphoproliferation
LPS	Lipopolysaccharide
MS	Mass spectrometry
MTT	3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyl tetrazolium
NIST	National Institute of Standards and Technology
OC	Organochlorine
4-OHA	4-androstenoldione
PCBs	Polychlorinated biphenyls
PCV	Packed cell volume or hematocrit
PDB	Phorbol 12,13-dibutyrate
PHA	Phytohemagglutinin
RBC	Red blood cell
SCL	Straight carapace length
SD	Standard deviation
SI	Stimulation index
SRBC	Sheep red blood cell
SRM	Standard Reference Material
TEQ	Dioxin toxic equivalents
VTG	Vitellogenin
WBC	White blood cell

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CHAPTER ONE

Sea Turtles as a Case Study for Integrating Wildlife Toxicology and Conservation Biology

ABSTRACT

Endangered sea turtles are a timely case study for integrating environmental toxicology and conservation biology. Most populations of sea turtles have suffered dramatic declines. Many have partially recovered after conservation efforts were established, but some are still in decline. Contaminants may have contributed to past and current declines, but until now, the effects of contaminants on sea turtles have not been thoroughly studied. This review examines evidence suggesting that organochlorine (OC) contaminants affect a variety of wildlife. An emphasis is placed on those effects that directly influence the three vital rates that concern conservation biologists: survival, reproduction, and growth. Effects that directly relate to survival include early life-stage mortality, cancer, and immunotoxicity. Reproduction may be reduced by developmental abnormalities, altered sex ratios, and reproductive failure. Growth rates may be influenced by contaminants that alter thyroid actions. Highlighted effects include the immunosuppression and/or endocrine disruption seen in birds and snapping turtles from the Great Lakes, alligators from Lake Apopka, a contaminated lake in Florida, and beluga whales from the St. Lawrence estuary. Sea turtle life history traits are explained in the context of how each life stage may be susceptible to these contaminant effects. In addition, a comprehensive list of polychlorinated biphenyls (PCBs), 4,4'-DDE, and chlordane concentrations measured in sea turtle tissues is provided. The concentrations found in sea turtle blood, adipose, and eggs are then compared to those of other reptiles, birds, and mammals. The occurrence of OC contaminants in sea turtle tissues and the abundant evidence linking

OCs to effects in other wildlife warrant investigation into the effects of OCs on sea turtle populations.

NEED FOR INTEGRATING WILDLIFE TOXICOLOGY AND CONSERVATION BIOLOGY

In 1962, Rachel Carson shocked society with descriptions of how environmental contaminants affected wildlife populations (Carson 1962). She blamed the death of song birds on the heavy-use of pesticides and other pollutants. DDT decimated bald eagle (*Haliaeetus leucocephalus*) and double-crested cormorant (*Phalacrocorax auritus*) populations driving some populations to near extinction (see reviews of Grasman *et al*. 1998; Bowerman *et al*. 1995). Still today, the effects of contaminants on wildlife are apparent as are population declines. While these observations are likely linked, we rarely integrate environmental toxicology and conservation biology. The authors of a recent editorial that was concurrently published in *Environmental Toxicology and Chemistry* and *Conservation Biology* called for the integration of these two fields (Hansen and Johnson 1999a, 1999b). They stated that "Integrating these two fields is crucial to meeting the long-term goals of each."

Toxicologists often focus on effects at the subcellular to individual levels of biological organization, while conservation biologists are concerned with the population to ecosystem levels. Many toxicological effects seen at the individual level directly relate to population level consequences (Fig. 1.1). For example, suppression of the immune system by contaminants may lead to greater susceptibility to disease

and ultimately to increased mortality. Similarly, altered endocrine functions may increase the incidence of developmental abnormalities which may result in reduced reproduction.

Conservation biologists are concerned with trends in populations, whether they are increasing or decreasing in numbers. They often perform population viability analyses (PVA) in order to predict the extinction or recovery of a species. To construct these models, three vital rates are needed: survival probabilities, reproductive potential, and growth rates. Contaminants are known to affect all three of these parameters and should be factored into PVAs. To do this, however, is not simple. One must first know whether the species in question is sensitive to environmentally relevant concentrations of contaminants.

WHY USE SEA TURTLES AS A CASE STUDY?

Sea turtles are a timely case study for integrating these disciplines. Populations of all seven species around the world have declined in numbers and are now protected by national agencies and international treaties. All species, except the Australian flatback sea turtle (*Natator depressus*), inhabit U.S. waters and have protected status under the U.S. Endangered Species Act of 1973. Specifically, the loggerhead sea turtle (*Caretta caretta*) is listed as a threatened species. Conservation efforts, including the use of turtle excluder devices (TEDs) by fishermen, have helped in the recovery of loggerheads, but one subpopulation has not improved. For unknown reasons, this population that nests from northern Florida to North Carolina has been declining by 2 to 3 % per year (TEWG 2000). Because the loggerhead has

not recovered, the U.S. National Marine Fisheries Service is currently considering an increase in its status to endangered. It is not known whether environmental contaminants contributed to the past and current declines of sea turtle populations.

Very few studies have investigated the effects of contaminants on sea turtles, probably due to the logistical problems of working with these animals. Their protected status restricts experimentation and sampling to only non-invasive methods. Their complex life history also creates major challenges for researchers (Fig. 1.2). Because they are long-lived, highly migratory, and rare marine species, little is known about their basic biology. Additional difficulties include the inability to determine the sex of juveniles or the age of live sea turtles from external morphology.

LOGGERHEAD SEA TURTLE LIFE HISTORY

Although the life history of sea turtles creates difficulties for researchers, its complexity and uniqueness may provide for interesting toxicological studies. On ocean-facing beaches along the U.S. Atlantic coast, adult female loggerhead turtles return to their natal beach every 2 to 3 years to lay eggs (Fig. 1.2). In one nesting season, a loggerhead turtle will lay an average of 4 clutches of 112 eggs each. An egg is approximately the size of a ping-pong ball and weighs 33 g (Miller 1997). That calculates to roughly 15 kg of egg production per female every other or every third year. If contaminants are maternally transferred to sea turtle eggs as has been shown in other turtles (Pagano *et al*. 1999), this large egg production could facilitate the dumping of a significant portion of the adult female contaminant load.

After the eggs have been deposited into a nest chamber, they incubate for approximately 60 days. The incubation duration as well as the sex of the embryo is dependent upon the incubation temperature. Many reptile species, and most turtle species, share this developmental characteristic called temperature-dependent sex determination (TSD). In sea turtles, the embryonic sex is determined near the middle third of development. Females result from warmer temperatures and males from cooler temperatures with a pivotal temperature of 28.7 ^{o}C for loggerhead sea turtles (Ackerman 1997). Temperature influences the expression of a steroid-synthesizing enzyme, cytochrome P450 aromatase, which converts testosterone to estrogen (Desvages *et al*. 1993). In this way, external temperature is thought to direct the steroid hormone production and consequently differentiation of the gonads. Contaminants, especially those that have estrogenic properties, may override temperature signals and alter sex ratios (Willingham and Crews 2000; Bergeron *et al*. 1994).

Hatching success is typically 80 % or higher for sea turtle nests, except when disturbed by predators, flooded, or invaded by plant roots or microbes (Ackerman 1997), but the effect of contaminants on hatching success is not known. Turtles emerge as a group at night and head directly towards the sea. During their first 24 hours the hatchlings swim in a frenzy to reach the Gulf Stream which sweeps them northward as they begin their pelagic juvenile stage.

In the pelagic juvenile stage, loggerheads circum-navigate the entire North Atlantic Ocean with the help of the North Atlantic gyre. They grow from 4 cm to 45 cm straight carapace length (SCL) while feeding among floating Sargassum. They

inhabit the open ocean and coastal areas around the Azores, Madeira, Spain, Africa, and the Canary Islands (Musick and Limpus 1997). Some even enter the Mediterranean Sea (Laurent *et al*. 1998). This poorly understood stage is thought to last approximately 10 years.

At roughly 45 cm SCL, loggerhead turtles return to U.S. coastal waters and estuaries to begin the benthic juvenile stage, named for their dietary preferences of benthic crustaceans and molluscs. In this stage of development, the turtles grow from 45 cm to 80 cm SCL over possibly another decade, and they make seasonal migrations each year. Presumably their nearshore benthic feeding strategy makes them more susceptible to exposure to organic contaminants that typically accumulate in sediments and nearshore fauna. Moreover, this pubertal stage may be particularly sensitive to contaminant effects, because the reproductive organs are beginning to mature and secondary sexual characteristics are beginning to form.

Adult loggerhead turtles relocate to poorly described adult foraging areas and return to U.S. coastal waters during the mating and nesting season. Although there is debate over the age at maturity, loggerheads become sexually mature somewhere around 25 to 35 years of age (Chaloupka and Musick 1997; Snover 2002) and the average SCL of nesting females has been measured at 87 cm (Miller 1997) and 92 cm (Frazer and Ehrhart 1985). In non-nesting years, turtles build their energy reserves and undergo vitellogenesis for the next breeding season in which they deposit vitellogenin into follicles as an egg yolk precursor. As mating season approaches, dramatic changes in plasma testosterone and estradiol concentrations coincide with the

timing of migration to the nesting beach (Wibbels *et al*. 1990; Owens 1997). Mating occurs near the nesting beaches about one month prior to the first nest being laid.

The loggerhead life history is very complex and makes them susceptible to the effects of contaminants at several life stages. Contaminants have been shown to affect each of these life stages in other organisms. Even subtle effects at a sensitive life stage or effects compounded over an entire lifetime may ultimately contribute to population declines.

ORGANOCHLORINE CONTAMINANTS

Organochlorine (OC) contaminants are lipophilic, aromatic compounds. Their chlorination makes them persistent in the environment. Compounds categorized as OCs include the polychlorinated biphenyls (PCBs), dioxins, furans, and organochlorine pesticides, such as dichlorodiphenyltrichloroethane (DDT), chlordanes, mirex, dieldrin, lindane, and heptachlor. Once heavily-used, most of these compounds have been banned or restricted in developed countries.

The production of PCBs began in 1929 under many trade names, one being Aroclor (Safe 1993). Aroclor mixtures were used for many industrial purposes, such as flame retardants, coolants, and dielectric fluids. In 1960s, PCBs were first detected in environmental samples and a realization of their toxicity quickly followed. In 1979, PCB production for industrial use was banned in the U.S.

From the 1950s to 1970s, DDT was sprayed heavily as an agricultural and municipal insecticide. It was also banned in the 1970s because of its ability to bioaccumulate and negatively affect non-target animals, including the bald eagle and

other birds. DDT continues to be used in developing countries to fight mosquito-born diseases such as malaria. Still today OC contaminants are found in sediments and animal tissues because they are highly persistent, bioaccumulate into organic matrices, and biomagnify up the food web.

The ban of these compounds, along with most of the other OC pesticides, came after discovering their toxicity to a multitude of systems. OCs are known to cause cancer, developmental abnormalities, reproductive impairments, liver damage, immunotoxicity, and endocrine disruption in laboratory rodents (see reviews by Safe 1993; Crisp *et al*. 1998). Evidence of these effects has also been shown in humans and wildlife (see reviews by Colborn *et al*. 1993; Fox 2001a; Keller *et al*. 2000).

EFFECTS OF OCS ON WILDLIFE WITH AN EMPHASIS ON THE THREE VITAL RATES

As mentioned previously, OC contaminants may contribute to wildlife population declines by negatively affecting the three vital rates that concern conservation biologists. Contaminants have resulted in reduced survivorship, reduced reproductive output, and altered growth rates of wildlife populations.

Survival

Contaminants may decrease survival by many mechanisms, including acute mortality, cancer, and suppression of the immune system. Acute mortality was observed in birds during heavy use of OC contaminants (Carson 1962). It is rarely seen anymore in adult wildlife because it requires very high concentrations, but early life-stage mortality has been documented recently. A large percentage of first-born

offspring of marine mammals do not survive. Mothers are known to transfer larger doses of their accumulated OC compounds into their first-born than subsequent offspring (Lee *et al*. 1996; Beckman *et al*. 1999; Ylitalo *et al*. 2001). These high levels of OC contaminants are believed to contribute to the death of the young animals (Schwacke *et al*. 2002). In the Great Lakes, which are heavily contaminated with PCBs, embryonic birds and fish often suffer from edema and deformities and subsequently die (Gilbertson *et al*. 1991; Wright and Tillitt 1999). Complete brood mortality was seen more often in offspring of American kestrels (*Falco sparverius*) that were exposed to PCBs *in ovo* than controls (Fernie *et al*. 2001). Early life-stage mortality has also been documented in reptilian wildlife. American alligators (*Alligator mississippiensis*) from Lake Apopka, a highly contaminated lake in Florida, had reduced hatching success and greater mortality 10 days after hatching than alligators from reference lakes (Guillette *et al*. 1994).

Adult sea turtles may not accumulate lethal concentrations of these contaminants since they live in open ocean habitats and feed lower on the food chain than fish-eating marine mammals. However, some venture into the mouths of rivers and inhabit nearshore coastal waters and estuaries where localized high concentrations of contaminants may reach their food sources. Although sea turtles may not accumulate such high levels, their sensitivity to these contaminants is completely unknown.

The transfer of a lifetime of accumulated contaminants from an adult female into eggs may reach lethal levels for a developing embryo, especially into the eggs of

first-time nesters. Maternal transfer and the effect of contaminants on early life stages should be studied.

OC contaminants are known carcinogens in laboratory rodents and possibly humans (see Safe 1993; Colborn *et al*. 1993). Cancer is rarely observed in wildlife because it usually occurs in older animals past their reproductive age. However, contaminant-induced cancers can be seen in some wildlife populations. Beluga whales (*Delphinapterus leucas*) that live in the highly polluted St. Lawrence estuary accumulate such high levels of PCBs and pesticides that their carcasses are considered toxic waste according to Canadian regulations. This population was driven to near extinction and in 1995 only approximately 500 individuals remained (see De Guise *et al*. 1995). The beluga whales of the St. Lawrence estuary suffer many health problems, including cancer which is not seen in the less exposed Arctic population. Cancer is a major cause of death in these animals and was found in 27% of the adult whale carcasses (Martineau *et al*. 2002). The brown bullhead catfish (*Ameiurus nebulosus*) is also susceptible to carcinogenic compounds, and those that live in OC-contaminated sites had significantly increased prevalence of hepatic and skin tumors (Pinkney *et al*. 2001).

It is difficult to link cancer to negative population consequences. Cancer may not necessarily lead to death, and it usually occurs post-reproduction when survival is not as important for the population. For example, cancer rates are high in adult humans, but this does not affect the population growth rate. To our knowledge, only one sea turtle has been documented with cancer (a multicentric lymphoblastic lymphoma, Oros *et al*. 2001), but a common and spreading disease among sea turtles

is fibropapillomatosis. Fibropapillomas are non-cancerous tumors, but they are often lethal as they can cover the eyes, mouth, or anus and interrupt foraging. The disease is thought to be caused by a herpes virus but researchers have suspected that OC contaminants may promote the tumors by suppressing the immune system (Aguirre *et al*. 1994; Schumacher *et al*. 1998).

The immune system is very sensitive to contaminants. OCs have been shown to suppress the immune system (immunosuppression), increase it (immunoenhancement), or cause it to recognize and attack self molecules (autoimmunity). OC contaminants have been shown to suppress the immune systems of wild fish, birds, and mammals (see review by Keller *et al*. 2000). Caspian tern (*Sterna caspia*) chicks from OC-contaminated sites in the Great Lakes showed suppressed immune responses (Grasman *et al*. 1996) and blood PCB concentrations in the terns significantly correlated to reduced T-lymphocyte responses (Grasman and Fox 2001). Several immune parameters were reduced in harbor seals (*Phoca vitulina*) that were fed fish from the OC-contaminated Baltic Sea (Ross *et al*. 1996). In addition, increasing blood OC concentrations correlated with suppressed immune function in bottlenose dolphins (*Tursiops truncatus*; Lahvis *et al*. 1995). It is thought that OC contaminants contributed to massive mortality events of marine mammals by weakening their immune systems (Hall *et al*. 1992; Aguilar and Borrell 1994; Borrell *et al*. 1996). Recently, immunoenhancement has been observed in animals exposed to OC contaminants. Male American kestrels exposed to PCB mixtures exhibited an increased PHA-skin response (Smits *et al*. 2002). Alligators from Lake Apopka had elevated mitogen-induced lymphoproliferative (LP) responses compared to alligators

from reference lakes (Rooney 1998). Likewise, seals with higher tissues levels of OCs and in herring gulls (*Larus argentatus*) inhabiting OC-contaminated sites demonstrated greater LP responses (Shaw *et al*. 2002; Croisant and Grasman 2002).

Little is known about the sea turtle immune system or about diseases that they encounter. As in other reptiles, their immune system is expected to fluctuate with seasonal changes, such as temperature and photoperiod. Diseased animals typically have weakened immune systems. For example, immunosuppression was seen in green sea turtles (*Chelonia mydas*) afflicted with fibropapillomatosis, but it is not known whether the disease caused the immunosuppression or prior immunosuppression caused the disease (Work *et al*. 2001; Cray *et al*. 2001).

Sea turtles must fight many pathogens and parasites, including parasitic trematodes and the virus that is suspected to cause fibropapillomatosis. We still do not have good knowledge of many of their illnesses, and most turtles that are found dead or dying cannot be attributed to a particular cause. For example, 66 loggerhead sea turtles were admitted to the Karen Beasley Sea Turtle Rescue and Rehabilitation Center from 1996 to 2002 (KBSTRRC 2002). Many of the turtles had boat-related injuries or were cold-stunned, but 32% were ill from unknown causes. Many of the ill turtles were extremely lethargic and emaciated. We need to better understand the sea turtle immune system, diseases, and how contaminants may affect these.

Reproduction

Reduced reproduction in an already threatened species can result in severe population declines. OC contaminants can impair this vital rate via a multitude of mechanisms, but most involve a disruption of the endocrine system. Endocrine-

disrupting OC contaminants have resulted in developmental abnormalities, altered sex ratios, and reduced reproductive output in wildlife populations.

Developmental abnormalities that last into adulthood may result in reduced future reproduction. High rates of limb deformities were observed in adult mudpuppies (*Necturus maculosus*), an amphibian salamander, living in a highly OC-polluted site of the Great Lakes (Gendron *et al*. 1994). Atrazine, a heavily-used herbicide, at concentrations that are found in surface and ground waters demasculinized the larynx of male tadpoles (*Xenopus laevis*) and resulted in hermaphrodites (Hayes *et al*. 2002a). An hermaphroditic beluga whale was found in the highly contaminated St. Lawrence estuary (De Guise *et al*. 1994). Birds in OC-contaminated sites have shown bill abnormalities, club feet, missing eyes, and defective feathering (Gilbertson *et al*. 1991). Deformities in snapping turtle (*Chelydra serpentina*) embryos and hatchlings were associated with sites in the Great Lakes region that were more contaminated with OCs (Bishop *et al*. 1998). Deformities included limb malformations, tail deformities, undeveloped or malformed carapace, missing eyes, and recessed lower jaws. Male snapping turtles from these sites had feminized secondary sexual characteristics (de Solla *et al*. 1998). Similarly, alligators from Lake Apopka, which have higher blood concentrations of OC pesticides compared to reference lakes (Guillette *et al*. 1999), exhibited altered steroid hormone profiles and shorter penises (Guillette *et al*. 1994; 1996), while turtles (*Chrysemys nelsoni*) in this lake developed ovotestes (Guillette *et al*. 1996). These endocrine effects of OC compounds are thought to have suppressed reproductive success and contributed to the decline of this alligator population (Guillette *et al*. 1994).

Embryonic development of sea turtles has been well characterized (Miller 1985). Developmental abnormalities such as albinos, crossed beaks, and scute malformations are common in sea turtle embryos that do not survive incubation, but the causes are unknown. Very little is known about pubertal development, such as the timing of reproductive maturation, or the onset of secondary sexual characteristics, or even plasma estrogen levels of immature turtles. The extent to which contaminants affect these developmental stages of sea turtles is a complete unknown.

Proper sex ratios are vital to the stability of a population. Too few females may mean fewer offspring, while too few males may mean fewer mating pairs and decreased fertilization. Female-skewed sex ratios and feminization of herring gulls were found in the Great Lakes in the 1970s, and gonadal abnormalities associated with OC contaminants were still seen in gull chicks in the late 1990s (see review by Fox 2001b). Sex ratios of fish from the Great Lakes were also highly skewed towards females in 1967 and returned to equal males and females in the late 1980s (see review by Fox 2001a). This sex ratio recovery was significantly correlated with the declining tissue concentrations of 4,4'-DDE.

Sex ratios in many reptiles and most turtles with TSD are governed by the delicate balance of nest temperature and steroid synthesis. In embryos with TSD, the incubation temperature signals the embryo to become male or female, however developing turtles can become female when incubated at male temperatures and exposed to estrogen or estrogenic contaminants (Wibbels *et al*. 1991, Bergeron *et al*. 1994, Willingham and Crews 1999). Sex reversal in turtles is very sensitive to OC contaminants, such as PCBs and 4,4'-DDE and may not follow the "threshold dose

concept" (Sheehan *et al*. 1999). In theory, one molecule of a potent contaminant could reverse the sex of a developing turtle. The mechanism controlling sex reversal is not fully known but involves, at least in part, cytochrome P450 aromatase. Aromatase converts testosterone to estradiol and is expressed at greater levels in embryonic gonads and brains incubated at female-producing temperatures (Willingham *et al*. 2000; Place *et al*. 2001; Milnes *et al*. 2002). By altering the steroid synthesis of aromatase, OC contaminants may override temperature signals and reverse the sex of turtles thereby altering sex ratios. PCBs are known to alter aromatase activity in rodents and embryonic red-eared slider turtles (*Trachemys scripta*) exposed in the laboratory (Gertenberger *et al*. 2000, Hany *et al*. 1999; Willingham and Crews 2000). OCs are also associated with possible changes in aromatase activity in wild amphibians and reptiles (Hayes *et al*. 2002b; Crain *et al*. 1997).

Sexual differentiation of all 7 species of sea turtles is governed by temperature. Desvages *et al* (1993) showed that aromatase activity is influenced by temperature in the leatherback sea turtle (*Dermochelys coriacea*). Warmer, female-producing temperatures increased aromatase activity in the leatherback gonad. Estrogen exposure reversed the sex of olive ridley sea turtles (*Lepidochelys olivacea*) that were incubated at male temperatures (Merchant-Larios *et al*. 1997). However, 4,4'-DDE failed to reverse the sex of green sea turtle embryos (Podreka *et al*. 1998). Additional compounds, singly and in mixtures, different concentrations and incubation temperatures, and other sea turtle species need to be tested before general conclusions can be made about sea turtle embryonic sensitivity to OC contaminants.

This particular effect of contaminants may be a serious and timely issue for sea turtle populations. Sex ratios are already skewed towards female, because the beach temperatures along the southeast U.S. coast are generally warmer than the pivotal temperature. The few nests laid on North Carolina beaches, at the northern extreme of their nesting range, may be primarily responsible for the production of males. If sea turtles are as sensitive as freshwater turtles to sex reversal by estrogenic contaminants, then it is possible that North Carolina beaches are producing even fewer males than predicted.

In addition to the endpoints mentioned above (decreased survival, increased deformities, and altered sex ratios), contaminants can also directly affect the number of offspring produced by an individual. Reproductive failure has been noted in seals living in the PCB-contaminated region of the Wadden Sea (Reijnders 1986). Moreover, seals fed a diet of fish from an OC-contaminated region produced fewer pups than those fed less contaminated fish (Reijnders 1986). The lack of recovery of the beluga whale population in the St. Lawrence estuary is thought to be due in part to contaminant-related decreases in reproduction (De Guise *et al*. 1995). Even though PCB concentrations have decreased in the Great Lakes, lake trout (*Salvelinus namaycush*) still cannot naturally reproduce in the lower lakes because they do not survive past early life stages (Wright and Tillitt 1999). Historically, reproductive failure of the bald eagle and cormorant populations around the Great Lakes caused populations to plummet mainly due to eggshell thinning by DDT (see review by Grasman *et al*. 1998), but this effect has not been documented in reptiles. Bird population declines were also correlated with OC exposure in Long Island Sound,

Puget Sound, San Francisco Bay, and San Diego Bay (see review by Fry 1995). American kestrels exposed to PCBs *in ovo* had a higher risk of complete failure to lay eggs, delayed egg laying, and smaller clutch sizes than controls (Fernie *et al*. 2001).

Reproductive failure of adult sea turtles has not been investigated due to the logistical difficulties of working with large, rare animals that remain at sea for most of their life. Adult sea turtles are rarely encountered except when the females nest on the beach. Turtles with reproductive failure may not come onto the beach and therefore they may not be sampled. A few in-water sampling projects that capture adult animals may offer valuable samples.

The number of nesting female sea turtles has declined in certain populations. These declines were originally blamed on poaching and the incidental catch of turtles by fisheries. Even after conservation measures were established, certain populations are still in decline, such as the northern subpopulation of loggerhead sea turtles (TEWG 2000). Contaminants should be investigated as a potential risk for these reduced nesting populations.

Growth

Proper growth rates are critical to the survival and reproduction of individuals. Like a balancing act, growth must be fast enough to avoid size-specific predation, but not too fast to cause physiological problems. If OC contaminants slow growth rates, then individuals may be more susceptible to predators or be forced to remain in a physically challenging environment for longer periods. Slower growth may also cause a delay to the age of reproduction. Delaying reproduction can significantly affect the stability of a population. Willingham (2001) observed changes in growth of turtles

exposed to OC pesticides *in ovo*. Trans-nonachlor and 4,4'-DDE resulted in weight loss during the first 28 days after hatching. During this time, the turtles were not fed, so the positive growth of the control turtles was attributed solely to the energy reserves in the resorbed yolk sac. During the next 14 days, when turtles were fed, growth was greater in the turtles exposed to chlordane and trans-nonachlor than the controls. These changes in growth are hypothesized to be due to a disruption of the thyroid endocrine actions.

Thyroid hormones and retinol are intimately involved in growth as well as development, metabolism, immunity, and reproduction. Abundant and consistent evidence links OC contamination to reduced thyroid hormone and retinol levels and to thyroid histological alterations in wildlife populations, including marine mammals, birds, and fish (see review by Rolland 2000). Brouwer *et al*. (1998) reviewed many of the possible mechanisms of OC-induced thyroid alterations, including competition with transport proteins and alteration of thyroid hormone synthesis and metabolism. Hydroxylated PCBs, are especially potent competitors with thyroxine (T_4) and retinol for binding to transthyretin, a blood transport protein. The displacement of these natural ligands increases their degradation and reduces their concentrations in blood (see review by Rolland 2000). Altered thyroid and retinol functions can result in developmental malformations, altered growth, altered amphibian metamorphosis, decreased reproductive success, altered immune system, skin disease, and possibly altered migration, courtship, and breeding behaviors (see reviews by Rolland 2000 and Colborn 2002).

Hatchling sea turtles and small juveniles are extremely vulnerable to predation, therefore it is advantageous for them to grow quickly into large juveniles when their predation rates drop substantially. Growth rates, slowed by OC contaminants, could increase predation and reduce reproduction by extending the juvenile stage and delaying the age at maturity.

Thyroid hormones, including T_4 and triiodothyronine (T_3) are measurable in sea turtle plasma, and T_3 has been shown to influence the lung development of embryonic green sea turtles (Owens 1997; Sullivan *et al*. 2001). Blood levels of thyroid hormones in other species have been shown to bind and be transported by vitellogenin (VTG), an egg yolk precursor protein, but VTG did not influence thyroid hormones in Kemp's ridley sea turtles (*Lepidochelys kempii*; Heck *et al*. 1997). The effect of contaminants on thyroid functions in sea turtles, or turtles in general, have yet to be studied.

ORGANOCHLORINE CONCENTRATIONS IN SEA TURTLE TISSUES

OC contaminants have been detected in sea turtle tissues (see reviews by Meyers-Schone and Walton 1994; Pugh and Becker 2001). A comprehensive list of PCB, DDT, and chlordane concentrations in sea turtle tissues are reported in Table 1.1 (on a wet mass basis) and Table 1.2 (on a lipid-normalized basis). Mirex, heptachlor epoxide, HCB, and dieldrin have been detected in some studies but at very low concentrations, therefore they were not included in these tables. These measurements are so widely scattered in time, space, species, and tissue type that few general conclusions can be made. No long-term monitoring system is in place to track spatial

and temporal trends. OCs have been measured in tissues of only 4 of the 7 sea turtle species, and egg concentrations have only been measured in 2 species. Geographical regions include mainly the U.S. eastern coast, the Mediterranean Sea, and a few other locations.

Until recently, only eggs or tissues from carcasses have been utilized. Contaminant levels in eggs may offer information for two life stages, the embryo and the adult females, since contaminants are transferred to the egg from the mother during vitellogenesis (Pagano *et al*. 1999). The other life stages, namely juveniles or adult males, have been sporatically sampled only from carcasses. This biased sampling may lead to values that do not reliably represent those in the average live turtle. Decomposing tissues may become cross-contaminated with the external environment or with other decaying tissues. These tissues may also lose their lipid content, thereby affecting lipid-normalized contaminant concentrations. In addition, dead turtles may be biased towards diseased individuals. Diseased marine mammals are known to have higher levels of OCs than healthy, surviving animals (Hall *et al*. 1992, Aguilar and Borrell 1994, Borrell *et al*. 1996). Thus, this opportunistic sampling method may bias towards higher levels of OCs. The use of blood may offer a non-invasive alternative for long-term monitoring, since OC compounds were shown to be measurable in sea turtle blood (Keller *et al*. submitted), and the concentrations found in blood correlated with those in adipose tissue (Keller *et al*. in prep a).

Species differences are apparent from these sea turtle studies. Adipose concentrations of OCs were consistently higher in loggerhead and Kemp's ridley sea turtles than leatherback and green turtles. These differences have been explained

previously as trophic level differences (Meyers-Schone and Walton 1999; McKenzie *et al*. 1999). Loggerhead and Kemp's ridley turtles feed higher on the food chain. They eat benthic invertebrates such as mollusks and crabs, while the leatherback feeds primarily on jellyfish and the green turtle is herbivorous. The low concentrations measured in green sea turtles are likely caused by their low trophic status. On the other hand, the blubber of the leatherback turtle had higher OC concentrations than any other sea turtle adipose tissue (Keller *et al*. in prep a). Leatherback blubber is a lipid-rich structural component of the carapace which insulates these deep diving turtles and allows them to inhabit cooler waters. Because of these important functions, lipids in the blubber may not be mobilized as often as those in body fat and OCs may continually accumulate.

Differences among tissues are large on a wet mass basis (Table 1.1). Wet mass OC concentrations in adipose are higher than liver, kidney, muscle, heart, lung, and then blood. This pattern generally follows lipid content in each tissue (Storelli and Marcotrigiano 2000) and is similar to the pattern seen in snapping turtles (Bryan *et al*. 87; Meeks 1968). Once normalized for lipid (Table 1.2), blood and adipose concentrations in the loggerhead became very similar (Keller *et al*. in prep a), as did all tissues of loggerhead turtles from the Mediterranean Sea (Storelli and Marcotrigiano 2000). OC concentrations correlated between liver and adipose, between blood and adipose, and between eggs and chorioallantoic membranes (Lake *et al*. 1994; Keller *et al*. in prep a; Cobb and Wood 1997). These findings suggest that OC contaminants partition into each tissue based on lipid content, and that OCs in these tissues are in equilibrium with each other. Compared to other sea turtle tissues,

OC concentrations in loggerhead eggs from the southeast U.S. coast were relatively high (Alam and Brim 2000; Cobb and Wood 1997).

OC concentrations in sea turtles are compared to levels in blood components, adipose tissues, and eggs of other species in Tables 1.3 to 1.5. (For more comprehensive reviews of OC concentrations in tissues of reptiles in general see the appendix tables in Sparling *et al*. 2000; of turtles in general see Meyers-Schone and Walton 1994; of lizards and snakes see Campbell and Campbell 2002; and of marine mammals see Aguilar *et al*. 2002.) OC concentrations were generally lower in sea turtles than many other species. These species differences are likely due to differences in trophic level and geographic location. Top predator snakes and fish-eating birds and marine mammals feed higher on the food chain than the crab and mollusk-feeding loggerhead sea turtle. Reptiles inhabiting the Great Lakes, Hudson River, and Lake Apopka had higher OC levels than sea turtles. These differences may be explained by the proximity of these sites to point sources of OC contamination compared to the open ocean or even coastal regions.

Blood OC levels in the loggerhead turtle were comparable to those in snapping turtles and alligators from reference lakes, but were one to two orders of magnitude lower than reptiles in contaminated sites where effects have been seen, such as Hamilton Harbor in Lake Ontario and Lake Apopka in Florida (Table 1.3). The loggerhead blood OC levels were generally two to three orders of magnitude lower than concentrations in birds and marine mammals, but similar to those of humans.

Similarly, adipose concentrations in sea turtles were generally orders of magnitude lower than reptiles from contaminated sites, birds, and marine mammals

(Table 1.4). Even animals that live in similar habitats as the loggerhead sea turtle, but feed higher on the food chain (fish-eaters) had much higher concentrations than the loggerhead sea turtle. For example, the OC levels in juvenile bottlenose dolphins from Beaufort, NC (near Core Sound where the loggerhead turtles were sampled) were 150 fold higher and in albatrosses (*Diomedea immutabilis*) from a mid-oceanic island were 10 fold higher than the levels found in loggerhead turtles.

On the other hand, the wet mass OC concentrations in loggerhead eggs from the southeastern U.S. coast were higher than those measured in snapping turtle and alligator eggs from reference sites (Table 1.5). Some loggerhead eggs even had concentrations of PCBs and 4,4'-DDE that exceeded the mean level in snapping turtle eggs from the most contaminated site in the Great Lakes. PCB concentrations also exceeded the mean level of alligator eggs from three lakes in Florida. Sea turtle eggs generally had lower OC concentrations than those measured in birds from the Great Lakes, but higher concentrations of PCBs than oceanic albatross eggs.

The relatively high OC concentrations measured in sea turtle eggs should be investigated for their potential impact on embryonic and hatchling development. Additional sea turtle nesting populations should be analyzed to determine the range of exposure among different species and locations. Loggerhead eggs from North Carolina should be analyzed for OC contaminants and examined for sex reversal, since these nests are expected to produce the majority of males. The Kemp's ridley sea turtle, the most endangered of all sea turtles species which nests on only one beach in Mexico, has never been analyzed for egg concentrations of OCs. This species may be of concern, because Kemp's ridley turtles generally accumulate the highest adipose

concentrations among all the sea turtle species. Many spend their juvenile phase in the Gulf of Mexico which receives large masses of OC contaminants from the Mississippi River (Rostad 1997).

Even though juvenile sea turtles accumulate less OCs than most wildlife, we do not know whether they are affected by these levels of contaminants. Differential sensitivity between species is becoming more apparent, and we are beginning to realize that it is difficult to determine threshold concentrations. For example, atrazine, a commonly-used herbicide, is found at ppb concentrations in many surface and ground waters. These concentrations were thought to be safe until a recent study showed that tadpoles became hermaphrodites at these concentrations (Hayes *et al*. 2002a, 2002b). Additionally, sex reversal of developing reptiles with temperature-dependent sex determination (TSD) appears to be highly sensitive to estrogenic contaminants (Sheehan *et al*. 1999). As we study compounds at lower concentrations and examine more sensitive species and life stages, it is likely that we will see effects below so-called safe concentrations.

EFFECTS OF CONTAMINANTS ON SEA TURTLES

Extremely little is known about how contaminants may affect sea turtles. Only a handful of studies have investigated this issue. Most deal with the effects of oil or petroleum products (Lutcavage *et al*. 1995; Vargo *et al*. 1986). For example, crude oil caused sloughing of skin, anemia, increased white blood cell counts, and decreased plasma glucose levels in loggerhead sea turtles (Lutcavage *et al*. 1995). Only two studies have examined the effects of OC compounds on sea turtles. Podreka *et al*.

(1998) showed that DDE failed to reverse the sex of embryonic green sea turtles. Another study analyzed tissues of green sea turtles from Hawaii to compare OC concentrations between turtles with and without fibropapillomas (Aguirre *et al*. 1994). This disease affects several populations and species of sea turtles and has been associated with areas of urban development (George 1997). Because of high detection limits, Aguirre and coauthors were not able to detect any OC compound in either group, nor would they have been able to detect the higher OC levels typically found in loggerhead sea turtles. Thus, more research is needed to determine whether OC compounds in green turtles are contributing to this disease. The abundant evidence that OCs are affecting wildlife populations and the occurrence of OC contaminants in sea turtles warrants investigation into their sensitivity to contaminants.

OBJECTIVES OF THE CURRENT STUDY

The overall goal of the current study was to address whether the current levels of contaminants in juvenile sea turtle tissues could potentially harm two vital rates, survival and reproduction. Within that context, this study had three major objectives:

1. to investigate the use of blood as a non-invasive sampling technique to measure OCs in sea turtles;
2. to examine relationships between OC concentrations and indicators of poor health and immunotoxicity; and
3. to assess potential endocrine disruption in sea turtles by OC contaminants.

Additionally, this study investigated the use of *in vitro* cell culture models in order to experimentally examine the sensitivity of the sea turtle immune and endocrine systems to OCs.

Table 1.1. Mean (SD) organochlorine contaminant concentrations (ng/g wet mass unless stated otherwise) in tissues of sea turtles.

Species	Stage/ Sex[a]	Status[b]	Tissue[c]	Year[c]	Location	PCB 153	Sum PCBs	4,4'-DDE	Sum DDTs	Chlordanes[d]	N	Reference
Loggerhead	BJ	L	plasma	1998 - 2001	NC	2.10 (1.61)	7.13 (4.94)	0.575 (0.294)	0.578 (0.294)	0.238 (0.155)	5	Keller *et al* . submitted
Loggerhead	BJ	L	RBC	1998 - 2001	NC	0.479 (0.394)	1.72 (1.35)	0.448 (0.329)	0.457 (0.320)	0.135 (0.135)	5	Keller *et al* . submitted
Loggerhead	BJ	L	blood	1998 - 2001	NC	1.50 (1.27)	5.14 (3.95)	0.576 (0.305)	0.583 (0.307)	0.260 (0.182)	5	Keller *et al* . submitted
Loggerhead	BJ	L, ill	blood	1999 - 2002	NC		127 (191)		14.9 (22.3)	5.83 (9.06)	3	Keller *et al* . in prep b
Loggerhead	BJ	L, HL	blood	1999 - 2002	NC		5.17 (4.58)		0.582 (0.508)	0.209 (0.168)	47	Keller *et al* . in prep b
Loggerhead	BJ F	L, VTG	blood	2000	NC - SC	1.59	6.94	0.512	0.541	0.128	2	Keller *et al* . in prep c
Loggerhead	BJ F	L, HL, no VTG	blood	2000 - 2001	NC - FL	1.06 (0.759)	4.54 (3.61)	0.519 (0.502)	0.534 (0.507)	0.171 (0.113)	36	Keller *et al* . in prep c
Loggerhead	BJ	L	blood	2000 & 2001	NC	1.40 (1.35)	5.56 (5.28)	0.650 (0.704)	0.661 (0.704)	0.223 (0.192)	44	Keller *et al* . in prep a
Loggerhead	BJ	L	adipose	2000 & 2001	NC	80.9 (86.4)	256 (269)	64.4 (64.8)	67.0 (68.7)	26.9 (21.3)	44	Keller *et al* . in prep a
Loggerhead	BJ & A	D & EU	adipose	1991 - 1992	VA - NC	146(120)[e]	551 (473)	195 (266)	206 (268)		20	Rybitski *et al* . 1995
Loggerhead	J	D	adipose	1993	NE Italy	87.93 (31.15 - 134.46)	334 (179)				4	Corsolini *et al* . 2000
Loggerhead	J, AM, AF	D	adipose	1994 - 1995	Cyprus, Greece	241 (17.7)	840 (60.0)	509 (173)	528 (185)	19.7 (11.6)[f]	3	Mckenzie *et al* . 1999
Loggerhead	NR	D	adipose	1986	NR		647	300		61[g]	1	Lake *et al* . 1994
Loggerhead	BJ & A	D & EU	liver	1991 - 1992	VA - NC	49.8 (51.7)[e]	145 (158)	47.5 (104)	48.4 (105)		18	Rybitski *et al* . 1995
Loggerhead	J	D	liver	1993	NE Italy	26.92 (17.82 - 45.50)	119 (60)				4	Corsolini *et al* . 2000
Loggerhead	4J & 1AM	D	liver	1994 - 1995	Cyprus, Greece & Scotland	23.8 (6.3)	99 (39.6)	78.2 (41.3)	79.6 (42.2)	2.97 (1.77)[f]	5[h]	Mckenzie *et al* . 1999
Loggerhead	J	D	liver	NR	East FL		64 (61)[i]	22 (33)			8	McKim and Johnson 1983
Loggerhead	NR	D	liver	1986	NR		370[j]	110[j]			1	Lake *et al* . 1994
Loggerhead	NR	D	liver	1988	NR		110[j]	50[j]			1	Lake *et al* . 1994
Loggerhead	AF	D	liver	1990 - 1991	SE Italy		33.5 (17.0 - 50.0)	20.9 (15.8 - 26.0)			2	Storelli and Marcotrigiano 2000[k]
Loggerhead	AM	D	liver	1990 - 1991	SE Italy		415 (379 - 452)	95.9 (63.0 - 129)			2	Storelli and Marcotrigiano 2000[k]
Loggerhead	BJ	D	kidney	1991	VA	0.89 (1.54)[e]	1.61 (2.78)	ND	ND		3	Rybitski et al. 1995
Loggerhead	AF	D	kidney	1990 - 1991	SE Italy		36 (34.1 - 38.0)	13.1 (12.4 - 13.9)			2	Storelli and Marcotrigiano 2000[k]
Loggerhead	AM	D	kidney	1990 - 1991	SE Italy		198 (123 - 273)	25.4 (23.1 - 27.8)			2	Storelli and Marcotrigiano 2000[k]
Loggerhead	BJ	D	muscle	1991	VA	ND[c,e]	ND	ND	ND		5	Rybitski *et al* . 1995
Loggerhead	J	D	muscle	1993	NE Italy	2.99 (2.01 - 4.24)	15 (4)				4	Corsolini *et al* . 2000
Loggerhead	J	D	muscle	NR	East FL		13 (13)[i]	8.0 (14.4)			9	McKim and Johnson 1983
Loggerhead	AF	D	muscle	1990 - 1991	SE Italy		3.07 (1.65 - 4.49)	2.01 (1.10 - 2.93)			2	Storelli and Marcotrigiano 2000[k]
Loggerhead	AM	D	muscle	1990 - 1991	SE Italy		39.8 (33.9 - 45.6)	2.25 (2.15 - 2.34)			2	Storelli and Marcotrigiano 2000[k]

Table 1.1. continued

Species	Stage/ Sex[a]	Status[b]	Tissue[c]	Year[c]	Location	PCB 153	Sum PCBs	4,4'-DDE	Sum DDTs	Chlordanes[d]	N	Reference
Loggerhead	AF	D	heart	1990 - 1991	SE Italy		9.35 (9.05 - 9.65)	5.09 (4.02 - 6.15)			2	Storelli and Marcotrigiano 2000[k]
Loggerhead	AM	D	heart	1990 - 1991	SE Italy		98.9 (81.1 - 117)	15.6 (5.43 - 25.7)			2	Storelli and Marcotrigiano 2000[k]
Loggerhead	AF	D	lung	1990 - 1991	SE Italy		15.7 (7.20 - 24.2)	7.03 (6.00 - 8.05)			2	Storelli and Marcotrigiano 2000[k]
Loggerhead	AM	D	lung	1990 - 1991	SE Italy		82 (74.2 - 89.7)	11.2 (9.09 - 13.3)			2	Storelli and Marcotrigiano 2000[k]
Loggerhead	H	D	whole	1995	Cyprus, Greece	7.08 (7.95)	40.3 (23.1)	43.9 (44.2)	47.8 (47.4)	3.68 (2.99)[f]	4	Mckenzie *et al* . 1999
Loggerhead	E	UH, UD	whole	1995	Cyprus, Greece	29	89	154	155	1.8[f]	1	Mckenzie *et al* . 1999
Loggerhead	E	UH, V	egg contents	1992	NW FL		240-3720	ND	ND - 800[l,m]	ND	20[n]	Alam and Brim 2000
Loggerhead	E	NR	NR	NR	SC - GA				58-305		NR	Hillestad *et al* . 1974
Loggerhead	E	L day 43-52	egg contents	1976	FL		78 (32-201)[o]	66 (21; 18-200)[p]		ND - 9[g]; ND -17[q]	9 (9)[r]	Clark and Krynitsky 1980
Loggerhead	E	UH, IN	egg contents	1976	FL			247 (23)[s]			19 (3)[r]	Clark and Krynitsky 1980
Loggerhead	E	L day 0 to 61	egg	1979	FL			99 (56-150)[o]		ND - 8[q]	56 (1)[r]	Clark and Krynitsky 1985
Kemp's ridley	J	D	blood	1999	MA	0.761 (0.793)	3.33 (3.48)	0.688 (0.562)	0.717 (0.568)	0.321 (0.312)	8	Keller *et al* . in prep a
Kemp's ridley	J	D	yellow fat	1998 - 2000	MA & NC	161 (173)	701 (893)	99.6 (76.4)	101 (77.9)	103 (95.5)	9	Keller *et al* . in prep a
Kemp's ridley	J	D	brown fat	1998 - 2000	MA & NC	135 (127)	525 (545)	90.9 (71.9)	92.8 (73.6)	84.2 (70.8)	10	Keller *et al* . in prep a
Kemp's ridley	J	D	adipose	1991	VA - NC	189 (96.4)[e]	660 (333)	194 (98.2)	223 (106)		3	Rybitski *et al* . 1995
Kemp's ridley	J	D	adipose	1985	NY	384 (289)	1250 (985)	386 (250)	454 (298)	129 (112)[g]	7	Lake *et al* . 1994
Kemp's ridley	J	D	adipose	1989	NY	161 (95.6)	476 (273)	232 (157)	261 (176)	48.9 (29.4)[g]	6	Lake *et al* . 1994
Kemp's ridley	J	D	liver	1991	VA - NC	151 (108)[e]	375 (225)	55.5 (1.3)	64.2 (2.13)		3	Rybitski *et al* . 1995
Kemp's ridley	J	D	liver	1980	NY	222	655	195	263	75.4[g]	1	Lake *et al* . 1994
Kemp's ridley	J	D	liver	1985	NY	238 (190)	738 (737)	253 (162)	300 (207)	86.0 (78.1)[g]	8	Lake *et al* . 1994
Kemp's ridley	J	D	liver	1986	NY	217	680	173	213	53.6[g]	1	Lake *et al* . 1994
Kemp's ridley	J	D	liver	1987	NY	77.9 (37.8)	218 (127)	176 (97.6)	205 (122)	29.6 (20.1)[g]	6	Lake *et al* . 1994
Kemp's ridley	J	D	liver	1989	NY	95.3 (42.8)	272 (126)	137 (85.3)	156 (99.2)	27.5 (12.6)[g]	6	Lake *et al* . 1994
Leatherback	AF	EU	blubber	1999	NC	664	2330	288	292	336	1	Keller *et al* . in prep a
Leatherback	AF	EU	adipose	1999	NC	41.0	129	13.2	13.2	15.8	1	Keller *et al* . in prep a
Leatherback	AM	D	adipose	1993 - 1996	Wales & Scotland	42.3 (32.7)	152 (94.3)	45.0 (30.8)			3	Godley *et al* . 1998
Leatherback	AM	D	adipose	1993 + 1995	Scotland	26.9 (7.8 - 46)	113 (47 - 178)	33.5 (10 - 57)	36 (14 - 58)	17 (12.0 - 22)[f]	2	Mckenzie *et al* . 1999
Leatherback	AM	D	liver	1993 + 1995	Scotland	1.4 (1.3 - 1.5)	3.4 (3.1 - 3.7)	4.1 (1.7 - 6.5)	11.6 (9.1 - 14)	2.3 (2.3 - 2.3)[f]	2	Mckenzie *et al* . 1999
Green	JM	D	adipose	2000	NC	28.7	81.1	15.0	15.0	15.3	1	Keller *et al* . in prep a
Green	J	D	adipose	1995	Cyprus, Greece	15.3 (16.0)	136 (113)	9.13 (8.73)	12.4 (9.93)	ND[f]	3	Mckenzie *et al* . 1999
Green	JM, AF, AM	D	adipose	1992 - 1993	Hawaii	59 (79)[l,j]	285 (330)[l]				3	Miao *et al* . 2001

Table 1.1. continued

Species	Stage/ Sex[a]	Status[b]	Tissue[c]	Year[c]	Location	PCB 153	Sum PCBs	4,4'-DDE	Sum DDTs	Chlordanes[d]	N	Reference
Green	J	D	adipose	1992	Hawaii		22.9 (21.3)	4.97 (8.82)			5	Rybitski 1993
Green	J	D & EU	liver, adipose, kidney	NR	Hawaii		<1000	<100		< 50[q]	12	Aguirre *et al* . 1994
Green	J	D	liver	1995 - 1996	Cyprus, Greece	6.42 (4.37)	33.6 (22.3)	4.83 (6.44)	6.03 (6.97)	0.8 (1.51)[f]	9[t]	Mckenzie *et al* . 1999
Green	JM, AF, AM	D	liver	1992 - 1993	Hawaii	9.3 (3.5)[l,j]	51.6 (6.7)[l]				3	Miao *et al* . 2001
Green	J	D	liver	NR	East FL		65 (16)[i]	3 (5)			4	McKim and Johnson 1983
Green	J	D	liver	1992	Hawaii		3.43 (6.85)	1.26 (2.52)			5	Rybitski 1993
Green	J	D	muscle	NR	East FL		6.8 (1.8)[i]	0.5 (0.6)			4	McKim and Johnson 1983
Green	H	D	whole	1995	Cyprus, Greece	0.4 (0.6)	4.7 (7.2)	0.2 (0.3)	2.0 (3.3)	0.1 (0.2)[f]	3	Mckenzie *et al* . 1999
Green	E	UD, UH	whole	1995	Cyprus, Greece	ND	6.1	2.3	4.3	ND[f]	1	Mckenzie *et al* . 1999
Green	E	L, stage 21	albumin, fluids, yolk & embryo	1995	Queensland, Australia			1.3 - 2.4			15 (4)[r]	Podreka *et al* . 1998
Green	E	L day 43-52	egg contents	1976	FL	ND	ND	ND-5	ND - 47	ND[u]	2 (2)	Clark and Krynitsky 1980
Green	E	UD ~day 30	yolk	1972	Ascension Island		76 (64)[v]	3 (3)			10 (4)[r]	Thompson *et al* . 1974

[a] BJ = Benthic juvenile; J = juvenile; A = adult; M = male, F = female; E = eggs; H = hatchling.

[b] L = live; ill = visibly ill (lethargic and emaciated); HL = healthy; VTG = abnormally expressing VTG; no VTG = no abnormal expression of VTG; D = dead; EU = euthanized; UD = undeveloped; UH = unhatched; IN = infertile; V = various stages of development; stage or day of incubation is listed for eggs.

[c] RBC = red blood cells; NR = not reported; ND = not detected within the limits of detection of each study.

[d] sum of *trans*-, *cis*-chlordane, *trans*-, *cis*-nonachlor, and oxyclordane.

[e] 153 plus minor congener of 132.

[f] sum of heptachlor, heptachlor epoxide, *trans*-, *cis*-chlordane, *trans*-nonachlor, and oxychlordane.

[g] *trans*-nonachlor only.

[h] N=3 for chlordanes and sum DDT.

[i] sum of 4 Aroclors mixtures (1242, 1248, 1254, 1260), see Eganhouse and Gossett (1991) for problems associated with this method.

[j] approximation of values taken from a graph.

[k] back calculated values using mg/kg lipid concentrations and % lipid measured in each individual sample.

[l] Values were reported as ng/g dry weight.

[m] p,p'-DDD was detected in only 2 sites at 753 and 800 ng/g dry weight, no other OC pesticides were detected.

Table 1.1. continued

[n] authors pooled 4-10 eggs per nest. 20 composite samples or 20 clutches were analyzed.
[o] geometric mean (range); total PCBs were measured as Aroclor 1260.
[p] arithmetic mean (standard error; range).
[q] oxychlordane only.
[r] total # of eggs analyzed (out of a total # of clutches).
[s] average of the mean concentrations measured in the 3 most contaminated clutches (4 to 10 eggs from each clutch).
[t] N=8 for chlordanes.
[u] sum of *cis*-chlordane, *trans*-, *cis*-nonachlor, and oxychlordane.
[v] Total PCBs were calculated as either Aroclor 1254 only or an average of 1248 and 1254.

Table 1.2. Mean (SD) organochlorine contaminant concentrations (ng/g lipid) in tissues of sea turtles.[a]

Species	Stage/ Sex[b]	Status[c]	Tissue[d]	Year	Location	PCB 153	Sum PCBs	4,4'-DDE	Sum DDTs	Chlordanes[e]	N	Reference
Loggerhead	BJ	L	plasma	1998 - 2001	NC	861 (662)	2890 (2020)	243 (158)	244 (158)	101 (73.0)	5	Keller *et al*. submitted
Loggerhead	BJ	L	RBC	1998 - 2001	NC	170 (150)	610 (509)	150 (93.3)	154 (89.8)	45.9 (47.2)	5	Keller *et al*. submitted
Loggerhead	BJ	L	blood	1998 - 2001	NC	474 (373)	1600 (1130)	193 (141)	195 (140)	85.4 (64.8)	5	Keller *et al*. submitted
Loggerhead	BJ	L, ill	blood	1999 - 2002	NC		316000 (500000)		36400 (58700)	14500 (23600)	3	Keller *et al*. in prep b
Loggerhead	BJ	L, HL	blood	1999 - 2002	NC		2140 (1980)		234 (186)	86.0 (63.6)	47	Keller *et al*. in prep b
Loggerhead	BJ F	L, VTG	blood	2000	NC - SC	1060	4270	331	358	87.4	2	Keller *et al*. in prep c
Loggerhead	BJ F	L, HL, no VTG	blood	2000 - 2001	NC - FL	530 (549)	2360 (2980)	231 (229)	239 (233)	79.8 (58.5)	36	Keller *et al*. in prep c
Loggerhead	BJ	L	blood	2000 - 2001	NC	634 (975)	2490 (3700)	300 (578)	305 (577)	102 (151)	44	Keller *et al*. in prep a
Loggerhead	BJ	L	adipose	2000 - 2001	NC	736 (1170)	2010 (2960)	445 (643)	452 (643)	246 (412)	44	Keller *et al*. in prep a
Loggerhead	BJ & A	D & EU	adipose	1991	VA - NC	542 (944)[f]	1890 (3240)	385 (578)	414 (611)		5	Rybitski et al. 1995
Loggerhead	J, AM, AF	D	adipose	1994 - 1995	Cyprus, Greece	637 (408)	2310 (1690)	1260 (599)	1310 (631)	45.1 (21.6)[g]	3	Mckenzie *et al*. 1999
Loggerhead	BJ & A	D & EU	liver	1991	VA - NC	365 (629)[f]	1100 (1920)	304 (460)	307 (459)		5	Rybitski *et al*. 1995
Loggerhead	4J & 1AM	D	liver	1994 - 1995	Cyprus, Greece, Scotland	294 (46.8)	1270 (621)	1110 (796)	1130 (811)	24.0 (24.9)[g]	5[h]	Mckenzie *et al*. 1999
Loggerhead	J	D	liver	1990-1991	SE Italy		5010(1220)	1420(250)			5	Storelli and Marcotrigiano 2000
Loggerhead	AF	D	liver	1990-1991	SE Italy		350(210)	410(250)			6	Storelli and Marcotrigiano 2000
Loggerhead	AM	D	liver	1990-1991	SE Italy		2800 (2650 - 3010)	700 (420 - 900)			2	Storelli and Marcotrigiano 2000
Loggerhead	J	D	kidney	1990-1991	SE Italy		4200(830)	1230(360)			5	Storelli and Marcotrigiano 2000
Loggerhead	AF	D	kidney	1990-1991	SE Italy		340(70)	220(190)			6	Storelli and Marcotrigiano 2000
Loggerhead	AM	D	kidney	1990-1991	SE Italy		2180 (1700 - 2650)	300 (270 - 320)			2	Storelli and Marcotrigiano 2000
Loggerhead	J	D	muscle	1990-1991	SE Italy		2030(960)	540(190)			5	Storelli and Marcotrigiano 2000
Loggerhead	AF	D	muscle	1990-1991	SE Italy		160(60)	180(100)			6	Storelli and Marcotrigiano 2000
Loggerhead	AM	D	muscle	1990-1991	SE Italy		1930 (1740 - 2120)	110 (100 - 120)			2	Storelli and Marcotrigiano 2000
Loggerhead	J	D	heart	1990-1991	SE Italy		3520(1600)	1130(360)			5	Storelli and Marcotrigiano 2000
Loggerhead	AF	D	heart	1990-1991	SE Italy		250(100)	130(50)			6	Storelli and Marcotrigiano 2000

Table 1.2. continued

Species	Stage/ Sex[b]	Status[c]	Tissue[d]	Year	Location	PCB 153	Sum PCBs	4,4'-DDE	Sum DDTs	Chlordanes[e]	N	Reference
Loggerhead	AM	D	heart	1990-1991	SE Italy		2370 (2240 - 2500)	350 (150 - 550)			2	Storelli and Marcotrigiano 2000
Loggerhead	J	D	lung	1990-1991	SE Italy		2950(970)	480(240)			5	Storelli and Marcotrigiano 2000
Loggerhead	AF	D	lung	1990-1991	SE Italy		250(100)	140(80)			6	Storelli and Marcotrigiano 2000
Loggerhead	AM	D	lung	1990-1991	SE Italy		1860 (1450 - 2270)	245 (230 - 260)			2	Storelli and Marcotrigiano 2000
Loggerhead	H	D	whole	1995	Cyprus, Greece	102 (114)	571 (319)	560(523)	610 (557)	46.8 (31.5)[g]	4	Mckenzie *et al* . 1999
Loggerhead	E	UD, UH	whole	1995	Cyprus, Greece	483	1480	2570	2580	30[g]	1	Mckenzie *et al* . 1999
Loggerhead	E	UH, late stage	egg contents	1993	SC		1188 (311)[i]				16 (16)[j]	Cobb and Wood 1997
Loggerhead	E	UH, late stage	CAM	1993	SC		10100 (5466)[i]				16 (16)[j]	Cobb and Wood 1997
Loggerhead	E	UH, UD	egg contents	1993	SC		2556 (1202)[i]				4 (4)[j]	Cobb and Wood 1997
Kemps ridley	J	D	blood	1999	MA	216 (248)	985 (1250)	166 (147)	172 (147)	77.2 (81.6)	8	Keller *et al* . in prep a
Kemps ridley	J	D	yellow fat	1998 - 2000	MA & NC	247 (244)	1050 (1220)	154 (111)	156 (113)	158 (138)	9	Keller *et al* . in prep a
Kemps ridley	J	D	brown fat	1998 - 2000	MA & NC	377 (512)	1110 (1030)	254 (332)	257 (332)	240 (331)	10	Keller *et al* . in prep a
Leatherback	AF	EU	blubber	1999	NC	2570	9040	1120	1130	1300	1	Keller *et al* . in prep a
Leatherback	AM	D	blubber	1988	Wales		1200				1	Davenport *et al* . 1990
Leatherback	AF	EU	adipose	1999	NC	3500	11000	1120	1120	1340	1	Keller *et al* . in prep a
Leatherback	AM	D	adipose	1993 - 1996	Wales & Scotland	93.6 (83.6)	327 (250)	97.8 (77.5)			3	Godley *et al* . 1998
Leatherback	AM	D	adipose	1993 + 1995	Scotland	51.3	210	63.8	67.5	26.9[g]	2	Mckenzie *et al* . 1999
Leatherback	AM	D	liver	1993 + 1995	Scotland	11.5	27.3	35.6	96.1	18.7[g]	2	Mckenzie *et al* . 1999
Green	JM	D	adipose	2000	NC	64.4	182	33.6	33.6	34.2	1	Keller *et al* . in prep a
Green	M	D	adipose	1998	NE Australia	70	171	45	53	15	1	Vetter *et al* . 2001
Green	J	D	adipose	1995	Cyprus, Greece	47.5 (50.2)	432 (344)	28.5 (25.6)	38.9 (28.8)	ND[g]	3	Mckenzie *et al* . 1999
Green	J	D	liver	1995 - 1996	Cyprus, Greece	42.8 (27.3)	222 (131)	28.0 (27.1)	39.6 (29.8)	3.64 (7.28)[g]	9[k]	Mckenzie *et al* . 1999
Green	H	D	whole	1995	Cyprus, Greece	4.53 (7.84)	58.4 (88.7)	2.06(3.56)	24.8(40.5)	1.65(2.85)[g]	3	Mckenzie *et al* . 1999

Table 1.2. continued

Species	Stage/ Sex[b]	Status[c]	Tissue[d]	Year	Location	PCB 153	Sum PCBs	4,4'-DDE	Sum DDTs	Chlordanes[e]	N	Reference
Green	E	UD, UH	whole	1995	Cyprus, Greece	ND[l]	87.1	32.9	61.4	ND[g]	1	Mckenzie *et al*. 1999
Green	E	UD ~day 30	yolk	1972	Ascension Island		939 (698)[m]	36 (33)			10 (4)[j]	Thompson *et al*. 1974

[a] values either came directly from the published study or were lipid-normalized from wet mass concentrations using % lipid values.
[b] BJ = Benthic juvenile; J = juvenile; A = adult; M = male, F = female; E = eggs; H = hatchling.
[c] L = live; ill = visibly chronically ill; HL = healthy; VTG = abnormally expressing VTG; no VTG = no abnormal expression of VTG; D = dead; EU = euthanized; UD = undeveloped; UH = unhatched; stage or day of incubation is listed for eggs.
[d] RBC = red blood cells; CAM = chorioallantoic membrane.
[e] Sum of trans-, *cis*-chlordane, *trans*-, *cis*-nonachlor, and oxychlordane unless otherwise stated.
[f] 153 plus minor congener of 132.
[g] Sum of heptachlor, heptachlor epoxide, *trans*-, *cis*-chlordane, *trans*-nonachlor, and oxychlordane.
[h] N=3 for chlordanes.
[i] mean (standard error).
[j] total # of eggs analyzed (out of a total # of clutches).
[k] N=8 for chlordanes, N=8 for Sum DDTs.
[l] ND = not detected with each studies detection limits.
[m] Sum PCBs were calculated as either Aroclor 1254 only or an average of 1248 and 1254.

Table 1.3. Comparison of sea turtle blood OC concentrations to other species.[a]

					ng/g wet mass		ng/g lipid			
Species	Stage/ Sex[b]	Tissue[b]	Year[b]	Location[c]	Sum PCBs	4,4'-DDE	Sum PCBs	4,4'-DDE	N	Reference
Loggerhead	JF & JM	Blood	2000-2001	North Carolina	5.56 (5.28)	0.650 (0.704)	2490 (3700)	300 (578)	44	Keller *et al* . in prep a
American alligator	JM	Serum	1995	**Lake Apopka, FL**	1.2 (0.5)[ef]	7.35 (2.41)[e]			6	Guillette *et al* . 1999
American alligator	JF	Serum	1995	**Lake Apopka, FL**	2.4 (0.3)[ef]	17.98 (5.35)[e]			7	Guillette *et al* . 1999
American alligator	JM	Serum	1995	Lake Woodruff, FL	1.25 (0.15)[ef]	0.92 (0.06)[e]			14	Guillette *et al* . 1999
American alligator	JF	Serum	1995	Lake Woodruff, FL	2.1 (0.5)[ef]	1.28 (0.33)[e]			6	Guillette *et al* . 1999
American alligator	JM	Serum	1995	Orange Lake, FL	1.5 (0.1)[ef]	0.92 (0.06)[e]			11	Guillette *et al* . 1999
American alligator	JF	Serum	1995	Orange Lake, FL	1.2 (0.3)[ef]	0.77 (0.03)[e]			7	Guillette *et al* . 1999
American alligator	JF & JM	Serum	1995	all FL lakes	1.54 (0.12)[e]				51	Guillette *et al* . 1999
Snapping turtle	AM	Plasma	1995	**Hamilton Harbor, L. Ont**	414.8 (351.7)	10.1 (4.3)			9	de Solla *et al* . 1998
Snapping turtle	AM	Plasma	1995	**Lynde Creek, L. Ont**	263.3 (116.2)	21.7 (3.0)			2	de Solla *et al* . 1998
Snapping turtle	AM	Plasma	1995	Jack L.	17.8 (7.8)	0.7 (0.9)			10	de Solla *et al* . 1998
Snapping turtle	AM	Plasma	1995	L. Sasajewun	18.2 (12.5)	0.2 (0.4)			4	de Solla *et al* . 1998
Lake Erie water snake	AM	Plasma	1998	W Lake Erie	167	5	47851	1433	4 pooled	Bishop and Rouse 2000
Northern water snake	AM	Plasma	1998	E Lake Ontario	3	2	344	229	4 pooled	Bishop and Rouse 2000
Northern water snake	M	Plasma	1998	Little Lake, central Ontario	12	3	1875	469	4 pooled	Bishop and Rouse 2000
Diamondback water snake	NR	Blood	1994-1995	Texas, ORS[d]		733 (140 - 3830)[g]			5	Clark *et al* . 2000
Diamondback water snake	NR	Blood	1994-1995	Texas, PL[d]		ND			5	Clark *et al* . 2000
Cotonmouth	NR	Blood	1994-1995	Texas, ORS[d]		759 (260 - 2220)[g]			5	Clark *et al* . 2000
Red-eared slider turtle	NR	Blood	1994-1995	Texas, ORS[d]		124 (56.1 - 274)[g]			5	Clark *et al* . 2000
Red-eared slider turtle	NR	Blood	1994-1995	Texas, RPL[d]		ND - 19			5	Clark *et al* . 2000
Red-eared slider turtle	NR	Blood	1994-1995	Texas, ML[d]		ND			5	Clark *et al* . 2000
Caspian terns	prefledg	Plasma	1997-1999	N. Channel, L. Huron	22 - 80[fh]	7.8 - 19[fh]			15-30	Grasman and Fox 2001
Caspian terns	prefledg	Plasma	1997-1999	**Saginaw Bay, L. Huron**	110 - 300[fh]	23 - 90[fh]			15-30	Grasman and Fox 2001
Bald eagle	chicks	Plasma	1990-1996	N. Lake Erie	129.5 (9.9 - 326)[i]	22.4 (3.6 - 139.4)[i]			30	Donaldson *et al* . 1999
Bald eagle	chicks	Plasma	1993	Lake Nipigon	47.1 (21.0 - 197.2)[i]	23.9 (12.6 - 85.0)[i]			7	Donaldson *et al* . 1999
Bald eagle	chicks	Plasma	1992	Lake of the Woods	149.8 (114.6 - 195.7)[i]	54.0 (40.1 - 72.6)[i]			2	Donaldson *et al* . 1999
Bald eagle	chicks	Plasma	1993	Upper Michigan	56.0 (11.1)	9.93 (4.02)	9824 (3995)	1718 (812)	4	Kumar *et al* . 2002
Bald eagle	chicks	Plasma	1993-1994	British Columbia	1.9 - 114	0.6 - 86			50	Elliott and Norstrom 1998
American kestrel	M & F	Plasma	1975	Idaho & Colorodo		182 (266)			28	Henny and Meeker 1981
Bottlenose dolphin	JM & AM	Blood	1991	**Sarasota, FL**	402 (323, 26.3 - 752)[j]	237 (220, 12.7 - 536.3)			5	Lahvis *et al* . 1995
Harbor seal[m]	pups	Blood	1994	Atlanic Ocean	7109	788[k]	3090870[l]	342609[kl]	11 pooled	de Swart *et al* . 1995
Harbor seal[m]	pups	Blood	1994	**Baltic Sea**	15062	2779[k]	6846364[l]	1263182[kl]	11 pooled	de Swart *et al* . 1995
Grey seal	pups	RBC	1991	**Norway**	4.6 (7.6, 1 - 32)	2.4 (3.3, 0.3 - 12)	2820 (2957)	1349 (1477)[k]	17	Jenssen *et al* . 1994

Table 1.3. Continued

Species	Stage/ Sex[b]	Tissue[b]	Year[b]	Location[c]	ng/g wet mass		ng/g lipid		N	Reference
					Sum PCBs	4,4'-DDE	Sum PCBs	4,4'-DDE		
Polar bear	AF with cub	RBC	1990-1994	Svalbard	12.92[n]	0.096[n]	6460 (2710 - 11200)[o]	48 (<40 - 170)[o]	11	Bernhoft *et al* . 1997
Polar bear	J	RBC	1990-1994	Svalbard			2900 - 40100	< 40 - 710	39	Bernhoft *et al* . 1997
Harp seal	AF fat	Blood	1998	Greenland Sea	1.13[l]	0.28[kl]	201 (55)[f]	50 (20)[fk]	10	Lydersen *et al* . 2002
Harp seal	AF lean	Blood	1999	Greenland Sea	8.1[l]	2.24[kl]	1447 (800)[f]	400 (150)[fk]	7	Lydersen *et al* . 2002
Northern fur seal	AF mom	Blood	1996	St. George Island, Alaska	14.5 (2.6)	1.4 (0.9)	7250[l]	700[l]	19	Beckman *et al* . 1999
Northern fur seal	pups	Blood	1996	St. George Island, Alaska	20.6 (6.9)	7.8 (9.2)	6867[l]	2600[l]	48	Beckman *et al* . 1999
Human (fish eaters)	AF	Plasma	1992	Ontario	3.4 (0.7 - 23.0)[op]	2.9 (0.2 - 26.0)[o]	465.2 (117.1 - 2428.8)[op]	364.1 (22.4 - 3031.6)[o]	51	Kearney *et al* . 1999
Human (no fish)	AM	Plasma	1992	Ontario	3.9 (1.1 - 12.0)[op]	2.3 (0.4 - 16.0)[o]	442.5 (154.0 - 1484.3)[op]	292.8 (72.1 - 1855.3)[o]	45	Kearney *et al* . 1999
Human (fish eaters)	AM	Plasma	1992	Ontario	5.5 (0.9 - 21.0)[op]	3.8 (0.5 - 51.0)[o]	613.6 (142.4 - 2562.6)[op]	383.1 (73.7 - 4611.4)[o]	101	Kearney *et al* . 1999
Human (Canadian Inuit)	M & F	Blood	1992	N. Quebec	15.2 (1.19 - 65.9)[i]				30	Sandau *et al* . 2000
Human	pooled	Blood	NR	S. Quebec	0.488				pool	Sandau *et al* . 2000
Human (no fish)	AM	Plasma	NR	SE Sweden	1.5 (0.6 - 3.5)[q]	2.4 (0.6 - 5.1)	470 (220 - 760)[q]	750 (290 - 1100)	9[r]	Asplund *et al* . 1994
Human (moderate)	AM	Plasma	NR	SE Sweden	1.8 (0.8 - 3.3)[q]	3.5 (0.7 - 11)	580 (330 - 860)[q]	1200 (300 - 1900)	14[r]	Asplund *et al* . 1994
Human (high)	AM	Plasma	NR	SE Sweden	3.4 (0.7 - 6.6)[q]	14 (1.8 - 33)	1000 (400 - 2400)[q]	4500 (1300 - 14000)	14[r]	Asplund *et al* . 1994

[a] Concentrations are means (SD or range) unless otherwise stated.
[b] A = adult; J = juvenile; S = subadult; M = male; F = female; NR = not reported; RBC = red blood cells.
[c] Sites in bold indicate that effects were noted in those animals.
[d] ORS = Old River Slough, RPL = Research Park Lake; PL = Private Lake; ML = Municipal Lake, all within 17 km of Texas A&M, College Station, TX.
[e] mean (SE); ng/ml.
[f] estimated values from a graph.
[g] geometric mean (95% confidence intervals).
[h] range of the mean concentrations for each year.
[i] geometric mean (range).
[j] sum of tri- through deca-chloro PCBs.
[k] sum DDTs.
[l] back calculated using mean and mean %lipid.
[m] pups from NE Scotland were fed fish from either the Atlanic Ocean or Baltic Sea for 1 year.
[n] back calculated using median and median %lipid.
[o] median (range).
[p] PCB is Aroclor 1260.
[q] PCB 153 only.
[r] N for lipid normalized values are 8 for no, 7 for moderate, and 11 for high fish intake.

Table 1.4. Comparison of sea turtle adipose OC concentrations (ug/g) to other species.[a]

					ug/g wet mass		ug/g lipid			
Species[b]	Stage/Sex[b]	Tissue[b]	Year[b]	Location[c]	Sum PCBs	4,4'-DDE	Sum PCBs	4,4'-DDE	N	Reference
Loggerhead	BJ	adipose	2000 - 2001	North Carolina	0.256 (0.269)	0.0644 (0.0648)	2.01 (2.96)	0.445 (0.643)	44	Keller *et al* . in prep a
Leatherback	AF	adipose	1999	North Carolina	0.129	0.0132	11.0	1.12	1	Keller *et al* . in prep a
Leatherback	AF	blubber	1999	North Carolina	2.33	0.288	9.04	1.12	1	Keller *et al* . in prep a
Snapping turtle		adipose	1988	Grasse R. & Snye Marsh, St. Lawrence R.	258 - 1010				2	Hong *et al* . 1998
Snapping turtle	M	adipose	1988-1989	Lake Ontario	633.292	87.598			1	Olafsson *et al* . 1983
Snapping turtle	M	adipose	1988-1989	Hudson R, NY	3608	14.999			1	Olafsson *et al* . 1983
Snapping turtle		adipose	NR	NY	4.2 - 1600				2	Bryan *et al* . 1987
Snapping turtle		adipose	NR	Hudson R, NY	750				1	Rappe *et al* . 1981
Snapping turtle	F	adipose	NR	6 sites in NY	98.651 (207.187)[d]	0.119 (0.197)[d]	196.993 (351.199)[d]	0.60 (0.453)[d]	6	Pagano et al. 1999
Snapping turtle		adipose	1976-1978	Hudson R, NY			2990 (2990)	104 (215)	11[l]	Stone *et al* . 1980
Snapping turtle		adipose	1976-1978	Other NY waters			464 (716)	115 (264)	9	Stone *et al* . 1980
Snapping turtle	M	adipose	1981-1982	MD			41.2 (37.24)	0.390 (0.310)	7	Albers *et al* . 1986
Snapping turtle	F	adipose	1981-1982	MD			36.17 (81.16)	0.100 (0.160)	6	Albers *et al* . 1986
Snapping turtle	M	adipose	1981-1982	NJ Brackish			291.13 (304.82)	0.160 (0.290)	8	Albers *et al* . 1986
Snapping turtle	F	adipose	1981-1982	NJ Brackish			34.07 (15.56)	0.260 (0.440)	3	Albers *et al* . 1986
Snapping turtle	M	adipose	1981-1982	NJ Freshwater			23.55 (11.19)	2.03 (1.24)	8	Albers *et al* . 1986
Snapping turtle		adipose	1981	Minnesota	2.14 (1.89)		265 (223)		15	Helwig and Hora 1983
Snapping turtle	F	adipose	1974	Iowa		ND			1	Punzo *et al* . 1979
Midland painted turtle	M	adipose	1974	Iowa		0.018			1	Punzo *et al* . 1979
Midland painted turtle		adipose	NR	Tennessee		2.672 (0.589)[e]			2	Owen and Wells 1976
Red-eared slider turtle		adipose	NR	Tennessee		4.558 (3.886)[e]			3	Owen and Wells 1976
American alligator	neonate	adipose	NR	Bear Island, SC			0.75 (0.57)[f]	7.542 (NR)[f]	13	Bargar *et al* . 1999
Northern water snake	F	adipose	1974	Iowa		0.079 (ND - 0.300)			13	Punzo *et al* . 1979
Northern water snake	M	adipose	1974	Iowa		0.215 (ND - 0.625)			5	Punzo *et al* . 1979
Red-sided garter snake	F	adipose	1974	Iowa		0.036			1	Punzo *et al* . 1979
Smooth green snake	F	adipose	1974	Iowa		ND			1	Punzo *et al* . 1979
Western plains garter snake	F	adipose	1974	Iowa		0.112			1	Punzo *et al* . 1979
Western plains garter snake	M	adipose	1974	Iowa		0.01			2	Punzo *et al* . 1979
Python	A	adipose	1999	NE Australia			> 0.380	0.03	1	Vetter *et al* . 2001
Laysan albatross	A	fat	1993-1994	Midway Atoll, N Pacific	2.11 - 2.75				2 pools of	Jones *et al* . 1996
Bald eagle	JF	fat	2000	Michigan	8.1 - 17	1.6 - 2.3	9.85 - 22.6	1.95 - 3.06	2	Kumar *et al* . 2002
Mallard	NR	fat	1989	Raquette R.	9.2				1	Hong *et al* . 1998
Herring gull	NR	fat	1992-1994	Korea			7.818 (1.8 - 24.0)	2.675 (1.136 - 5.100)[g]	7	Choi *et al* . 2001
Bottlenose dolphin	J	blubber	1995	Beaufort, NC	38.33 (14.01)				21	Schwacke *et al* . 2002
Bottlenose dolphin	AM	blubber	1995	Beaufort, NC	70.27 (7.92)				2	Schwacke *et al* . 2002
Bottlenose dolphin	AF	blubber	1995	Beaufort, NC	4.24 (1.18)				4	Schwacke *et al* . 2002

Table 1.4. Continued

					ug/g wet mass		ug/g lipid			
Species[b]	Stage/Sex[b]	Tissue[b]	Year[b]	Location[c]	Sum PCBs	4,4'-DDE	Sum PCBs	4,4'-DDE	N	Reference
Bottlenose dolphin	J	blubber	1992	Matagorda Bay, TX	86.24 (74.43)				19	Schwacke *et al* . 2002
Bottlenose dolphin	AM	blubber	1992	Matagorda Bay, TX	91.21 (53.60)				7	Schwacke *et al* . 2002
Bottlenose dolphin	AF	blubber	1992	Matagorda Bay, TX	10.27 (14.50)				7	Schwacke *et al* . 2002
Bottlenose dolphin	J	blubber	1997-1999	Sarasota Bay, FL	76.78 (62.94)				19	Schwacke *et al* . 2002
Bottlenose dolphin	AM	blubber	1997-1999	Sarasota Bay, FL	76.18 (84.03)				6	Schwacke *et al* . 2002
Bottlenose dolphin	AF	blubber	1997-1999	Sarasota Bay, FL	5.07 (4.27)				11	Schwacke *et al* . 2002
Bottlenose dolphin	AM	blubber	1990	Matagorda Bay, TX			13.7 - 41.4		10	Finklea *et al* . 2000
Bottlenose dolphin	AF	blubber	1995 & 1999	NE Australia			0.794 - 1.722	0.420 - 1.683	2	Vetter *et al* . 2001
Bottlenose dolphin	M	blubber	1996-1997	NE Australia			6.601 - 25.524	11.303 - 52.416	2	Vetter *et al* . 2001
Bottlenose dolphin	AF, SM, AM	blubber	1992	NE Italy	584 (456)	170 (190)[g]	1000 (750)	330 (390)[g]	7	Corsolini *et al* . 1995
Risso's dolphin	AM & SF	blubber	NR	W Italy	20 - 610	5.2 - 400[g]	42 - 1000	11 - 670[g]	2	Corsolini *et al* . 1995
Common dolphin	JF	blubber	1995	NE Australia			0.627	0.396	1	Vetter *et al* . 2001
Striped dolphin	mixed, E	blubber	1990-1992	**Mediter. Sea**			855.9 (569)		30	Borrell *et al* . 1996
Striped dolphin	mixed, E	blubber	1990	**Mediter. Sea**			~750[h]		72	Aguilar and Borrell 1994
Striped dolphin	mixed, L	blubber	1987-1991	Mediter. Sea			~300[h]		109	Aguilar and Borrell 1994
Dall's porpoise	AM	blubber	1984	**NW Pacific**	9.02 (3.88)	11.0 (3.08)			12	Subramanian *et al* . 1987
Harbor seal	mixed, E	blubber	1988	**U.K.**	21.1 (5.03 - 54.9)	2.03 (0.040 - 4.60)	57.5 (7.62 - 337)	4.86 (0.108 - 25.3)	34	Hall *et al* . 1992
Harbor seal	mixed, L	blubber	1989	U.K.	13.5 (0.273 - 99.7)	1.04 (0.30 - 10.0)	32.7 (0.496 - 348)	2.34 (0.055 - 17.8)	54	Hall *et al* . 1992
California sea lion	AF (Pre)	blubber	1970	**San Miguel Island, CA**	112.4 (85 - 145)	824.4 (626 - 1039)[g]	133.8[i]	981.4[ig]	6	DeLong *et al* . 1973
California sea lion	AF (F-T)	blubber	1970	San Miguel Island, CA	17.1 (12 - 25)	103.2 (51 - 203)[g]	20.1[i]	121.4[ig]	4	DeLong *et al* . 1973
Beluga whale	F	blubber	1987-1990	**St. Lawrence estuary**			29.6 (8.82 - 83.3)[j]	10.4 (1.736 - 52.3)[j]	21	Muir *et al* . 1996
Beluga whale	M	blubber	1987-1990	**St. Lawrence estuary**			78.9 (8.33 - 412)[j]	47.07 (2.1 - 249)[j]	15	Muir *et al* . 1996
Killer whale	mixed, R	blubber	1994-1999	Kenai Fjords/Prince William Sound, AK	3.9 (4.5)	3.1 (4.1)	14 (13)	11 (12)	64	Ylitalo *et al* . 2001
Killer whale	mixed, T	blubber	1994-1999	Kenai Fjords/Prince William Sound, AK	59 (43)	71 (54)	230 (130)	280 (180)	13	Ylitalo *et al* . 2001
Polar bear	AM	adipose	1996	N Alaska	3.59 (1.44)	0.0616 (0.0403)	4.34 (1.83)	0.0739 (0.0482)	5	Kucklick *et al* . 2002
Dugong	mixed	blubber	1996-1999	NE Australia			> 0.069 - 0.209	ND - 0.161	8	Vetter *et al* . 2001

[a] Concentrations are means (SD or range) unless otherwise stated.
[b] E = died during epizootic; L = live before or after epizootic; A = adult; J = juvenile; S = subadult; M = male; F = female; P = pregnant; L = lactating; Pre = females had premature pups; F-T = females that had full-term pups; R = resident; T = transient; NR = not reported.
[c] BI = Bear Island, SC. Sites in bold are locations in which effects were noted.
[d] all sites were averaged, mean lipid concentrations were converted to wet mass concentrations using mean lipid at each site.
[e] mean (SE) from control turtles, not from turtles exposed in the laboratory.
[f] values were estimated from graphs, PCB 153 only, and DDE plus minor PCB 85.
[g] total DDTs.
[h] estimated medians from graph.
[i] back calculated using mean and mean % lipid.
[j] geometric mean (range).

Table 1.5. Comparison of sea turtle egg OC concentrations to other species.[a]

			ng/g wet mass		ng/g lipid			
Species[b]	Year[b]	Location[bc]	Sum PCBs	4,4'-DDE	Sum PCBs	4,4'-DDE	N[d]	Reference
Loggerhead	1992	NW Florida	240-3720				80+ (20)[e]	Alam and Brim 2000
Loggerhead	1976	Florida	78 (32-201)[f]	66 (21; 18-200)[g]			9 (9)	Clark and Krynitsky 1980
Loggerhead	1993	South Carolina			1188 (311)[g]		16 (16)	Cobb and Wood 1997
Snapping turtle	1998	**Hamilton Harbor, L. Ont**	2956.28 (1448.36)	135.14 (85.67)	43157.37[h]	1972.85[h]	70 (14)	de Solla *et al* . 2002
Snapping turtle	1998	Akwesasne, St. Law. R.	3377.00 (3265.34)	10.00 (6.56)	46579.31[h]	137.93[h]	15 (3)	de Solla *et al* . 2002
Snapping turtle	1998	Algonquin Park	20.33 (10.79)	1.67 (0.58)	379.29[h]	31.16[h]	15 (3)	de Solla *et al* . 2002
Snapping turtle	1998	**Akwesasne, St. Law. R.**	95289 (259577)	117.63 (296.86)	1033503[h]	1275[h]	40 (8)	de Solla *et al* . 2001
Snapping turtle	1989	Algonquin Park	17.9 (8.8)	1.8 (0)			35 (7)	Bishop *et al* . 1998
Snapping turtle	1989	Rondeau Park, L. Erie	616.8 (404.1)	36.9 (26.4)			30 (6)	Bishop *et al* . 1998
Snapping turtle	1989	Big Creek, L. Erie	388.3 (245.4)	54.7 (28.9)			25 (5)	Bishop *et al* . 1998
Snapping turtle	1989	**Hamilton Harbor, L. Ont**	2082.4 (919.4)	311.9 (154.0)			35 (7)	Bishop *et al* . 1998
Snapping turtle	1990	**Hamilton Harbor, L. Ont**	3574.9 (2089.5)	388.8 (251.6)			60 (12)	Bishop *et al* . 1998
Snapping turtle	1991	Lynde Creek, L. Ontario	1429.6 (484.9)	231.8 (108.6)			40 (8)	Bishop *et al* . 1998
Snapping turtle	1991	Cranberry Marsh, L. Ontario	241.4 (173.5)	31.6 (21.8)			15 (3)	Bishop *et al* . 1998
Snapping turtle	1989	Trent River, L. Ontario	834.9 (759.6)	71.3 (69.8)			20 (4)	Bishop *et al* . 1998
Snapping turtle	1990	Akwesasne, St. Law. R.	3945.8 (1425.4)	67.8 (22.9)			35 (7)	Bishop *et al* . 1998
Snapping turtle	1976-1978	Hudson River, NY	23400 (12100)	151 (267)			6 (6)[i]	Stone *et al* . 1980
Snapping turtle	1974	Iowa		nd			1 (1)	Punzo *et al* . 1979
Snapping turtle	1974	Rondeau Park, L. Erie	1390 - 1920[j]	150 - 210	31591 - 36923[jh]	3409 - 4038[h]	2	Bishop and Gendron 1998
Snapping turtle	NR	6 sites in NY	3277 (4775)	7.3 (7.5)	70333 (121163)	118 (119)	6 (6)	Pagano *et al* . 1999
American alligator	1984	**Lake Apopka, FL**	170 (ND - 450)[k]	5800 (3400 - 7600)[k]			6 (3)	Heinz *et al* . 1991
American alligator	1984	Lake Okeechobee, FL	ND	870 (380 - 3200)[k]			14 (7)	Heinz *et al* . 1991
American alligator	1984	Lake Griffin, FL	80 (ND - 670)[k]	450 (100 - 2400)[k]			12 (6)	Heinz *et al* . 1991
American alligator CAM	NR	BI, SC			711 (250)[g]		15 (3)	Cobb *et al* . 1997
American alligator CAM	NR	YWC, SC			1671 (483)[g]		14 (7)	Cobb *et al* . 1997
American alligator E	NR	BI, SC			386 (81)[g]		19 (3)	Cobb *et al* . 1997
American alligator E	NR	YWC, SC			3763 (1175)[g]		19 (7)	Cobb *et al* . 1997
American alligator	NR	BI, SC			333.0 (171.7 - 645.8)[l]		19 (7)	Cobb *et al* . 2002
American alligator	NR	YWC, SC			3176 (1970-5120)[l]		19 (7)	Cobb *et al* . 2002
American alligator	NR	RWR, LA			218.3 (94.0-507.0)[l]		5 (5)	Cobb *et al* . 2002
American alligator	NR	BI, SC			250 (150)[m]	2632 (NR)	13 (NR)	Bargar *et al* . 1999
Chrysemys picta marginata	1974	L. Huron & L. Erie	1190 (1455)[j]	195 (153)	25783 (31617)[jh]	4088 (3158)[h]	6	Bishop and Gendron 1998
Apalone spiniferus	1974	Rondeau Park, L. Erie	5680[j]	720	71000[jh]	9000[h]	1	Bishop and Gendron 1998
Graptemys geographica	1974	Canadian Great L. Basin	1408 (1398)[j]	275 (253)	22408 (17063)[jh]	4499 (2836)[h]	4	Bishop and Gendron 1998
Emydoidea blandingi	1974	Long Point, L. Erie	330 - 340[j]	160 - 160	4250 - 5156[jh]	2000 - 2500[h]	2	Bishop and Gendron 1998
American crocodile	1997	Turneffe Atoll		111 (22)			12 (5)	Wu *et al* . 2000a
Morelet's crocodile	1997	S Belize		103 (31)			11 (4)	Wu *et al* . 2000a

Table 1.5. Continued

Species[b]	Year[b]	Location[bc]	ng/g wet mass		ng/g lipid		N[d]	Reference
			Sum PCBs	4,4'-DDE	Sum PCBs	4,4'-DDE		
Morelet's crocodile	1998	Gold Button Lagoon, N Belize		67 (52)			17 (8)	Wu *et al* . 2000b
Morelet's crocodile	1998	New R. Lagoon, N Belize		71 (26)			6 (1)	Wu *et al* . 2000b
American crocodile	1977-1978	Everglades, FL	520 (450)[n]	1160 (830)[n]			23 (8)	Hall *et al* . 1979
Northern water snake	1974	Iowa		60 (nd - 274)			9 (9)	Punzo *et al* . 1979
Red-sided garter snake	1974	Iowa		52			1 (1)	Punzo *et al* . 1979
Western plains garter snake	1974	Iowa		31			1 (1)	Punzo *et al* . 1979
Northern water snake	1993	Twelve Mile Creek	4460 (4410)				6 (6)	Fontenot *et al* . 2000
Northern water snake	1993	Reference L. Issaqueena	180 (160)				3 (3)	Fontenot *et al* . 2000
Herring gull	1992	N. Channel, L. Huron	6670	4030			1 pool of 12	Grasman *et al* . 1996
Herring gull	1992	**Saginaw Bay, L. Huron**	27450	7780			1 pool of 12	Grasman *et al* . 1996
Caspian tern	1992	N. Channel, L. Huron	4310	930				Grasman *et al* . 1996
Caspian tern	1992	**Saginaw Bay, L. Huron**	7540	3120				Grasman *et al* . 1996
Caspian tern	1997-1999	N. Channel, L. Huron	3550 (2000 - 5540)	1090 (590 - 2080)			39[o]	Grasman and Fox 2001
Caspian tern	1997-1999	**Saginaw Bay, L. Huron**	5690 (3450 - 8550)	1240 (980 - 1510)			39[o]	Grasman and Fox 2001
Bald eagle	1974-1980	Lake Erie	84000 (63100 - 139400)	24400 (13800 - 35800)			6	Donaldson *et al* . 1999
Bald eagle	1989-1994	Lake Erie	26400 (11700 - 43700)	10800 (2700 - 22200)			6	Donaldson *et al* . 1999
Bald eagle	1993-1996	Lake of the Woods	3270 (1300 - 12100)	3300 (900 - 12600)			7	Donaldson *et al* . 1999
Brown pelican (S)	1971-1972	SC	5500	1770			11 (11)	Blus *et al* . 1974
Brown pelican (U)	1971-1972	**SC**	7940	3230			26 (26)	Blus *et al* . 1974
Laysan albatross	1993	Midway Atoll, N. Pacific	177 - 220				20[p]	Jones *et al* . 1996
Black-footed albatross	1993	Midway Atoll, N. Pacific	688				1 pool of 10	Jones *et al* . 1996

[a] Concentrations are means (SD or range) unless otherwise stated.
[b] CAM = chorioallantoic membrane; E = egg tissues; S = successful nest; U = unsuccessful nest; NR = not reported; YWC = Yawkey Wildlife Center; BI = Bear Island, RWR = Rockefeller Wildlife Refuge.
[c] Sites in bold are locations in which effects were noted.
[d] total # of eggs analyzed (out of a total # of clutches).
[e] pooled 4-10 eggs per nest. 20 clutches were analyzed.
[f] geometric mean (range); total PCBs were measured as Aroclor 1260.
[g] arithmetic mean (standard error; range).
[h] back calculated using mean and mean % lipid.
[i] N = 5 (5) for DDE.
[j] total PCBs are Aroclor 1254:1260.
[k] geometric mean (range).
[l] means were calculated from log-transformed nest means (95 % cofidence limits).
[m] PCB 153 only, estimated from graph.
[n] mean of the mean concentration per clutch.
[o] 3 pools of 13 eggs (1 pool/year); mean (range) of 3 years.
[p] 2 pools of 10 eggs each.

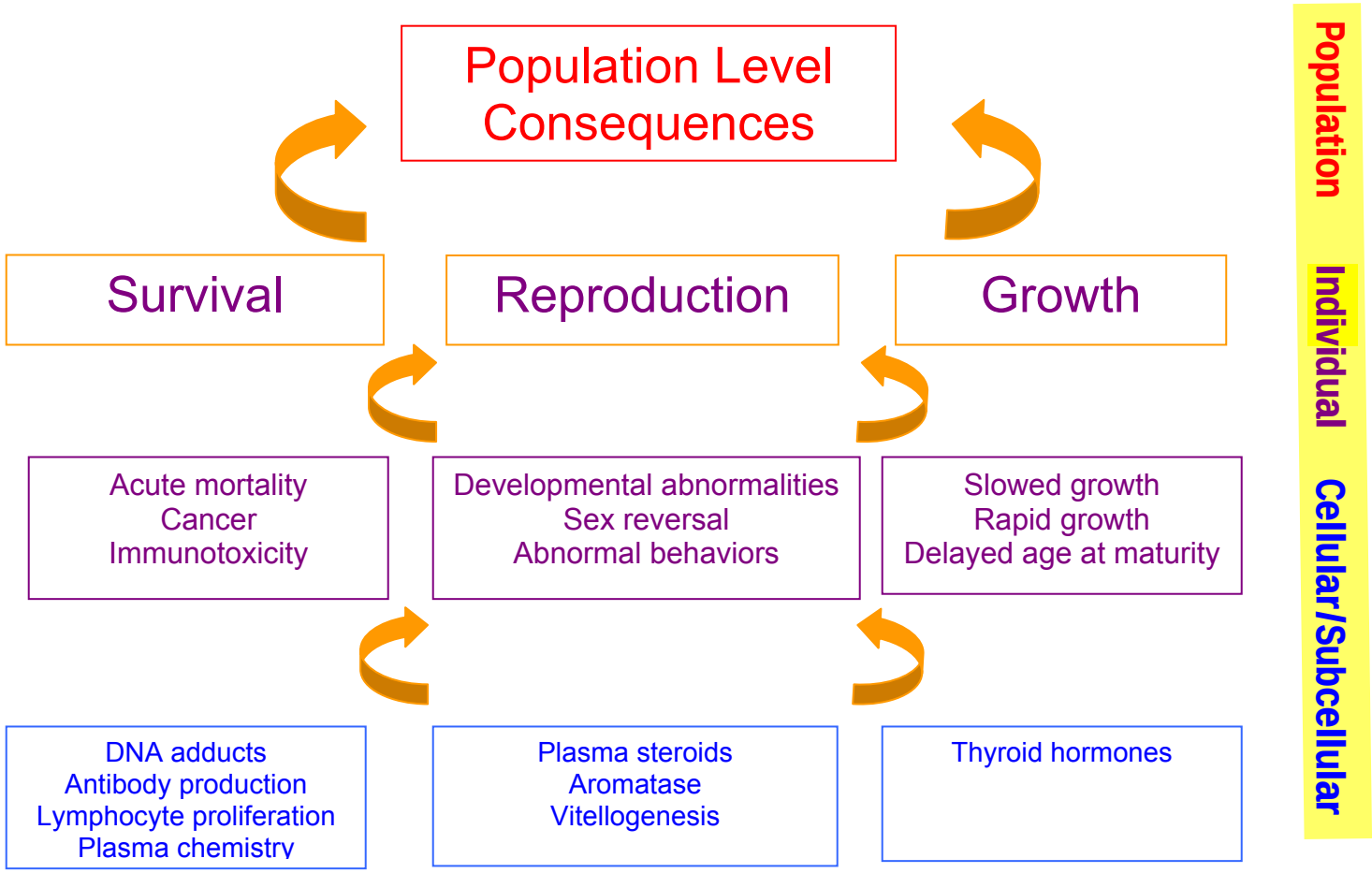


Figure 1.1. Flow chart showing the increasing level of biological organization and a variety of toxicological endpoints at the subcellular to individual levels that may affect vital rates of populations. Toxicologists typically focus on subcellular to individual levels of organization, but those toxicological effects can cause consequences at higher levels, including reduced survival, reproductive failure, and altered growth. All of which can ultimately affect populations.

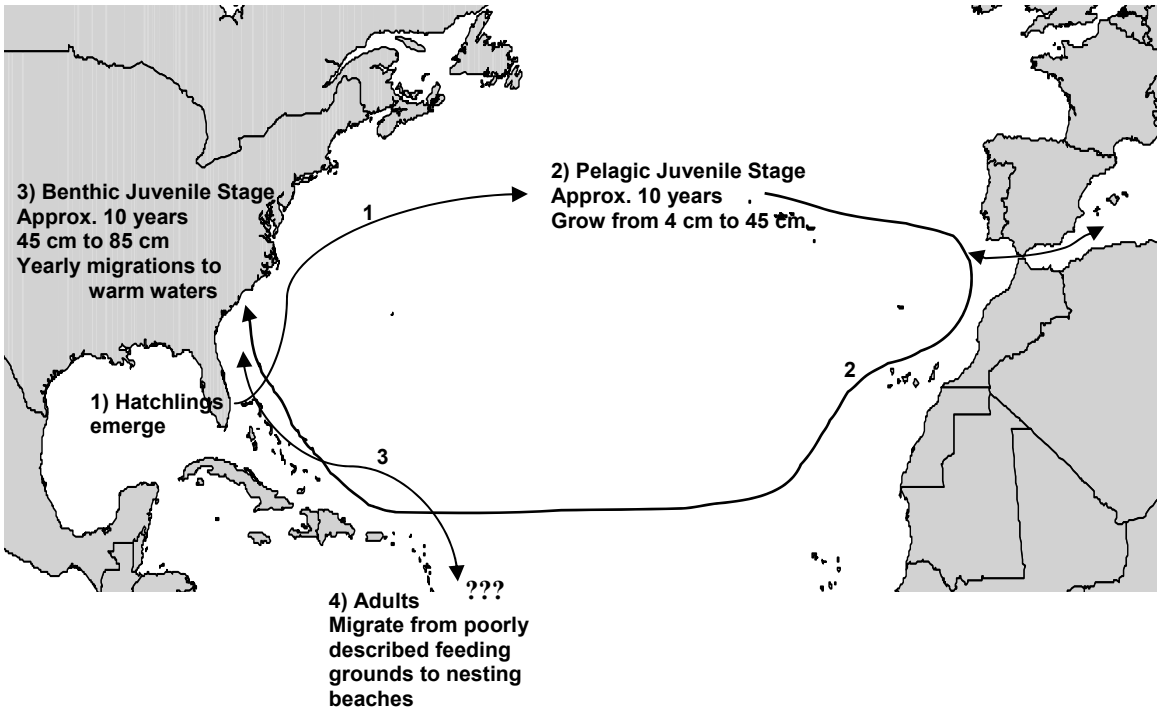


Figure 1.2. Diagram of the life history of loggerhead sea turtles that nest along the southeast coast of the U.S. The approximate duration and growth of each stage is provided. Turtle lengths (cm) are straight carapace length from the nuchal notch to the notch in the posterior marginal scute. Arrows indicate general migration patterns of each life stage. Numbers above each arrow are provided for the ease of following the chronological stages of loggerhead turtle development.

CHAPTER TWO

Organochlorine contaminants in loggerhead sea turtle blood: extraction techniques and distribution among plasma and red blood cells

ABSTRACT

Few studies have described the organochlorine (OC) contaminant concentrations found in sea turtle tissues. These studies have relied on the opportunistic sampling of either eggs or tissues from stranded carcasses. In this study, the use of whole blood samples as well as both blood components (plasma and red blood cells) were examined as a non-destructive alternative for monitoring OCs in free-ranging loggerhead sea turtles (*Caretta caretta*). Blood samples were collected from juvenile loggerhead sea turtles (N=12) captured in Core Sound, North Carolina, USA and analyzed for 55 polychlorinated biphenyl (PCB) congeners and 24 OC pesticides by gas chromatography with electron capture detection and mass spectrometry. Using pooled loggerhead sea turtle whole blood, three different liquid:liquid extraction techniques were compared. Results were similar in terms of recovery of internal standards, lipids, and OC concentrations. An extraction technique, employing formic acid and 1:1 methyl-*tert*-butyl-ether:hexane, was found to be satisfactory. This method was applied to the extraction of OCs from whole blood, plasma, and red blood cell (RBC) samples from five loggerhead sea turtles. Plasma contained the highest OC concentrations on a wet mass basis, followed by whole blood and RBCs. The majority of each OC compound was found in the plasma rather than the RBCs, suggesting that OC compounds preferentially partition into the plasma. On average (SD), 89.4 % (3.1 %) of total PCBs, 83.4 % (11.9 %) of total chlordanes, 74.3 % (15.1 %) of mirex, 72.6 % (4.8 %) of total DDTs, and 80.1 % (16.6 %) of dieldrin were found in the plasma. The concentrations of total PCBs, mirex, total chlordanes, and total DDTs measured in both fractions of the blood

significantly correlated to those in whole blood. These are the first reported OC concentrations in the sea turtle blood. They were found to be similar to previously reported levels in blood components of humans and of reptiles from relatively clean sites, but lower than those measured in blood of fish-eating birds and marine mammals. The results indicate that blood, preferably plasma, can be used to detect and monitor OC contaminants in loggerhead sea turtles.

INTRODUCTION

Polychlorinated biphenyls (PCBs) and organochlorine (OC) pesticides, such as DDT, have contaminated the global environment (Iwata *et al.* 1994). Once widely used in industry and agriculture, most of these compounds were banned from use in developed countries starting in the 1970s mainly because of their reproductive toxicity and carcinogenicity. However, OC contaminants continue to be detected in environmental samples and animal tissues due to their persistence, their ability to bioaccumulate through the food chain, and their continued use in some underdeveloped countries (i.e.; Iwata *et al*. 1994, Letcher *et al.* 1995, Rybitski *et al*. 1995).

Because of their toxicity and environmental persistence, it is important that OC contaminants be monitored in wildlife populations. This is especially critical for species that are threatened with extinction, as these compounds may produce detrimental health effects and contribute to population declines in these vulnerable species. All species of sea turtles are listed as threatened or endangered under the U.S. Endangered Species Act or the Convention of International Trade of Endangered

Species (Pritchard 1997). There is little information available on the levels of contaminants in sea turtle tissues, and currently no long-term monitoring projects are in place for these animals (for review see Pugh and Becker 2001).

OC contaminants are generally measured in fatty tissues since they are highly lipophilic. In previous studies, fatty tissues from sea turtles, such as fat, liver, muscle, and eggs have been collected opportunistically from either unhatched eggs or stranded turtle carcasses in order to measure OCs (Corsolini *et al.* 2000, Rybitski *et al*. 1995, Cobb and Wood 1997, Mckenzie *et al*. 1999). Recently, however, researchers have started to use blood in wildlife monitoring studies (i.e.; Henny and Meeker 1981, Jenssen *et al*. 1994, Elliott and Norstrom 1998, Reddy *et al*. 1998, Bishop and Rouse 2000, Sandau *et al*. 2000). Blood offers several benefits over traditional tissue sampling. It can be collected easily and relatively non-destructively from free-ranging populations and facilitates the repeated collection of larger numbers of samples, which improves both the monitoring of OC levels and the assessment of toxicological effects. Blood OC concentrations have been used as a surrogate for concentrations in fatty tissues. For example, blood OC concentrations were significantly correlated to concentrations measured in the blubber of marine mammals, in brains of birds, and in fat biopsies of humans (Reddy *et al*. 1998; Henny and Meeker 1981; Mes 1992). However, it is difficult to compare the results of these past studies because they differ in their choice of extraction techniques or blood fractions used in the analysis of contaminants.

Prior studies on OCs in blood have employed everything from whole blood to any one of its components. Whole blood can be separated into a liquid fraction

(plasma or serum) and a packed red blood cell (RBC) fraction by simple centrifugation. Serum is collected after the blood is allowed to clot, while plasma is collected from blood that is mixed with an anti-coagulant, so it retains fibrinogen and other clotting factors. The liquid fractions contain lipoproteins and other proteins, such as albumin, which are known to bind and transport OC contaminants (see Norén *et al*. 1999). OCs also bind to membranes and hemoglobin in the RBC fraction (Moss and Hathway 1964). The goals of our study were to determine whether OCs could be reliably detected in the blood of loggerhead sea turtles (*Caretta caretta*), to describe an effective method for extracting OCs from their whole blood, and to establish the physical partitioning of OCs within their blood.

MATERIALS AND METHODS

Samples

Twelve juvenile loggerhead sea turtles were captured between June 1998 and July 2001 as bycatch in the pound net fishery in Core Sound, North Carolina, USA. Seven of the turtles were female, three were male, and two were of unknown sex. Turtles ranged in size from 62.1 cm to 79.6 cm straight carapace length (notch to tip). Based on their size, these turtles may be estimated at somewhere between 10 and 30 years of age (Snover 2002). Two 10 mL blood samples were collected from each turtle within 15 min of capture from the dorsocervical sinus using double-ended needles and blood collection tubes containing sodium heparin (Becton Dickinson, Franklin Lakes, NJ). Whole blood was kept on ice until frozen at -20 ^{o}C. The second blood sample from each turtle was centrifuged at 400 x g for 5 min to separate the

plasma and red blood cells (RBCs). The outside of each blood tube was marked at the meniscus of the plasma and at the top of the packed RBCs. The plasma was transferred to a hexane-rinsed glass vial using hexane-rinsed glass transfer pipets. The plasma and remaining RBCs were frozen at -20 ^{o}C. Once the RBCs were removed from the original tubes for contaminant analysis, the volume of total blood and RBCs were determined. Packed cell volume (PCV) was estimated as the volume of packed RBCs divided by the total blood volume.

Blood extraction techniques

Three different extraction techniques (A, B, and C) were compared to determine the relative efficiency of extracting OC contaminants from loggerhead sea turtle whole blood (Fig. 2.1). Blood pooled from 7 juvenile loggerhead sea turtles was sonicated and divided into 5 g aliquots. Three replicate blood tubes and a blank tube containing 5 mL deionized water were then assigned to each extraction technique (A, B, or C). An internal standard solution (0.2 g) of isooctane was added to each tube resulting in the addition of 35 ng of each of the following compounds: 4,4'-DDT-d_8, 4,4'-DDE-d_8, 4,4'-DDD-d_8, Endosulfan I-d_4, PCB 103 and PCB 198. Tubes were mixed well and allowed to set on ice for at least 2 h.

Extraction techniques were chosen based upon published methods and personal communications. Technique A was used by the National Institute of Standards and Technology (NIST) to determine the certified values for Standard Reference Material (SRM) 1589a (PCBs, Pesticides, and Dioxins/Furans in Human Serum) and was similar to another published method (Dr. Michele Schantz, personal communication, Hovander *et al*. 2000). Technique A (Fig. 2.1) was carried out by adding 5 mL of

hexane-rinsed 98 % formic acid followed immediately by the addition of 5 mL of 1:1 (v/v) methyl-*tert*-butyl ether (MTBE):hexane. These tubes were shaken by vortex for 2 min. Technique B (Fig. 2.1) was modified from a protocol developed for measuring dioxins in human whole blood (Dr. David J. Brown, personal communication). These tubes (technique B) received 25 mL of acetone and were mixed by shaking. Then, they received 1 mL 98 % formic acid and were mixed by inversion. These tubes were extracted by the addition of 10 mL 1:1 MTBE:hexane. Technique C (Fig. 2.1) was a method reported in a study measuring OCs in Antarctic seabird blood (van den Brink *et al*. 1998). This technique employed 25 mL acetone followed by an addition of 10 mL of 1:1 MTBE:hexane.

Emulsions formed from each extraction technique were broken by adding another 5 mL of 1:1 MTBE:hexane to the tubes. Organic layers from all extraction tubes were transferred to clean tubes and the blood samples were re-extracted with 10 mL 1:1 MTBE:hexane. Organic layers were combined with the previous fractions and a third extraction was carried out. For the third extraction, tubes in technique A received 10 mL of hexane, while those in techniques B and C received 10 mL of 1:1 MTBE:hexane. The organic layers were combined with the previous fractions. As each organic layer from techniques B and C was transferred, they were washed three times with 5 mL of aqueous 1 % KCl (mass:volume) to remove water. The combined final extracts were reduced by evaporation with purified N_2 (Turbovap II, Zymark, Hopkinton, MA).

Lipid content was determined gravimetrically for all blood samples and NIST SRM 1589a. Approximately 5 % to 10 % of each extract by weight was removed and

transferred to a tared aluminum weighing boat. The solvent in the weighing boat was allowed to evaporate at room temperature for 6 h to 12 h. The mass of dried lipid residue was measured to the nearest 0.0001 mg.

Four calibration solutions were prepared from 5 NIST SRMs: 2261 (Chlorinated Pesticides in Hexane), 2275 (Chlorinated Pesticides in Hexane II), 2262 (Chlorinated Biphenyl Congeners in 2,2,4-trimethylpentane), 2274 (PCB Congener Solution II), and a solution containing 14 additional PCB congeners. The calibration curve ranged from approximately 50 pg to 3,500 pg. An internal standard solution of isooctane was added to each calibration solution resulting in the addition of approximately 35 ng of each of the following compounds: 4,4'-DDT-d_8, 4,4'-DDE-d_8, 4,4'-DDD-d_8, Endosulfan I-d_4, PCB 103 and PCB 198. The calibration solutions were not extracted.

Sample Clean-up

High molecular mass compounds in the blood extracts were removed by gel permeation chromatography (GPC) on a 600 mm x 25 mm (10 μm particle size with 100 Å diameter pores) PLGel column (Polymer Labs, Amherst, MA) using CH_2Cl_2 according to Kucklick *et al*. (2002). Following separation, sample extracts were reduced in volume to approximately 0.5 mL using the Turbovap and the solvent was changed to hexane.

The sample extracts and the calibration solutions were fractionated into relatively lower and higher polarity fractions (F1 and F2, respectively) using a semi-preparative aminopropylsilane column (μBondapak NH_2, Waters) (Kucklick *et al.*

2002). Each fraction was amended with 5 ng PCB 14 prior to analysis in order to calculate percent recovery of internal standards. Compounds contained in F1 included PCBs, heptachlor, 2,4'-DDE, 4,4'-DDE, 2,4'-DDT, hexachlorobenzene (HCB), aldrin, mirex, and oxychlordane. Analytes in F2 included 4,4'-DDT, *cis*- and *trans*-chlordane, *cis*- and *trans*-nonachlor, α- β-, and γ-hexachlorocyclohexane (HCH), heptachlor epoxide, 2,4'-DDD, 4,4'-DDD, dieldrin, endrin, endosulfans I and II, and endosulfan sulfate.

Contaminant Analysis

OC compounds contained in F1 were identified by gas chromatography (GC) with dual micro-electron capture detectors (ECD) (Hewlett Packard 6890; Kucklick *et al.* 2002). Organochlorines were separated using a 60 m, 0.25 mm interior diameter, 0.25 μm film thickness, 5 % phenyl methyl-poly-siloxane capillary column (DB-5 column, J&W Scientific, Folsom, CA) and a 60 m, 0.25 mm interior diameter, 0.25 μm film thickness proprietary phase capillary column (DB-XLB column, J&W Scientific, Folsom, CA). The injector and detector temperatures were 220 ^{o}C and 325 ^{o}C, respectively. The carrier and makeup gasses were H_2 (constant velocity of 30 cm/s) and N_2 (30 mL/min), respectively. Samples were injected into the GC (2 μl, splitless injection), and the oven was programmed for 90 ^{o}C initially (1 min hold) to 170 ^{o}C at 18 ^{o}C /min, then 1 ^{o}C /min to 260 ^{o}C then ramped to 300 ^{o}C at 1.5 ^{o}C /min (107 min run time). The coplanar PCB congeners (PCB 77, PCB 126, and PCB 169) were not targeted for analysis, because the levels of these compounds in sea turtle blood would likely be well below the limits of detection (10 pg/g wet mass).

OC compounds in F2 were determined using a Hewlett Packard 6890/5973 GC-MS operating in the electron-impact (EI) mode and using selected ion monitoring (SIM) programs targeting only the analytes in this fraction. By quantifying the F2 compounds using GC-MS, the signal to noise ratio was greatly improved, which was not a problem with the F1, resulting in more reliable quantification of these compounds. Samples were injected (2 μl) onto a 60 m, 0.25 mm interior diameter, 0.25 μm film thickness, 5 % phenyl methyl-poly-siloxane capillary column (DB-5 MS, J&W Scientific, Folsom, CA). Helium was used as the carrier gas at a constant flow rate of 30 cm/s. The initial column temperature was 60 °C; the temperature was then ramped to 170 °C at 25 °C /min, then to 200 °C at 1 °C /min, then to 240 °C at 2 °C / min. The final ramp brought the temperature to 300 °C at 10 °C / min, with a 10 min hold. The ions used to determine the analytes are given in Table 2.1.

The amount of each compound in the unknowns was calculated using the mass of PCB 14 added and the slope and intercept of the four calibration solutions. PCB 14 was used to calculate percent recovery of internal standards and all values were corrected with those recovery estimates. The average recovery of PCB 103 and PCB 198 was used to correct all analytes in F1, except for 2,4'-DDE, 4,4'-DDE, and 2,4'-DDT where the percent recovery of 4,4'-DDE-d_8 was used. When the values from the GC-ECD were similar between columns, they were averaged. In cases where the values from the two columns disagreed, the smaller value of the two was reported. In F2, all compounds were corrected for the percent recovery of 4,4'-DDD-d_8, except 4,4'-DDT which was corrected using the recovery of 4,4'-DDT-d_8.

Analysis of NIST SRM 1589a (Human Serum) and plasma, red blood cells, and whole blood from individual turtles

Five bottles of NIST SRM 1589a (PCBs, Pesticides, and Dioxins/Furans in Human Serum) were analyzed for OC contaminants. Each bottle of freeze-dried serum was reconstituted with water according to the instructions in the Certificate of Analysis. Briefly, 10.0 mL of deionized water was added to each bottle. The bottles were mixed periodically over 1 h at room temperature.

OC contaminants were analyzed in plasma, RBCs, and whole blood samples from 5 individual loggerhead sea turtles (3 females and 2 males) all captured in July 2001. Analytical conditions for the NIST SRM 1589a and the turtle samples were identical to those described above except for the following modifications (Fig. 2.1). Extraction technique A was used to extract approximately 10 g of reconstituted human serum, 3 g of turtle plasma, 3 g turtle RBCs, and 4 g turtle whole blood. Approximately 3 ng of each internal standard compound was added to these samples prior to extraction. The tubes were mixed by sonication for 15 min and allowed to set for 2 h at room temperature. In order to reduce emulsions, the third re-extraction used 10 mL 1:1 MTBE:hexane instead of hexane. Remaining water was removed from the combined organic extracts with 5 g to 10 g of anhydrous Na_2SO_4. Sample clean-up was carried out using alumina columns prepared using the methods described in Holden and Marsden (1969). Briefly, alumina was activated by heating to 800 ^{o}C for 4 h and then deactivated by adding 5 % H_2O. Above glass wool, 2 g of alumina was

poured into a glass pipet and topped with Na_2SO_4. Hexane (30 mL) was used to elute the OC compounds from the column. After fractioning each sample and calibration solution on the aminopropylsilane column, approximately 5 ng of PCB 14 was amended to each fraction.

The percentage of each compound distributed into the plasma or RBCs was calculated using the following equation:

$$\% \text{ in fraction}_1 = (C_1P_1)/[(C_1P_1) + (C_2P_2)]*100$$

where C_1 is the concentration of a compound in fraction$_1$ of the blood on a wet mass basis, P_1 is the proportion of whole blood consisting of fraction$_1$ on a volume basis (calculated by PCV), and C_2 and P_2 are the same as above for fraction$_2$ of the blood.

Lab blanks (5 mL deionized water) and field blanks were processed with each sample batch. Field blanks were 5 mL deionized water pulled through a Vacutainer needle directly into a Vacutainer tube, both of which were previously taken into the field.

Statistics

All statistical tests were performed using Systat 8.0 (SPSS, Inc, Chicago, IL). Differences in percent recovery of internal standards, percent lipid, and final contaminant concentrations among the three extraction techniques were analyzed using analysis of variance (ANOVA) and the Tukey multiple comparison test. Pearson correlations were calculated to compare the contaminant concentrations between the different blood components.

RESULTS

The three extraction techniques resulted in similar percent recovery of the internal standards and resulted in similar lipid values from the pooled loggerhead sea turtle whole blood (Table 2.2). Significant differences were noted for only PCB 198 in which technique A extracted less than techniques B and C. Percent lipid determined in the pooled blood did not differ among the extraction techniques.

Fifty-five PCB congeners and 24 OC pesticides were targeted for analysis. Twenty-four PCBs and 7 pesticides were detected in the pooled loggerhead sea turtle whole blood (Table 2.3). Figure 2.2 shows a representative chromatogram of loggerhead sea turtle whole blood. Each of the extraction techniques allowed for the detection of the major compounds. Few differences in OC concentrations were observed among the techniques. Technique A resulted in the detection of slightly higher concentrations of PCB 99, PCB 158, PCB 163, dieldrin, *trans*-nonachlor, and total chlordanes than technique C. Extraction technique A resulted in the detection of lower concentrations of PCB 206 than techniques B or C. Although some significant differences were detected, the three techniques were generally similar, at most differing by only 24 %.

In order to validate the analytical method, OC contaminants were determined from five bottles of NIST SRM 1589a (Human Serum) using extraction technique A. The OC concentrations were compared to the certified and reference values (Table 2.4). The results differed from the mean certified values by less than 30 % for all compounds, except PCB 118, PCB 206, PCB 209, and *trans*-nonachlor. The measured concentrations of PCB 74, mirex, and 2,4'-DDE also substantially differed

from the mean reference values. Interfering, unknown compounds may have artificially increased the measured concentrations of PCB 118, PCB 206, PCB 209, *trans*-nonachlor, and mirex, while the reason for the lower observed concentrations of PCB 74 and 2,4'-DDE is unknown. The observed concentrations of 4,4'-DDT closely matched the reference value for SRM 1589a; however, the GC-MS peak for 4,4'-DDT in the turtle samples was abnormally shaped and did not consist of the correct target ions. The 4,4'-DDT concentrations, therefore, were not reported for the turtle samples nor were they included in the calculation of total DDTs for the turtle samples.

PCB and pesticide concentrations were compared between both components of the blood (plasma and RBCs) and whole blood from five individual loggerhead sea turtles (Table 2.5). Generally, plasma contained the highest concentrations of OCs on a wet mass basis, followed by whole blood and RBCs. In the turtles with lower levels of OCs, several compounds were below the limits of detection in the RBC fraction. As indicated by the large standard deviations, the blood OC concentrations varied greatly among individual turtles, suggesting that some turtles are exposed to or are accumulating higher levels than others.

The partitioning of OCs was calculated between plasma and RBCs. Estimated packed cell volume (PCV) averaged 35.2 % and ranged from 22.7 % to 40.7 %. Using these values we calculated the percentage of OC masses that were distributed between the plasma and RBCs. Plasma contained a larger percentage of OC contaminants than the RBC fraction (Fig. 2.3). Plasma comprised 65 % of loggerhead sea turtle blood and contained 81 % to 95 % of the individual congeners of PCBs and chlordanes. The plasma also contained approximately 80 % of the total masses of dieldrin and 75 % of

mirex and DDTs found in whole blood. Therefore, the majority of OC compounds preferentially partitioned into the plasma even though no differences in lipid content were observed between plasma, RBC, or whole blood. When comparing the OC concentrations measured in the two fractions to each other or to whole blood, significant correlations were observed for most compounds (Table 2.6). Dieldrin, however, did not correlate between the whole blood and the two fractions. The lack of correlation for dieldrin was most likely due to the fact that many of the samples were below the detection limit.

DISCUSSION

This study is the first to report OC concentrations in sea turtle blood and to provide comparative methods for extracting OC contaminants from their blood. Additionally, this study provides the first comparison of the distribution of OC contaminants among the plasma and cellular components of whole blood in a reptile species.

The extraction techniques selected for this study were compiled from several sources (see methods section) and differed mainly in their use of formic acid. Formic acid used in techniques A and B was expected to aid in the extraction of most compounds and decrease the amount of lipid and acid-sensitive dieldrin recovered from the samples. Interestingly, lipid recovery did not differ between the three techniques, and technique A resulted in the detection of higher dieldrin concentrations than the acid-free extraction employed by technique C. These results suggest that formic acid did not negatively affect lipid determinations nor acid-sensitive

compounds. Based on these data and in order to remain consistent with the methods currently used for the certification of SRM 1589a, technique A with slight modifications was chosen for future analyses.

Other techniques such as solid phase extraction (SPE) are available to extract OCs from blood components (Guillette *et al*. 1999). SPE can easily extract plasma and serum samples but not coarse tissues such as whole blood or RBC. Coarser samples must first be extracted using a liquid:liquid technique prior to employing the SPE (Volz *et al*. 2001).

Regardless of which technique is employed, insufficient sample volumes or high analytical costs often limit the number of replicates that can be analyzed for each sample. When each sample can only be analyzed once, which is often the case, it is important to extract replicates of a pooled sample or a standard material in order to estimate the variation in measured contaminant concentrations. This study provides two estimates of variation, a) the standard deviation observed from the bottles of human serum (SRM 1589a, Table 2.4) and b) the standard deviation of the three replicates of pooled loggerhead sea turtle blood for each extraction technique (Table 2.3). Among the three extraction techniques, the standard deviations for most compounds are very similar and relatively small (Table 2.3). Between loggerhead sea turtle blood (technique A) and the human serum, the standard deviations are also similar, except for those compounds that are found at a higher concentration in one than the other (i.e.; 4,4'-DDE). These known standard deviations could provide an estimate of variation for future analyses of sea turtle blood when only one replicate can be analyzed per sample. It should also be noted that analytical error in measuring

OCs in sea turtle blood (see SD of the pooled turtle blood in Table 2.3) is much smaller than the biological variation of OCs measured among 5 individual turtles (Table 2.5).

OC contaminants were detectable in both fractions of the blood (plasma and RBCs) as well as whole blood. The OC concentrations measured in the two fractions correlated to those detected in whole blood and to each other for the majority of the compounds. The same relationship has been observed for total PCB concentrations between human whole blood and plasma (Sandau *et al*. 2000) and for chlordanes, DDE, and total PCB concentrations between polar bear plasma and RBCs (Bernhoft *et al*. 1997). Future studies should examine the relationship between blood OC concentrations and those in fatty tissues of sea turtles to determine if blood can be used as a surrogate for those tissues.

OC contaminants preferentially partitioned into the liquid fraction of sea turtle blood, which is similar to observations in mammalian studies. Harbor seal blood is made up of approximately 33 % serum and 66 % RBCs, but 41 % of PCBs that were measured in whole blood were found in the serum, suggesting that substantial amounts of OC compounds bind to non-cellular molecules (Boon *et al*. 1987). Matthews and others (1984) injected Sprague-Dawley rats with eight PCB congeners to investigate the distribution of the PCBs between the plasma and RBC fractions. The larger proportion of each PCB congener (52 % to 83 %) was found in the plasma. Similarly, the majority of hexa- to octa-chlorinated PBCs, *trans*-nonachlor, and DDTs were found in human serum or plasma rather than the RBC fraction (Mes *et al.* 1992). OC contaminants are known to associate with plasma proteins, such as lipoproteins and

albumin (see Norén *et al*. 1999). This could explain the partitioning of OCs into the plasma.

The results observed with sea turtle blood suggest that any blood component may be used to measure OCs, but plasma offers significant advantages. Plasma contained the highest concentrations of OCs, therefore allowing for better detection of compounds. Operationally, plasma is easier to use because it resulted in fewer emulsions during the liquid:liquid extraction, and it can be passed directly through a solid phase extraction cartridge. However, for large-scale field projects that survey sea turtle populations, collecting whole blood is simpler and minimizes the potential of contaminating the sample in the field. If feasible, the blood components can be separated by centrifugation and the tubes then frozen in an upright position. Later, the blood can be thawed and plasma removed carefully from the underlying RBCs without contamination.

The blood OC concentrations (mean, and/or ranges on a wet mass basis) measured in the loggerhead sea turtles were similar to those reported for other reptile species. PCB concentrations measured in the blood of loggerhead sea turtles (mean = 5.14 ng/g; range = 1.01 ng/g – 11.0 ng/g) were similar to concentrations reported in blood components of the adult northern water snakes (3 ng/g – 12 ng/g) from the Great Lakes and juvenile American alligators (1.54 ng/mL) from lakes in central Florida (Bishop and Rouse 2000; Guillette *et al*. 1999). The loggerhead blood PCB concentrations were lower than those in plasma of snapping turtles from reference lakes (~ 18 ng/g) and contaminated sites (means ranged from 263 ng/g to 415 ng/g) in the Great Lakes (de Solla *et al*. 1998).

Compared to PCB concentrations in blood components of fish-eating or predatory birds, the loggerhead sea turtle has one to three orders of magnitude lower levels of PCB contaminants. For example, total PCBs in plasma of Caspian tern chicks from Lake Huron were reported to range from 20 ng/g to 400 ng/g (Grasman and Fox 2001). Total PCB concentrations in the plasma of bald eagle chicks from Lake Erie ranged from 9.9 ng/g to 326 ng/g (Donaldson *et al*. 1999), and from the Pacific coast of Canada they ranged from 1.9 ng/g to 114 ng/g (Elliott and Norstrom 1998).

PCB levels in loggerhead sea turtle blood were similar to those measured in RBC from grey seal pups from Norway (1 ng/g – 32 ng/g; Jenssen *et al*. 1994), but substantially lower than those in blood of bottlenose dolphins from Sarasota, Florida (26.3 ng/g – 752 ng/g; Lahvis *et al*. 1995). Compared to humans, PCB levels in loggerhead blood were similar to the mean plasma concentration of fish-eating men from Ontario, Canada (5.5 ng/g; Kearney *et al*. 1999) and slightly lower than those seen in Canadian Inuit blood (15.2 ng/g; Sandau *et al*. 2000).

Similar comparisons can be observed for 4,4'-DDE concentrations. Loggerhead blood concentrations of 4,4'-DDE (0.576 ng/g) were similar to alligator and snapping turtles from reference sites (means ranged from 0.2 ng/g to 1.28 ng/g), but lower than those animals from contaminated sites (7.35 ng/g – 21.7 ng/g; Guillette *et al*. 1999; de Solla *et al*. 1998). The loggerhead had three orders of magnitude lower 4,4'-DDE concentrations than blood of snakes and red-eared slider turtles from a site in Texas (56.1 ng/g – 3830 ng/g; Clark *et al*. 2000). DDE concentrations in the

birds and marine mammals mentioned previously were typically one to three orders of magnitude higher than those of loggerhead sea turtles.

In conclusion, OC concentrations are relatively low in sea turtle blood, but the sensitivity of sea turtles to these levels is unknown. OC contaminants were detected in loggerhead sea turtle blood components and a liquid:liquid extraction technique (technique A) employing formic acid and 1:1 MTBE:hexane was determined to be sufficient for future analyses. This analytical technique was validated using a similar tissue matrix, human serum (NIST SRM 1589a). Additionally, loggerhead sea turtle plasma was determined to contain the largest proportion of OC compounds found in the whole blood, suggesting that plasma may be the best blood component to use when monitoring OCs in sea turtles. Numerous studies, worldwide, survey sea turtle populations and many already collect blood for genetic research. This fact combined with the results and methods described in this study indicate that monitoring OCs may now be easily incorporated in these projects using non-destructive and selective blood sampling.

Table 2.1. Target ions for GC-MS used to identify compounds in fraction 2.

Compound	Major ion	Minor ion
PCB 14	222	--
ΣHCHs	219	217
4,4'-DDT	235	237
4,4'-DDT-*d8*	243	245
2,4'-DDD	235	237
4,4'-DDD	235	237
4,4'-DDD-*d8*	243	245
heptachlor epoxide	353	355
trans- and *cis* -chlordane	373	375
trans - and *cis* -nonachlor	409	407
dieldrin	263	265

Table 2.2. Mean (SD) percent recovery of internal standards and percent lipid content extracted from pooled whole blood of juvenile loggerhead sea turtles extracted with three different techniques.

	Technique A (%)	Technique B (%)	Technique C (%)	Differences[a, b]
PCB 103	41.1 (5.5)	44.2 (3.6)	46.6 (1.8)	NS
PCB 198	43.6 (5.9)	52.4 (1.9)	58.4 (3.4)	A < B < C
4,4'-DDE-d_8	42.8 (6.3)	46.8 (2.1)	51.2 (3.3)	NS
4,4'-DDD-d_8	49.1 (1.6)	46.2 (0.9)	49.6 (6.6)	NS
4,4'-DDT-d_8	59.8 (2.9)	60.3 (3.0)	60.8 (10.0)	NS
Percent lipid	0.200 (0.076)	0.267 (0.058)	0.175 (0.096)	NS

[a] Differences were determined by the Tukey Multiple Comparison Test ($p \leq 0.05$).
[b] NS, not significant.

Table 2.3. Mean (SD) organochlorine concentrations (pg/g wet mass) in pooled whole blood of seven juvenile loggerhead sea turtles extracted with three different techniques.

	Technique A (pg/g wet mass)	Technique B (pg/g wet mass)	Technique C (pg/g wet mass)	Differences[a,b]
PCB 66	48.3 (6.0)	44.1 (6.7)	39.2 (3.0)	NS
PCB 99	457 (19)	430 (8)	406 (13)	A > C
PCB 105	152 (14)	135 (7)	130 (9)	NS
PCB 118	392 (24)	385 (15)	367 (14)	NS
PCB 128	102 (9)	96.3 (3.4)	94.6 (6.7)	NS
PCB 138	2630 (53)	2560 (10)	2520 (76)	NS
PCB 146	342 (11)	333 (3)	325 (5)	NS
PCB 149	19.3 (2.2)	21.5 (5.8)	20.4 (2.9)	NS
PCB 153	2110 (190)	2130 (197)	2090 (144)	NS
PCB 156	49.4 (6.3)	49.7 (4.2)	43.7 (2.3)	NS
PCB 157	16.3 (3.7)	13.2 (2.3)	8.07 (7.15)	NS
PCB 158	73.7 (6.4)	63.9 (8.2)	56.3 (1.0)	A > C
PCB 163	329 (11)	312 (2)	302 (6)	A > C
PCB 170	136 (11)	136 (5)	130 (3)	NS
PCB 174	13.1 (12.3)	<10	<10	
PCB 180	762 (10)	821 (41)	805 (29)	NS
PCB 183	187 (6)	195 (8)	188 (7)	NS
PCB 187	639 (18)	657 (10)	644 (23)	NS
PCB 193	213 (6)	220 (11)	211 (8)	NS
PCB 194	56.2 (4.1)	61.9 (1.9)	64.2 (4.0)	NS
PCB 195	33.1 (4.9)	34.4 (1.5)	31.3 (0.9)	NS
PCB 201	41.0 (4.6)	39.9 (2.1)	34.2 (2.7)	NS
PCB 206	93.0 (1.8)	103 (4)	104 (4)	A < B = C
PCB 209	25.6 (2.8)	27.8 (1.7)	26.0 (1.0)	NS
Total PCBs	8920 (369)	8870 (272)	8640 (304)	NS
mirex	34.5 (5.7)	33.2 (2.4)	30.8 (3.1)	NS
dieldrin	72.8 (7.2)	66.4 (12.5)	46.8 (6.2)	A > C
trans -chlordane	45.0 (11.8)	35.1 (1.7)	36.2 (0.7)	NS
oxychlordane	63.9 (7.2)	58.6 (5.8)	53.6 (2.6)	NS
trans -nonachlor	120 (0)	112 (4)	106 (7)	A > C
cis -nonachlor	36.5 (4.8)	34.4 (1.9)	29.7 (3.1)	NS
Total chlordanes	265 (17)	240 (6)	225 (9)	A > C
4,4'-DDE	579 (33)	583 (45)	568 (10)	NS

[a] Differences were determined by the Tukey Multiple Comparison Test ($p \leq 0.05$).
[b] NS, not significant.

Table 2.4. Comparison of measured organochlorine concentrations (pg/g wet mass) to certified and reference values of NIST SRM 1589a Human Serum using extraction technique A.

	Certified values[a] (pg/g wet mass)	Reference values[a] (pg/g wet mass)	Measured values[b] (pg/g wet mass)
PCB 28			20.5 (5.2)
PCB 66			24.9 (4.2)
PCB 74		226 (5)	43.3 (9.1)
PCB 95		47 (4)	32.4 (6.6)
PCB 99	121 (10)		155 (20)
PCB 101+90	34 (9) for 101		38.5 (6.0)
PCB 105	29 (4)		37.2 (4.0)
PCB 110			25.3 (2.8)
PCB 118	119 (9)		162 (17)
PCB 138			331 (39)
PCB 146		76 (5)	90.0 (9.8)
PCB 149	56 (8)		54.0 (9.1)
PCB 151+82	28 (4) for 151		51.9 (18.9)
PCB 153	672 (35)		730 (49)
PCB 156	66 (4)		77.2 (22.1)
PCB 163			125 (15)
PCB 170		186 (4)	186 (28)
PCB 180	483 (29)		487 (65)
PCB 183	65 (5)		73.1 (7.9)
PCB 187	172 (25) for 187+182		212 (22)
PCB 193			34.8 (9.7)
PCB 194	98 (14)		123 (24)
PCB 195	22 (4)		28.7 (5.9)
PCB 206	40 (6)		64.0 (12.3)
PCB 209	25 (4)		41.3 (7.8)
Total PCBs			3280 (137)
PCB 138 + 163	483 (39) for 138+163+164		552 (64)
hexachlorobenzene		49 (9)	64.4 (11.3)
mirex		43 (4)	92.0 (17.1)
dieldrin		73 (7)	102 (26)
heptachlor epoxide	75 (9)		84.7 (18.1)
oxychlordane	157 (14)		112 (16)
trans -nonachlor	169 (29)		285 (25)
cis -nonachlor			40.6 (5.4)
Total chlordanes			438 (18)
2,4'-DDE		85 (5)	15.2 (2.6)
4,4'-DDE	6600 (1000)		7030 (204)
2,4'-DDT			144 (55)
4,4'-DDT		85 (10)	81.6 (21.6)
Total DDTs			7370 (133)

[a] Mean (the expected uncertainty) described in Certificate of Analysis.
[b] Mean (SD) obtained from 5 bottles of NIST SRM 1589a.

Table 2.5. Organochlorine concentrations (pg/g wet mass) in different components of blood from five juvenile loggerhead sea turtles using extraction technique A.

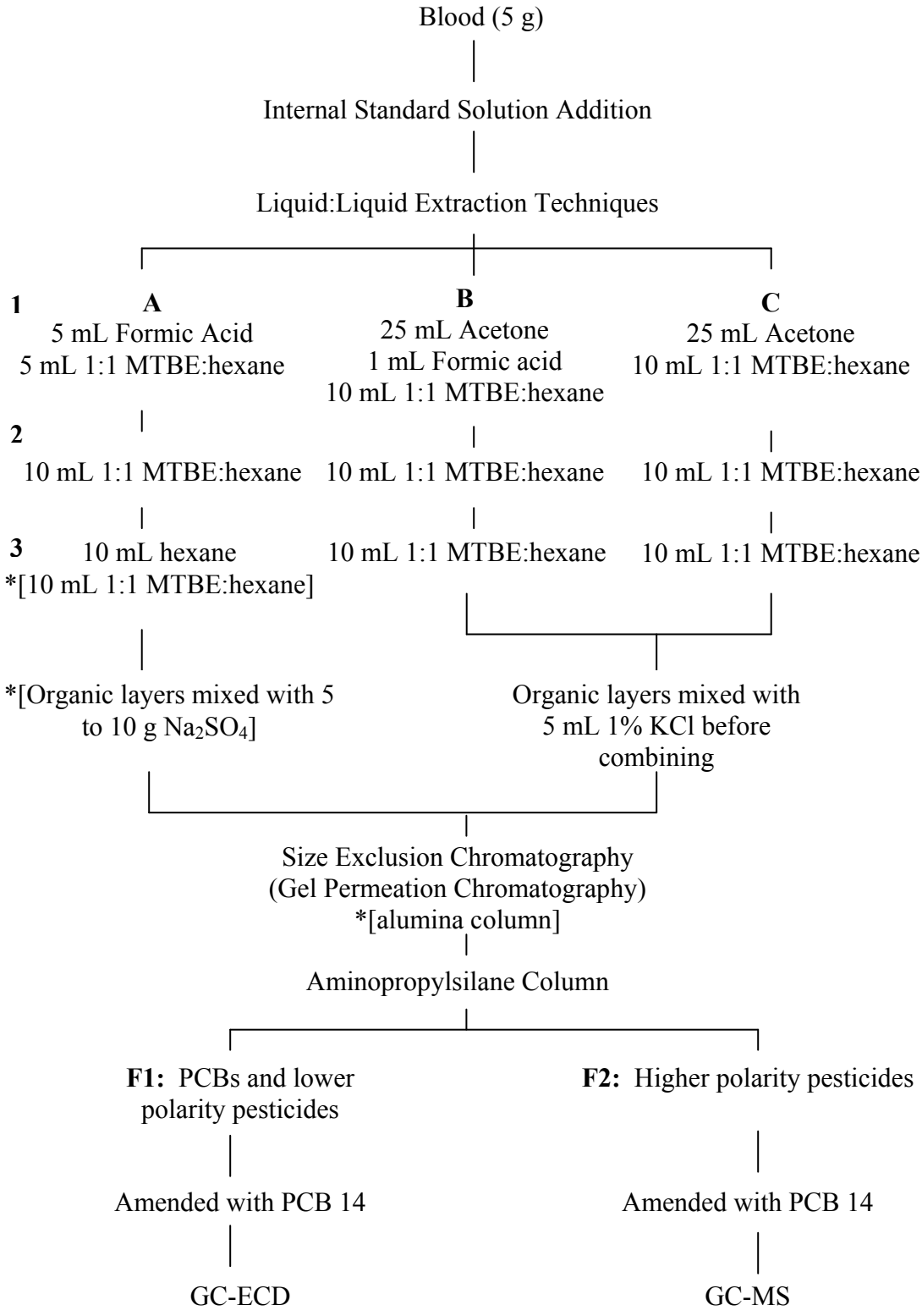
	Whole blood (pg/g wet mass)		Plasma (pg/g wet mass)		Red blood cells (pg/g wet mass)	
	Mean (SD)	Range	Mean (SD)	Range	Mean (SD)	Range
PCB 66	25.7 (27.6)	<10 - 56.9	34.3 (14.5)	17.6 - 47.4	16.4 (11.5)	<10 - 31.8
PCB 99	337 (280)	71.2 - 709	555 (478)	84.5 - 1120	99.1 (79.9)	15.1 - 190
PCB 105	96.4 (72.3)	25.9 - 205	122 (82.2)	32.1 - 239	33.5 (34.2)	<10 - 80.4
PCB 118	324 (264)	62.3 - 717	444 (327)	87.6 - 872	136 (103)	29.9 - 281
PCB 128	69.4 (49.3)	13.4 - 136	74.6 (54.9)	<10 - 138	28.1 (28.7)	<10 - 67.0
PCB 138	1050 (863)	271 - 2390	1550 (1140)	407 - 3130	303 (240)	63.8 - 635
PCB 146	129 (101)	28.8 - 291	199 (171)	35.8 - 486	29.1 (39.6)	<10 - 95.8
PCB 153	1500 (1270)	303 - 3560	2100 (1610)	405 - 4580	479 (394)	64.8 - 1070
PCB 163	143 (105)	21.3 - 253	186 (131)	26.3 - 353	67.3 (54.7)	<10 - 122
PCB 170	91.5 (66.4)	19.7 - 174	111 (66)	31.8 - 177	37.6 (36.9)	<10 - 74.5
PCB 180	509 (443)	52.2 - 1190	678 (450)	178 - 1330	177 (136)	43.4 - 372
PCB 183	138 (104)	25.6 - 296	164 (119)	25.5 - 339	46.3 (39.3)	<10 - 102
PCB 187 +182	320 (178)	97.5 - 570	412 (265)	105 - 822	106 (101)	<10 - 246
PCB 193	114 (98)	20.7 - 260	147 (106)	33.7 - 280	38.4 (38.3)	<10 - 89.3
PCB 194	58.2 (49.5)	<10 - 131	80.1 (43.6)	36.5 - 140	31.4 (30.5)	<10 - 68.7
PCB 206	71.0 (59.9)	<10 - 142	89.5 (61.2)	13.5 - 157	35.3 (34.7)	<10 - 75.4
Total PCBs[a]	5140 (3950)	1010 - 11000	7130 (4940)	1540 - 13700	1720 (1350)	231 - 3560
mirex	55.8 (74.8)	<10 - 178	36.5 (52.9)	<10 - 116	29.6 (47.1)	<10 - 108
dieldrin	45.9 (29.2)	<10 - 78.3	18.8 (19.1)	<10 - 44.3	8.90 (12.6)	<10 - 26.8
oxychlordane	111 (116)	17.7 - 307	121 (118)	39.6 - 322	64.8 (75.9)	<10 - 182
trans -nonachlor	109 (60)	44.2 - 176	103 (48)	45.4 - 148	51.0 (52.4)	<10 - 116
Total chlordanes	260 (182)	61.8 - 531	238 (155)	98.2 - 485	135 (135)	<10 - 292
4,4'-DDE	576 (305)	194 - 901	575 (294)	236 - 998	448 (329)	107 - 941
Total DDTs[b]	583 (307)	194 - 918	578 (294)	236 - 997	457 (320)	107 - 941
% lipid	0.341 (0.080)	0.209 - 0.427	0.269 (0.095)	0.166 - 0.426	0.281 (0.040)	0.238 - 0.343

[a] Totals include the major compounds listed in this table plus other minor detectable compounds.
[b] In parentheses are the number of samples out of 5 that had detectable concentrations.

Table 2.6. Pearson correlations of organochlorine contaminant concentrations (on a wet mass basis) between the different components of loggerhead sea turtle blood.

	Whole blood vs. Plasma				Whole blood vs. red blood cells				Plasma vs. red blood cells			
	R^2	p	slope	intercept	R^2	p	slope	intercept	R^2	p	slope	intercept
Total PCBs	0.985	0.002	0.789	-481	0.930	0.022	2.89	152	0.944	0.016	0.271	-206
mirex	0.983	0.003	1.43	0.657	0.894	0.041	1.63	3.78	0.827	0.084	0.868	-1.58
dieldrin	0.535	0.352	0.899	28.0	0.558	0.328	1.62	26.5	0.917	0.028	0.530	1.44
oxychlordane	0.989	0.001	0.987	-8.77	0.960	0.010	1.53	9.87	0.972	0.005	0.620	-9.24
trans -nonachlor	0.982	0.003	1.22	-16.2	0.873	0.053	1.20	45.0	0.922	0.026	0.983	-47.2
Total chlordanes	0.969	0.007	1.16	-16.6	0.936	0.019	1.28	85.7	0.977	0.004	0.795	-53.0
4,4'-DDE	0.955	0.011	0.990	6.53	0.955	0.011	0.794	220	0.908	0.033	0.756	13.7
Total DDTs	0.957	0.011	1.01	0.883	0.956	0.011	0.805	214	0.902	0.036	0.735	32.7

Figure 2.1. Analytical scheme for determining PCBs and organochlorine pesticides in loggerhead sea turtle blood. Original scheme was used for pooled loggerhead sea turtle blood to determine differences among the three extraction techniques (A, B, and C). *Shown in brackets are modifications made to technique A for extracting human serum (NIST SRM 1589a) and sea turtle plasma, red blood cells, and whole blood.


Blood (5 g)
Internal Standard Solution Addition
Liquid:Liquid Extraction Techniques
1
A
5 mL Formic Acid
5 mL 1:1 MTBE:hexane
B
25 mL Acetone
1 mL Formic acid
10 mL 1:1 MTBE:hexane
C
25 mL Acetone
10 mL 1:1 MTBE:hexane
2
10 mL 1:1 MTBE:hexane
10 mL 1:1 MTBE:hexane
10 mL 1:1 MTBE:hexane
3
10 mL hexane
*[10 mL 1:1 MTBE:hexane]
10 mL 1:1 MTBE:hexane
10 mL 1:1 MTBE:hexane
*[Organic layers mixed with 5 to 10 g Na2SO4]
Organic layers mixed with 5 mL 1% KCl before combining
Size Exclusion Chromatography
(Gel Permeation Chromatography)
*[alumina column]
Aminopropylsilane Column
F1: PCBs and lower polarity pesticides
F2: Higher polarity pesticides
Amended with PCB 14
Amended with PCB 14
GC-ECD
GC-MS

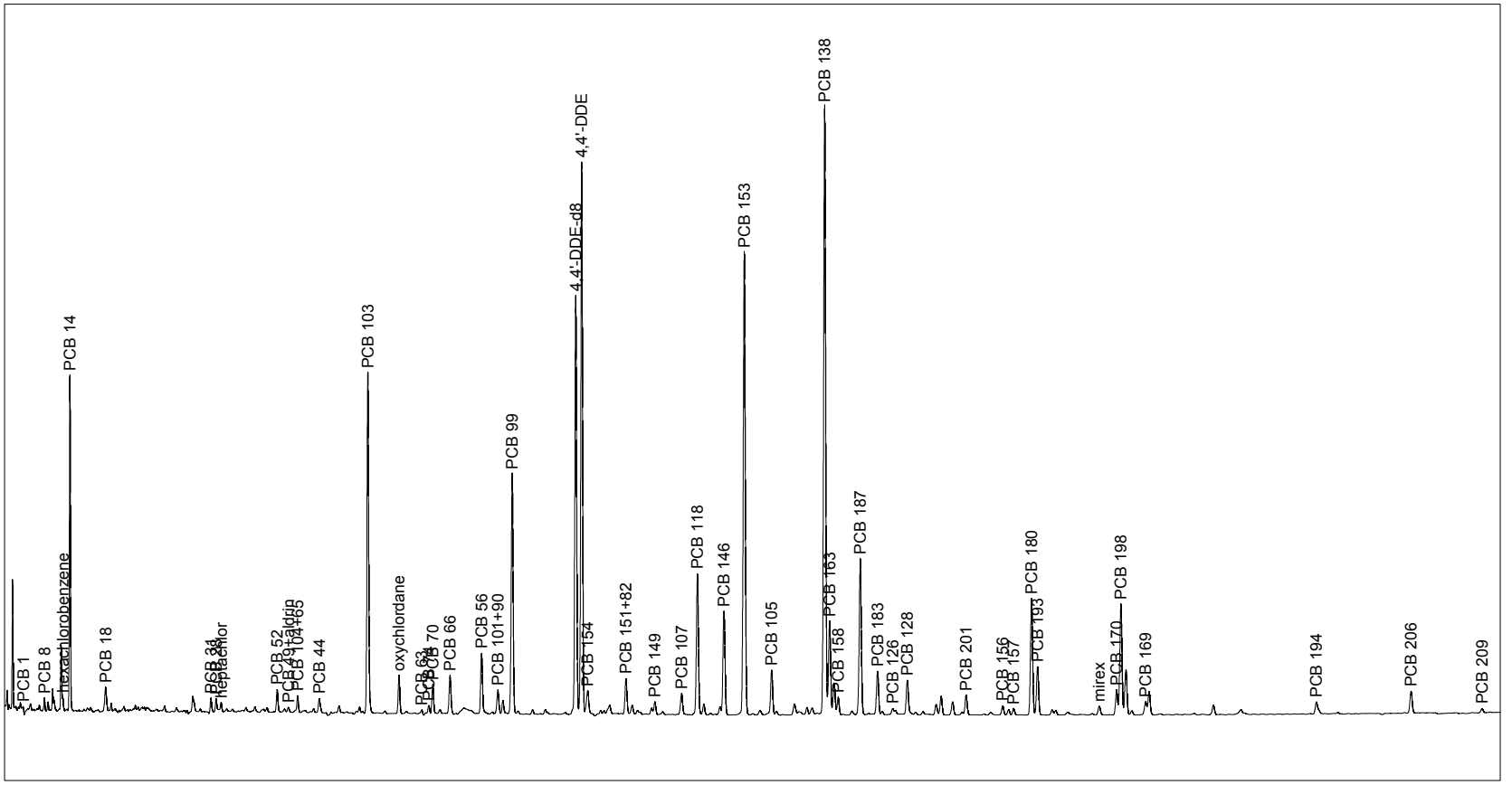


Figure 2.2. Representative chromatogram of PCBs and OC pesticides by GC-ECD in fraction 1 of a sample of loggerhead sea turtle whole blood. The blood was extracted using technique A and analyzed as described in the methods section.

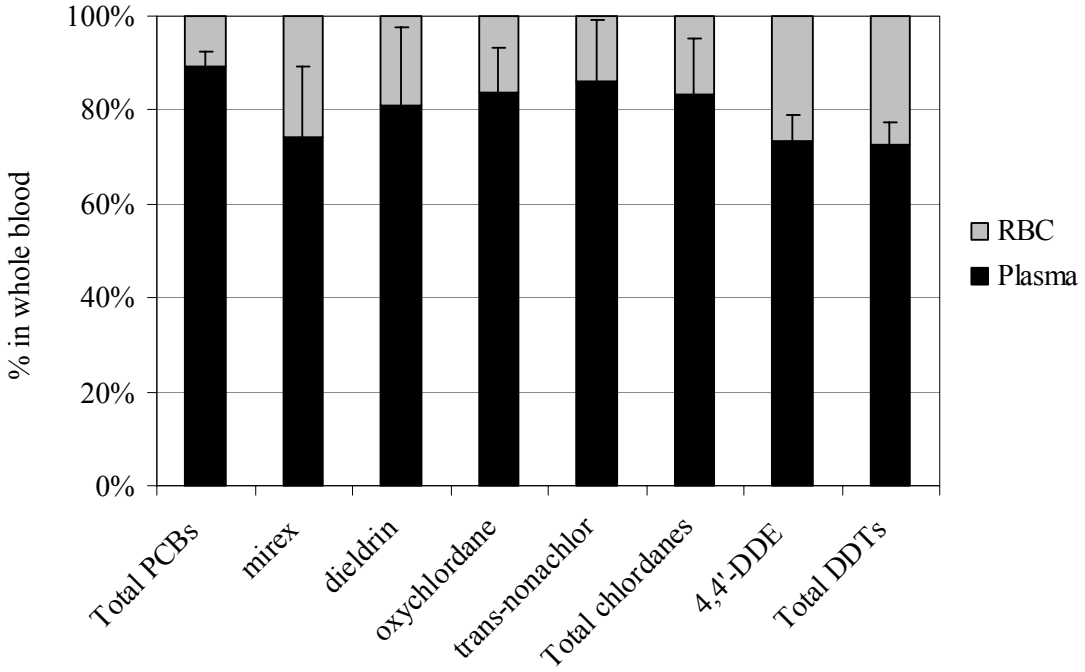


Figure 2.3. Distribution of organochlorine contaminants between loggerhead sea turtle plasma and red blood cells (RBCs). Paired samples of plasma and RBCs from five loggerhead sea turtles were extracted with technique A. The percentage of each compound found in each fraction was calculated and averaged among the 5 turtles. The error bars indicate standard deviation.

CHAPTER THREE

Organochlorine contaminants in sea turtles: correlations between whole blood and fat

ABSTRACT

Monitoring toxic organochlorine (OC) compounds is an important aspect in wildlife studies, especially in protected species such as sea turtles. The goal of this study was to determine whether blood OC concentrations can reliably predict those in adipose tissue of sea turtles. Blood offers many benefits for monitoring OCs. It can be collected non-destructively from live turtles and can be sampled repeatedly for continuous monitoring. OC concentrations in blood may better represent the exposure levels of target tissues, but blood concentrations may fluctuate more than those in fatty tissues following recent dietary exposure or lipid mobilization. Paired fat and blood samples were collected from 44 live, juvenile loggerhead sea turtles and 10 juvenile Kemp's ridley sea turtle carcasses. OCs were analyzed using gas chromatography with electron capture detection and mass spectrometry. For both species, lipid-normalized OC concentrations measured in the blood significantly correlated to levels found in the fat samples. This result suggests that sea turtle blood is a suitable alternative to fatty tissues for measuring OCs, because blood concentrations reasonably represent those observed in the paired fat samples. However, blood OC concentrations calculated on a wet mass basis were significantly and inversely correlated to lipid content in the fat samples. Therefore, caution should be used when monitoring spatial or temporal trends, as OC levels may increase in the blood following mobilization of fat stores, such as during long migrations, breeding, or disease events.

INTRODUCTION

Organochlorine (OC) compounds, such as pesticides and polychlorinated biphenyls (PCBs), are highly persistent, bioaccumulative, lipophilic contaminants. Most of these compounds were banned from use in the U.S. and Europe beginning in the 1970s; however, they continue to be detected in both biological and environmental samples worldwide (Safe 1993). OCs have been shown to affect a variety of biological systems at concentrations much lower than those responsible for acute toxicity. Effects have been demonstrated on the immune, endocrine, developmental, and reproductive systems (i.e.; Ross *et al*. 1996; Grasman and Fox 2001; Guillette *et al*. 1999). The toxicity of OCs poses a considerable hazard for both wildlife and human populations.

The toxicological effects of OC contaminants create special problems for endangered and threatened species, such as sea turtles. All seven species of sea turtles are listed nationally or internationally as endangered or threatened (Pritchard 1997). The Kemp's ridley sea turtle (*Lepidochelys kempii*) is considered the most endangered sea turtle species in the world. The number of nesting females of this species plummeted in the 1960s and continued to decline until 1985 when fewer than 1000 nests were laid on their only known nesting beach at Rancho Nuevo, Mexico (TEWG 2000). Since then, the population has been increasing, largely due to conservation policies that have reduced poaching and entanglement in fishing gear. Similarly, the loggerhead sea turtle (*Caretta caretta*) is listed as threatened on the U.S. Endangered Species Act and has suffered population declines. While the loggerhead subpopulation that nests in southern Florida has recently been increasing in numbers,

the northern subpopulation that ranges from northern Florida to North Carolina has been decreasing by 2 % to 3 % per year (TEWG 2000). The cause of this decline is unknown, but it has prompted the U.S. National Marine Fisheries Service to consider uplisting the loggerhead sea turtle to the endangered status.

While sea turtles clearly face numerous man-made threats, the effects of environmental contaminants on their health, survival, and reproduction are completely undocumented. The life history of sea turtles creates a challenge for toxicological research as they are highly migratory and long-lived species. Loggerhead turtles circumnavigate the Atlantic Ocean as pelagic juveniles, spending time in the open ocean and near the Azores, Madeira, and the Canary Islands (Musick and Limpus 1997). Some even enter the Mediterranean Sea during this stage. They return to U.S. estuaries as large juveniles to feed on benthic crustaceans and molluscs. Kemp's ridley sea turtles spend their pelagic juvenile phase in the offshore waters of the Gulf of Mexico or the western North Atlantic Ocean. The age at which these turtles mature is not known, but has been estimated to be 25 to 35 years for the loggerhead and 7 to 16 years for the Kemp's ridley (Chaloupka and Musick 1997; Snover 2002). Their long migrations facilitate their exposure to diverse contaminant classes arising from a variety of sources, while their long lifespan allows time for them to accumulate persistent OC contaminants.

Studies reporting contaminant concentrations in sea turtle tissues are limited and widely scattered across contaminant types, geographic locations, species, and tissues (see Pugh and Becker 2001 for a review). Furthermore, none of these studies have analyzed sea turtle blood for contaminants. Since OCs possess a great ability to

bioaccumulate into lipid-rich tissues, past studies have primarily analyzed eggs or fatty tissues from dead sea turtles. As shown in snapping turtles and birds, egg concentrations reasonably represent the contaminant burdens of adult nesting females, as OC compounds are maternally transferred into eggs (Pagano *et al*. 1999; Bargar *et al*. 2001). Fatty tissues, such as fat and liver, can only be collected from live animals using invasive or logistically difficult sampling; therefore, these tissues have traditionally been collected from carcasses stranded on the beach. Samples from dead stranded turtles are solely opportunistic and may be biased towards diseased or partially decomposed animals. As shown with marine mammals, diseased animals may have higher contaminant concentrations than live, healthy organisms (Hall *et al*. 1992; Aguilar and Borrell 1994). These studies are further complicated, because decomposition can result in cross contamination from other tissues or surroundings. The loss or decomposition of lipids may increase the net tissue OC concentrations, as concentrations are typically normalized to lipid content. OC concentrations in collected tissue samples may decrease due to bacterial degradation of the compounds. An alternative tissue – blood – can be collected from live sea turtles in a relatively non-destructive and non-biased manner and has recently become a common tissue for measuring OC levels in humans and wildlife (i.e.; Reddy *et al*. 1998; Bishop and Rouse 2000; Sandau *et al*. 2000; Keller *et al*. submitted).

The use of turtle blood to measure accumulation of OC contaminants may offer many benefits; however, studies in other organisms have shown that the concentrations in blood can fluctuate temporally depending upon recent dietary exposure or mobilization of lipid stores. OC concentrations have been shown to

increase in the blood of humans and seals following dramatic weight loss, suggesting that contaminants stored in adipose tissue are released into the blood during lipid mobilization (Lydersen *et al*. 2002; Chevrier *et al*. 2000). However, a great number of studies examining birds, marine mammals, and humans found that blood OC concentrations were proportional to concentrations present in fatty tissues (Reddy *et al*. 1998; Henny and Meeker 1981; Bernhoft *et al*. 1997; Wolff *et al*. 1982; Mes 1992; Kanja *et al*. 1992). In all of these studies, statistically significant, positive correlations were observed between blood and fatty tissue OC contaminant levels. Boon *et al*. (1994) provides data on harbor seals that supports a kinetic model, in which OC concentrations in the blood are in a dynamic balance with other fatty tissues. Overall, these prior studies suggest that blood OC concentrations will fluctuate during lipid mobilization, but in many situations and with many wildlife species blood can be successfully used for monitoring contaminants.

Our study investigated OC concentrations in the blood of sea turtles and compared these to levels found in adipose tissue. Our goal was to determine if blood could be used to monitor OC contaminants in these endangered animals. Temporal changes were also investigated in blood OC concentrations by analyzing and comparing samples taken from loggerhead turtles that were captured at different times. Lastly, we compared blood OC concentrations to the lipid content in the adipose tissue. This allowed us to determine whether turtles with low fat lipid content, and possibly those that had mobilized their fat reserves, had higher levels of OCs in their blood.

MATERIALS AND METHODS

Samples

During August 2000 and July 2001, 44 free-ranging, juvenile loggerhead sea turtles were captured using the pound-net fishery in Core Sound, North Carolina, USA and were transported to the National Marine Fisheries Service in Beaufort, NC. The turtles ranged from 45.7 cm to 74.1 cm in straight carapace length (SCL, nuchal notch to posterior marginal notch) and from 14.4 kg to 56.6 kg in weight. Based on these sizes, the turtle ages may fall somewhere between 10 to 30 years of age (Snover 2002). Body condition was calculated as weight (kg) divided by the cube of SCL and multiplied by 10,000 as suggested by Bjorndal *et al*. (2000). The turtles were tagged, and a laparoscopy was performed to determine their sex. Thirty turtles were identified as females and 14 as males. Blood samples (10 mL) were collected from the dorsocervical sinus using Vacutainer double-ended needles inserted directly into Vacutainer blood collection tubes containing sodium heparin (Becton Dickinson, Franklin Lakes, NJ). Whole blood samples were kept on ice until frozen at -20 ^{o}C. Six of the loggerhead turtles were recaptured at a later date and a second blood sample was drawn.

Excisional biopsies of subcutaneous fat (0.4 to 4.0 g) were removed from the left inguinal region through a 3 cm transverse incision approximately 3 cm to 5 cm caudal to the plastron margin. Stainless steel surgical instruments were hexane-rinsed and sterile, and the surgical site was blocked prior to the procedure by infiltration of a lidocaine local anesthetic (Lidocaine Hydrochloride Injectable-2%, Phoenix Pharmaceutical, Inc., St. Joseph, MO). The incisions were closed with 4-O

polyglyconate (Maxon, Sherwood-Davis & Geck, Manati, Puerto Rico) in patterns according to surgeon preference, and cyanoacrylate tissue glue. Turtles were observed for a few hours and then released.

Fat samples were stored in hexane-rinsed aluminum foil and were frozen at -80 ^{o}C until analyzed for OC contaminants. A portion of a fat biopsy from one turtle was placed in 10 % neutral buffered formalin. Fixed tissue was processed routinely for paraffin sections and stained with hematoxylin and eosin for histological examination.

During a cold stunning event in November 1999 along Cape Cod, Massachusetts, USA, blood and fat samples were collected from eight juvenile Kemp's ridley sea turtles that died during rehabilitation. The turtles ranged from 21.9 cm to 31.1 cm in SCL and were estimated to be 1.25 to 2.25 years of age based on a skeletochronological method which counts the number of annually deposited layers of arrested growth in the humerus (Snover *et al*. 2000). Blood was drained from the heart directly into open Vacutainer tubes containing sodium heparin and was stored at –20 ^{o}C. Two types of fat were collected, yellow fat surrounding the internal organs and brown fat along the carapace margin and in the inguinal and shoulder regions. Fat samples were collected using hexane-rinsed stainless steel instruments and stored in hexane-rinsed foil at –80 ^{o}C. Both types of fat, but not blood, were also collected from 2 Kemp's ridley sea turtles (a female at 45.6 cm SCL, 4.75 years old (Snover 2002); and a male at 29.7 cm SCL, approximately 2 years old) that drowned in North Carolina in April of 1998 and July 2000, respectively.

In addition to the loggerhead and Kemp's ridley sea turtle samples, fat samples were also collected from one juvenile male green sea turtle (24.2 cm SCL) found dead

on Aug. 7, 2000 in Core Sound, NC and one adult female leatherback sea turtle (176.0 cm curved CL) that was euthanized following an injury inflicted by a boat propeller near Long Beach, NC on June 9, 1999. The blubber layer of the carapace was also sampled from the leatherback turtle along the right margin of the carapace and plastron.

Organochlorine Contaminant Analysis

Whole blood and fat samples were analyzed for 55 PCB congeners and 24 OC pesticides. All analyses were performed using cleaned and hexane-rinsed glassware, stainless steel instruments, and glass serological and transfer pipets. The fat samples were analyzed using a method modified from Kucklick *et al*. (2002). The blood samples were analyzed using a liquid:liquid extraction technique (technique A) described in Keller *et al*. (submitted).

Extractions of fat biopsies

Fat samples (0.4 g – 4 g) were weighed, minced using a hexane-rinsed scapel, mixed with 40 g anhydrous Na_2SO_4, and transferred to 33 mL pressurized fluid extractor (PFE) cells (Dionex, Salt Lake City, Utah). Five calibration solutions were prepared by combining and diluting National Institute of Standards and Technology (NIST) Standard Reference Materials (SRM): 2261 (Chlorinated Pesticides in Hexane), 2275 (Chlorinated Pesticides in Hexane II), 2262 (Chlorinated Biphenyl Congeners in 2,2,4-trimethylpentane), 2274 (PCB Congener Solution II), and a solution containing 14 additional PCB congeners. The diluted calibration solutions were added to individual PFE cells resulting in a calibration curve ranging from 0.3 ng to 400 ng. An internal standard solution was also added containing 70 ng of each of

4,4'-DDT-d_8, 4,4'-DDE-d_8, 4,4'-DDD-d_8, Endosulfan I-d_4, PCB 103 and PCB 198. NIST SRM 1945 Organics in Whale Blubber and a blank were also processed with each set of samples. Samples were extracted with dichloromethane on the PFE as described elsewhere (Kucklick *et al*. 2002). Extracts were reduced in volume using purified N_2 (Turbovap II, Zymark, Hopkinton, MA).

Extractions of blood samples

While Keller *et al*. (submitted) found that most OCs partition into the blood plasma, only whole blood was available for many of the samples in this study. Whole blood samples were extracted using the liquid:liquid extraction method (technique A) described in Keller *et al*. (submitted). Briefly, 3 g to 5 g of blood were mixed with 0.2 g of the internal standard solution described above and allowed to set at room temperature for 2 h. Samples were treated with formic acid then extracted 3 times with 1:1 (v/v) methyl-*tert*-butyl ether (MTBE):hexane. The organic phases were combined, reduced in volume, and dried with 10 g to 20 g anhydrous Na_2SO_4. NIST SRM 1589a PCBs, Pesticides, and Dioxins/Furans in Human Serum and blanks were processed with each sample batch.

Lipid Determination

Lipid content was determined gravimetrically for all fat biopsies, blood samples, NIST SRM 1945, and NIST SRM 1589a. Approximately 5 % to 10 % by weight of each extract was removed and transferred to a tared aluminum weighing boat. The solvent was allowed to evaporate at room temperature for 4 h to 12 h, and the dried lipid residue was reweighed to the nearest 0.00001 g for fat and 0.0001 mg for blood.

Sample Clean up

High molecular mass compounds in the fat samples were removed by gel permeation chromatography (GPC) according to Kucklick *et al*. (2002). The GPC was used for all of the Kemp's ridley blood samples and the loggerhead blood samples from 2000. An alumina column was used for the year 2001 loggerhead blood samples according to Holden and Marsden (1969) and modified by Keller *et al*. (submitted).

All sample extracts and the calibration solutions were fractionated into relatively lower and higher polarity fractions (F1 and F2, respectively) using a semi-preparative aminopropylsilane column (μBondapak NH_2, Waters) as detailed in Kucklick *et al*. (2002). Compounds contained in F1 included PCBs, heptachlor, 2,4'-DDE, 4,4'-DDE, 2,4'-DDT, hexachlorobenzene (HCB), aldrin, mirex, and oxychlordane. Analytes in F2 included 4,4'-DDT, *cis*- and *trans*-chlordane, *cis*- and *trans*-nonachlor, α- β-, and γ-hexachlorocyclohexane (HCH), heptachlor epoxide, 2,4'-DDD, 4,4'-DDD, dieldrin, endrin, endosulfans I and II, and endosulfan sulfate. Each fraction of the fat samples and the corresponding standards and blanks were reduced to approximately 0.5 mL. The blood samples, corresponding blanks, and standards were amended with 5 ng of PCB 14 prior to analysis, in order to calculate the recovery of internal standards (Keller *et al*. submitted). The blood extracts were reduced to between 0.05 mL and 0.1 mL using a stream of purified nitrogen prior to analysis.

Contaminant Analysis

Both the F1 and F2 fractions of fat samples were analyzed on a gas chromatograph (GC) with dual micro-electron capture detectors (ECD) according to Kucklick *et al*. (2002). The GC-ECD was used to analyze the F1 of the blood samples. For the F2 fraction of the blood samples, a GC-MS was used operating in the electron-impact (EI) mode and using selected ion monitoring (SIM) according to Keller *et al*. (submitted). The calibration standards used for the fat samples were extracted, but standards were not extracted for the blood samples. Thus, the concentrations in the blood were corrected for recovery of the internal standards.

Statistics

The contaminant data were not normally distributed even after log transformation; therefore, only non-parametric statistical tests were used (Systat 8.0 software; SPSS, Inc, Chicago, IL). All correlative relationships were tested using the Spearman rank correlation test. The Wilcoxon signed rank test was used to determine a difference in contaminant concentrations between the yellow and brown fat of the Kemp's ridley turtles. The Mann-Whitney test was used to compare mean fat concentrations of OCs in male to female loggerhead turtles. Only lipid-normalized OC concentrations (ng/g of lipid in the tissue) were used in statistical tests.

RESULTS

Validation of Methods

Histological examination was performed on a sample of loggerhead sea turtle fat to validate our fat biopsy sampling method. The tissue floated in formalin, as

expected, and histological examination verified our visual tissue identification (Fig. 3.1). The tissue section was composed of sheets of irregular polygonal adipose cells containing large clear vacuoles and small eccentric nuclei, interspersed with sparse fibrovascular stroma, typical of adipose tissue.

NIST SRMs representing tissue matrices similar to adipose and blood were analyzed for OCs alongside each batch of turtle samples to validate the chemical analysis. OC concentrations were measured in four samples of NIST SRM 1945 (Whale Blubber) and five bottles of NIST SRM 1589a (Human Serum). The values obtained from these SRMs were reported elsewhere (in Keller *et al*. (submitted) for SRM 1589a and in Appendix Table A1 for SRM 1945). For the majority of the OCs, the values we obtained differed from the certified values by less than 30 %.

Contaminant concentrations and lipid content

Organochlorine contaminant concentrations and lipid content in the tissues of loggerhead and Kemp's ridley sea turtles that we examined are listed in Tables 3.1 and 3.2, respectively. The patterns of PCB congeners in the blood and fat of both species are shown in Fig. 3.2. Lipid content in loggerhead adipose tissue was extremely variable among individuals (range = 0.255 % - 64.7 %), emphasizing the importance of normalizing the OC concentrations to lipid content for better comparisons of OC concentrations between individual turtles and between different tissues. The lipid content in the adipose tissues of the Kemp's ridley sea turtle varied only slightly, with the exception of one turtle. This turtle was extremely emaciated and had 0.521 % lipid in its brown fat and no apparent yellow fat. The blood lipid content varied less than the fat lipid content for both species.

Some OC compounds were detectable in one tissue and not in another (Tables 3.1 and 3.2). No blood sample from either loggerhead or Kemp's ridley turtles had detectable concentrations of HCHs, while low concentrations of HCHs were found in a few fat samples. No *trans*- or *cis*-chlordane was found in any loggerhead fat biopsy, but low concentrations were measurable in a few blood samples. These discrepancies are likely due to the difficulty in measuring these compounds at their low concentrations and because of the differing limits of detection (LOD) between the tissues. LOD were 1 ng/g wet mass for the fat and 10 pg/g wet mass for the blood samples.

Overall, the mean lipid-normalized blood OC concentrations were similar to the fat concentrations for both species. Likewise, the concentrations of all the major OC compounds in the Kemp's ridley yellow fat were not significantly different than those in the brown fat samples (Wilcoxon, $p > 0.05$; Table 3.2). The OC concentrations in the yellow fat were significantly correlated to those in the brown fat for all the major PCB congeners, total PCBs, and all of the major pesticides, including HCHs, mirex, dieldrin, heptachlor epoxide, total chlordanes, and total DDTs (Spearman Rank Correlation, $p < 0.05$; Table 3.3).

OC concentrations in fat versus blood

The OC concentrations in fat significantly correlated to blood concentrations for both species and for all of the major PCB congeners, total PCBs, and all of the major pesticides, including mirex, dieldrin, total chlordanes and total DDTs (Spearman Rank Correlation, $p < 0.05$; Table 3.3 and Fig. 3.3). Even when the one loggerhead turtle with the highest levels of OCs was removed from the comparisons,

the correlations between blood and fat concentrations remained statistically significant.

Blood OC concentrations versus lipid content in fat biopsies

Lipid content in the fat of the loggerhead sea turtles was not significantly correlated with body condition (Fig. 3.4A). This result suggested that emaciated turtles did not have lower lipid content in their fat. On the other hand, the percent lipid in the fat was inversely correlated to concentrations of blood OCs on a wet mass basis (Fig. 3.4B & C; r_s = -0.403 for total PCBs; -0.431 for heptachlor epoxide; -0.671 for total chlordanes; -0.551 for mirex; and –0.361 for total DDTs; all p values < 0.02). This suggested that turtles that mobilized their lipid stores had higher concentrations of OCs circulating in their blood.

Blood OC concentrations in recaptured turtles

To investigate the temporal variability of blood OC concentrations, we collected and analyzed a second blood sample upon recapturing six loggerhead turtles. The duration between sampling ranged from 17 to 403 days and all turtles grew over this time (Table 3.4). The blood lipid content changed on average (SD) between the first and second sampling times by 27.7 % (48.7 %) (Table 3.4). Only two turtles (ID #1 and 4) had relatively stable concentrations of OCs in their blood (Fig. 3.5). The blood OC concentrations of the other four turtles varied considerably, some nearly doubled or decreased by a half. OC concentrations increased in four of the six turtles, while a decreasing trend was observed in the other two turtles. The average percent difference between the first and second sampling times (SD) was an increase of 39.7 % (69.2 %) for total PCBs, 89.5 % (239 %) for mirex, 14.2 % (59.2 %) for total

chlordanes, and 4.6 % (57.3 %) for total DDTs. Changes in blood OC concentrations did not significantly correlate to duration between sampling events for any compound.

Are contaminant concentrations dependent upon sex and size?

The effect of sex and size (as a proxy for age) on contaminant concentrations was investigated in the loggerhead sea turtles. Male and female loggerhead sea turtles did not significantly differ in OC concentrations measured in their fat on a lipid-normalized or wet mass basis (data shown in Appendix Table A2). Since no differences were seen, the OC concentrations in both males and females were combined in Table 3.1. The only contaminants that showed significant, but weak, correlations to turtle length were fat concentrations of mirex and total chlordanes on wet mass basis (r_s = -0.370 and -0.422, respectively; $p < 0.05$), which were inversely related to turtle length. Total chlordanes also showed a weak, but significant, correlation to turtle weight (r_s = -0.299; $p < 0.05$). Interestingly, these correlations were negative indicating that larger turtles have lower concentrations of these compounds than smaller turtles.

DISCUSSION

Sea turtle OC concentrations: comparison to past studies

There have been some studies previously published on OC concentrations in the fat of sea turtles. We compare past findings to our current study as shown in Table 3.5 and discuss the data herein. The variation in OC concentrations among individual turtles is very large as seen in previous studies. In the current study, the large

variation seen in the concentrations of most of the contaminants could not be explained by sex or size of the turtles.

Kucklick *et al*. (1997) found that PAH concentrations in urban estuarine sediments were highly variable even among sites that were close in proximity. Hyland *et al*. (2000) showed that total PCB and total DDT concentrations in the sediment of NC estuaries range over two orders of magnitude depending on the sampling location. Therefore, spatial habitat choice may explain some of the variance observed in sea turtle tissue concentrations of OCs. Many juvenile loggerhead turtles captured at this study site (Core Sound, NC) show strong site fidelity (Avens *et al*. in press). They return to a particular area in successive years and remain in this area for extended parts of a season. This localized feeding may contribute to the variation seen in their contaminant levels. It is possible that one turtle may continually feed in a highly contaminated site, while another may feed in a less contaminated area. Additionally, these juvenile turtles use offshore habitats for their winter foraging grounds or follow a more coastal migratory route to southern, warmer waters (Epperly *et al*. 1995). Turtles that take the offshore route may accumulate lesser amounts of contaminants than those closer to the coast. A decreasing trend was observed for blood mercury levels in sea turtles caught farther offshore of South Carolina (Day *et al*. in press).

The total PCB and total DDT concentrations in loggerhead turtles from the current study were roughly half those measured in the fat from dead loggerhead turtles stranded in Virginia and North Carolina in the early 1990s (Rybitski *et al*. 1995) and a quarter of the concentration measured in loggerhead turtles stranded in the Mediterranean Sea in 1994 and 1995 (Mckenzie *et al*. 1999). These observed

differences in OC concentration may be the result of a number of factors, including spatial or temporal differences in OC environmental concentrations. Turtles inhabiting coastal waters of the more urban Mediterranean Sea or Chesapeake Bay may be exposed to higher levels of contaminants than turtles in the less urban Core Sound, NC. In addition, PCB concentrations along the U.S. coast have decreased over the past 9 years, as demonstrated by decreasing concentrations measured in bivalves through the National Oceanic and Atmospheric Administration Mussel Watch Program (O'Connor 1998).

Another potential cause for the observed difference between the loggerhead sea turtle OC concentrations in this study and previous studies may be sampling bias. All of the past studies utilized tissues from carcasses of stranded sea turtles, thereby biasing their sampling towards diseased or injured turtles. Animals dead from disease may contain higher concentrations of OCs than the live, apparently healthy turtles analyzed in our study. This relationship has been seen in other marine wildlife studies (Hall *et al*. 1992; Aguilar and Borrell 1994). Seals around Great Britain that died from a phocine distemper epizootic had significantly higher concentrations of total PCBs and total DDTs than those that survived (Hall *et al*. 1992). Likewise, striped dolphins that died from a morbillivirus epizootic in the Mediterranean Sea had nearly three fold higher concentrations than healthy dolphins in the same area (Aguilar and Borrell 1994). Therefore, using tissues from dead animals could complicate interpretation of contaminant data, and future studies should examine more closely the prospect that diseased turtles have higher levels of contaminants than healthy ones.

The PCB concentrations measured in dead Kemp's ridley turtles from Massachusetts in this study were extraordinarily similar to the findings of two previous studies on this species with only one exception (Table 3.5). PCB concentrations in Kemp's ridley turtles that stranded in New York in 1985 were roughly double those measured in the turtles analyzed in this study (Lake *et al*. 1994). Lake *et al*. (1994) also analyzed tissues from turtles that stranded 4 years later in 1989. The PCB concentrations in 1989 were half of those measured 1985 and very similar to the levels measured in the current study. The concentrations of DDE in this study were half those previously reported for Kemp's ridley sea turtles (Rybitski *et al*. 1995; Lake *et al*. 1994). These differences could be due to temporal or spatial trends in environmental OC concentrations or variations in analytical techniques.

Compared to other sea turtle species, the OC concentrations were higher in the fat of Kemp's ridley and loggerhead sea turtles than in green and leatherback sea turtles (Table 3.5). These species differences could be explained by differing trophic feeding levels. The crab and mollusc-eating loggerhead and Kemp's ridley sea turtles feed higher on the food chain than the herbivorous green sea turtles, which consume a variety of sea grasses and algae. The leatherback sea turtle feeds primarily on jellyfish.

An interesting finding highlighted during this study is the apparent difference between OC concentrations in the adipose tissue and the blubber of the leatherback sea turtle. The fat concentrations were similar to previous reports for leatherback adipose tissue (near 100 ng/g wet mass total PCBs), but the blubber concentrations were remarkably higher (2330 ng/g wet mass total PCBs) than reported in any fatty tissues

of any sea turtle species. Davenport *et al*. (1990) reported 1200 ng/g lipid total PCBs in a fatty tissue of a single leatherback turtle, but it was unclear whether the tissue was adipose or blubber. This previously anomalous value was more comparable to the concentrations measured in the blubber of the leatherback turtle from our current study (9040 ng/g lipid total PCBs).

The shell of the leatherback sea turtle differs from that of all the other sea turtle species. The bony structures of the shell are greatly reduced and the majority of this structure consists of a thick lipid layer, similar to the blubber layer in marine mammals. This insulating blubber layer is thought to help the leatherback turtle maintain its body temperature above the ambient water temperature and facilitate deep diving (Spotila *et al*. 1997).

Pattern of PCB congeners

The patterns of PCB congeners in the blood and fat of loggerhead sea turtles were very similar to those in the Kemp's ridley sea turtles (Fig. 3.2). The sea turtle congener profiles were also similar to previously reported patterns for these tissues in other species. As expected, the dominant congeners (PCB 138, 153, and 180) found in the loggerhead and Kemp's ridley sea turtles in our study were the dominant congeners in adipose tissue of green sea turtles from Hawaii (Miao *et al*. 2001) and in tissues of animals inhabiting the Great Lakes. Notably, these specific congeners were prevalent in plasma of Lake Erie water snakes and snapping turtles and in eggs of herring gulls and mudpuppies (Bishop and Rouse 2000). These congeners also dominated the PCB profile in fatty tissues of Alaskan marine mammals (Kucklick *et al*. 2002).

Interestingly, a difference was observed between the congener composition of blood and fat of the sea turtles. As shown in Figure 2, PCB 138 made up a greater percentage of the total PCBs in the blood than in the fat. This congener has only ortho-meta vicinal hydrogens and is metabolized by the methylcholanthrene (MC)-type enzyme (cytochrome P450 1A) subfamily in mammals (Kannan *et al*. 1995). The relatively high concentrations of this compound, along with the other PCB congeners that are metabolized by this enzyme subfamily (i.e.; PCBs 99, 118, 128, and 170), suggest that sea turtles may have weak P450 1A activity. The lack of congeners that are typically metabolized by phenobarbital (PB)-type enzymes (cytochrome P450 2B), including PCBs 52, 95, 101, 151, 149, 185, and 174, suggested that this enzyme activity may dominate the phase I metabolism of contaminants in sea turtles, as has been seen in polar bears (Kucklick *et al*. 2002). It should be noted that these enzymes have been well characterized in mammals, but little is known about their occurrence in reptiles; therefore, these conclusions should be viewed with caution. Four cytochrome P450 proteins have been partially isolated from the liver of a Kemp's ridley sea turtle, and although the identity of these isozymes has not been fully characterized, low levels of P450 1A activity were evident (Goldman and McClellan-Green 2001).

Contaminants versus age and sex

In other wildlife, it has been shown that contaminant concentrations are dependent upon age, sex, and nutritional status. Adult males typically contain higher concentrations of organochlorines than females, because females can release portions of their contaminant burden through their eggs, milk, or tissues of their offspring (Pagano *et al*. 1999; Bargar *et al*. 2001; Beckman *et al*. 1999). In addition, it has been

shown that older individuals possess higher concentrations of OCs through extended accumulation of the compounds (Tanabe *et al*. 1994). OC concentrations did not differ between male and female loggerhead turtles analyzed in the current study. A difference was not expected, because the juvenile females sampled in this study were not of breeding age and therefore had not yet transferred contaminants to their eggs.

Very few correlations were seen between OC concentrations and loggerhead size. Larger turtles unexpectedly had lower concentrations of chlordanes and mirex. A possible explanation for this finding may be growth dilution. Loggerhead sea turtles may accumulate these compounds at a higher rate during an earlier life stage. Lower exposure to the compounds and faster growth during the benthic juvenile stage would serve to dilute their tissue contaminant levels. During an earlier life stage, the pelagic juveniles inhabit not only open ocean habitat but also coastal areas of Madeira, the Azores and Canary Islands, Africa, Portugal, and the waters of the Mediterranean Sea. When the turtles return to the U.S. coast, their growth during the benthic juvenile stage may dilute their previous accumulation of these compounds. However, without knowing the migratory history and previous contaminant exposure of these animals, we can only offer this as a possible explanation.

At this time, it is very difficult to interpret age-dependent changes in contaminant concentrations for sea turtles. No non-invasive method is available to age live sea turtles. Size is the only available approximation of their age and the accuracy of this method is questionable as their growth rates can be very plastic (Snover 2002). Loggerhead sea turtle hatchlings begin life at 4 cm in straight carapace length and grow to more than 100 cm as reproducing adults (personal observation, JMK). The

single size class (45.7 to 74.1 cm) represented in this study, the benthic juvenile stage, is a mere portion of their life history. Future research should incorporate different age/size classes into these analyses, including eggs, hatchlings, pelagic juveniles, benthic juveniles, and adults. An alternative, but more difficult approach to this question, would entail repeated measurements of OC contaminants on individual turtles over time. Interestingly, the two turtles (ID# 5 and 6) that were recaptured after one year showed clear evidence of accumulation of OCs in their blood (Fig. 3.5). The second blood sample from both turtles had higher levels of all OCs than the first sample.

Yellow versus brown fat in Kemp's ridley sea turtle

Two types of fat, yellow and brown, were observed and analyzed for OCs in the Kemp's ridley sea turtles. The yellow fat surrounded the viscera, while the brown fat was located in the inguinal regions and along the margins of the plastron. To our knowledge, neither the lipid composition nor the functions of these two fat types have been described in sea turtles. Though brown in color, the brown fat of sea turtles differs from brown fat of mammals which contains adipocytes with multiple small vacuoles and serves a thermoregulatory function, particularly in hibernating animals (Dellman and Brown 1981). Histology on the brown fat from the loggerhead sea turtle showed that it more closely resembled mammalian white fat, which contains adipocytes with a single or few large lipid vacuoles. Histological examination was not performed on the Kemp's ridley brown or yellow fat.

The yellow and brown fat collected from the Kemp's ridley turtles in this study contained similar levels of lipid and all OC contaminants. These similarities suggest

that the deposition, mobilization, and possibly the function of these two fat stores are similar. Additionally, this indicates that either fat type may be used to monitor OC concentrations.

Previous sea turtle studies have shown significant correlations between OC concentrations between different tissue compartments. OC concentrations (total PCBs, 4,4'-DDE, and *trans*-nonachlor) in adipose tissue of Kemp's ridley sea turtles significantly correlated to levels in their liver (Lake *et al*. 1994). In eggs, the concentrations of total PCBs were correlated to levels in the chorioallantoic membrane (CAM) which is left behind after the turtle emerges (Cobb and Wood 1997). CAMs can therefore be sampled non-invasively to measure contaminants at the embryonic life stage; whereas the current study investigated the use of blood as a non-invasive sample to monitor OCs in later life stages.

Fluctuations in Blood OC Concentrations

In addition to size and sex, nutritional status may also influence OC concentrations, especially concentrations observed in the blood. Blood OC levels can fluctuate depending upon recent diet, hydration status, short-term changes in metabolism, or mobilization of lipid stores. Lipid mobilization is likely to occur in turtles during long migrations or when they are yolking eggs, fasting, fighting infections, or when food is scarce. Lydersen *et al*. (2002) found that harp seals had higher blood OC concentrations after fasting in the laboratory or after dramatic weight loss during lactation and molting in the wild. A rapid weight loss in humans has also been shown to result in increased blood and fat concentrations of OCs (Chevrier *et al*. 2000). Similarly, loggerhead turtles in this study with lower fat lipid stores had higher

levels of blood OCs, suggesting that these compounds were mobilized into the blood along with lipid stores. In addition, large changes in blood OC concentrations were observed in some of the six recaptured loggerhead turtles. The changes did not correlate to the length of time between sampling events and any conclusions based on the data from these 6 turtles are limited due to the small sample size.

Blood versus Fat OC Concentrations

The primary goal of this study was to determine if the high variability seen in blood OC concentrations of sea turtles could be explained by the accumulated concentrations found in the adipose tissue. Our data suggest that this is the case. Concentrations of all the major OC compounds in the blood correlated to those in the fat for both loggerhead and Kemp's ridley sea turtles. Similar relationships have also been noted in humans, marine mammals, and birds (Reddy *et al*. 1998; Henny and Meeker 1981; Bernhoft *et al*. 1997; Wolff *et al*. 1982; Mes 1992; Kanja *et al*. 1992; Boon *et al*. 1994). Reddy *et al*. (1998) observed a significant relationship between blubber and red blood cell concentrations of HCB, total DDTs, *trans*-nonachlor, and the dominant PCB congeners in captive bottlenose dolphins. Correlations were also seen between subcutaneous fat and plasma for chlordanes, DDE, HCB, HCHs, and total PCBs in polar bears (Bernhoft *et al*. 1997). Likewise, Henny and Meeker (1981) have shown that blood plasma concentrations of 4,4'-DDE were significantly related to brain concentrations in American kestrels. It is important to note that correlations of blood OCs to fat OCs observed in the current study were stronger than the correlations of blood OCs to fat lipid content. These results and previously

documented correlations in other species indicate that OC contaminants in blood are often in equilibrium with OCs in fatty tissues.

In conclusion, our data suggest that in most situations blood can be used to measure OC burden in sea turtles. This conclusion is based on findings of previous studies with other organisms and our finding that blood OC concentrations in two sea turtle species appear to be in equilibrium with levels present in the fatty tissues. Since blood carries contaminants to sensitive tissues and organs, blood OC concentrations may be the most relevant to toxicological studies. However, we caution the use of blood in studies that plan to monitor spatial or temporal changes in OC concentrations. Turtles undergoing nutritional changes, seasonal migrations, or disease events may be mobilizing their lipid stores that could cause drastic fluctuations in blood OC concentrations. Future studies may clarify contaminant concentrations in sea turtles that are undergoing these changes and provide insight as to how these events truly affect blood OC concentrations.

Table 3.1. Lipid-normalized organochlorine contaminant concentrations (ng/g lipid) in fat biopsies and blood from 44 juvenile loggerhead sea turtles.

	Fat Biopsies (ng/g lipid)			Whole blood (ng/g lipid)		
	Mean (SD)	N[a]	Median (Quartiles)	Mean (SD)	N[a]	Median (Quartiles)
Total PCBs	2010 (2960)	44	1010 (619 - 2360)	2490 (3700)	44	2030 (1020-2810)
α-HCH	0.270 (1.46)	2	<LOD[b]	<LOD	0	<LOD
β-HCH	0.358 (1.03)	5	<LOD	<LOD	0	<LOD
γ-HCH	3.27 (10.6)	7	<LOD	<LOD	0	<LOD
HCB	2.57 (6.40)	12	<LOD (<LOD - 1.85)	<LOD	0	<LOD
mirex	43.7 (62.1)	39	18.8 (9.05 - 42.7)	21.2 (39.2)	27	7.58 (<LOD - 20.5)
dieldrin	35.3 (87.2)	38	18.8 (7.00 - 30.3)	20.1 (25.8)	26	15.1 (<LOD - 27.4)
heptachlor epoxide	10.4 (12.2)	29	8.85 (<LOD - 15.1)	8.02 (15.9)	16	<LOD (<LOD - 10.6)
trans -chlordane	<LOD	0	<LOD	10.1 (15.7)	30	7.72 (<LOD - 12.8)
cis -chlordane	<LOD	0	<LOD	0.406 (1.56)	3	<LOD
trans -nonachlor	107 (147)	42	70.5 (43.6 - 103)	56.7 (99.4)	43	34.6 (22.4 - 61.0)
cis -nonachlor	5.17 (6.58)	25	3.72 (<LOD - 8.34)	9.05 (6.95)	37	8.61 (4.98 - 11.5)
oxychlordane	134 (277)	40	37.5 (26.8 - 163)	25.8 (35.7)	35	14.9 (8.21 - 37.1)
Total chlordanes	246 (412)	43	125 (83.2 - 262)	102 (151)	43	67.8 (42.3 - 124)
4,4'-DDD	<LOD	0	<LOD	4.09 (6.45)	20	<LOD (<LOD - 5.78)
4,4'-DDE	445 (643)	41	250 (144 - 476)	300 (578)	41	172 (99.8 - 333)
2,4'-DDT	7.03 (13.1)	11	<LOD (<LOD - 4.14)	0.286 (1.90)	1	<LOD
Total DDTs	452 (643)	41	254 (144 - 477)	305 (577)	42	192 (101 - 337)
Percent lipid	26.3 (20.6)		26.1 (4.68 - 42.6)	0.262 (0.0804)		0.257 (0.209 - 0.306)

[a] N = number of samples out of 44 that were above the limit of detection (1 ng/g wet mass for fat; 10 pg/g wet mass for blood).
[b] <LOD = below the limit of detection.

Table 3.2. Lipid-normalized organochlorine contaminant concentrations (ng/g lipid) in fat and blood samples from juvenile Kemp's ridley sea turtles.

	Yellow Fat (ng/g lipid)			Brown Fat (ng/g lipid)			Whole blood (ng/g lipid)		
	Mean (SD)	N[a]	Median (Quartiles)	Mean (SD)	N[b]	Median (Quartiles)	Mean (SD)	N[c]	Median (Quartiles)
Total PCBs	1050 (1220)	9	726 (206 - 1270)	1110 (1030)	10	887 (240 - 1540)	985 (1250)	8	404 (176 - 1240)
α-HCH	6.09 (4.59)	7	5.31 (3.54 - 8.17)	10.2 (15.8)	7	5.06 (0.638 - 11.3)	<LOD[d]	0	<LOD
β-HCH	50.8 (100)	5	3.90 (<LOD - 21)	41.4 (68.6)	8	9.45 (3.99 - 25.6)	<LOD	0	<LOD
γ-HCH	21.7 (35.1)	5	1.86 (<LOD - 28.6)	30.6 (46.2)	6	15.0 (<LOD - 43.5)	<LOD	0	<LOD
HCB	20.3 (14.7)	8	20.1 (10.7 - 32.4)	15.1 (13.2)	9	10.4 (3.94 - 26.1)	1.33 (2.55)	2	<LOD (<LOD - 1.02)
mirex	5.26 (6.82)	6	2.88 (<LOD - 7.02)	5.34 (6.13)	7	3.53 (0.783 - 6.62)	7.12 (13.5)	4	1.73 (<LOD - 6.06)
dieldrin	33.8 (22.3)	9	43.5 (13.6 - 43.7)	51.7 (66.2)	10	32.5 (14.9 - 53.3)	17.9 (16.3)	6	14.4 (8.49 - 25.1)
heptachlor epoxide	24.2 (30.5)	9	10.9 (8.31 - 26.5)	31.3 (38.0)	8	18.9 (3.27 - 37.5)	13.3 (10.6)	7	10.4 (6.09 - 20.9)
trans -chlordane	0.844 (2.53)	1	<LOD	2.80 (3.95)	5	0.720 (<LOD - 5.22)	2.74 (5.12)	3	<LOD (<LOD - 3.14)
cis -chlordane	1.37 (1.81)	4	<LOD (<LOD - 2.40)	1.12 (1.95)	3	<LOD (<LOD - 1.79)	0.816 (1.52)	2	<LOD (<LOD - 0.743)
trans -nonachlor	54.7 (46.9)	9	35.9 (23.7 - 79.1)	109 (173)	10	53.3 (24.4 - 102)	41.2 (50.2)	8	27.4 (18.7 - 36.8)
cis -nonachlor	10.4 (8.62)	8	6.64 (3.86 - 17.7)	9.77 (9.29)	8	5.64 (2.37 - 19.3)	11.8 (6.06)	8	10.3 (7.07 - 14.2)
oxychlordane	90.8 (98.9)	9	43.8 (16.7 - 123)	117 (162)	10	66.6 (27.6 - 135)	20.6 (24.3)	6	10.4 (5.49 - 29.0)
Total chlordanes	158 (138)	9	80.3 (49.6 - 234)	240 (331)	10	144 (60.7 - 266)	77.2 (81.6)	8	56.9 (36.3 - 73.5)
4,4'-DDD	2.76 (4.58)	4	<LOD (<LOD - 2.18)	2.96 (5.11)	5	0.795 (<LOD - 1.98)	5.97 (1.79)	8	5.74 (4.84 - 6.57)
4,4'-DDE	154 (111)	9	103 (54.8 - 256)	254 (332)	10	169 (69.9 - 276)	166 (147)	8	119 (76.7 - 178)
Total DDTs	156 (113)	9	103 (54.8 - 257)	257 (332)	10	175 (71.5 - 287)	172 (147)	8	124 (83.4 - 185)
Percent lipid	65.8 (10.6)		70.7 (57.7 - 74.8)	62.0 (23.9)		72.8 (57.6 - 74.3)	0.461 (0.313)		0.347 (0.289 - 0.463)

[a] Number of samples out of 9 that were above the limit of detection (LOD).
[b] Number of samples out of 10 that were above the LOD.
[c] Number of samples out of 8 that were above the LOD.
[d] <LOD = below LOD (1 ng/g wet mass for fat; 10 pg/g wet mass for blood).

Table 3.3. Spearman rank correlation coefficients (p-values) of lipid-normalized organochlorine contaminant concentrations between various tissues of sea turtles.

	Loggerhead fat biopsies vs. whole blood	Kemp's ridley brown fat vs. whole blood	Kemp's ridley yellow fat vs. whole blood	Kemp's ridley yellow fat vs. brown fat
Total PCBs	0.634 (<0.001)	0.976 (0.001)	0.893 (0.02)	0.929 (<0.002)
mirex	0.634 (<0.001)	0.762 (<0.05)	0.786 (0.05)	0.933 (0.001)
dieldrin	0.582 (<0.001)	0.786 (<0.05)	NS[a]	0.904 (<0.005)
heptachlor epoxide	0.436 (<0.005)	NS	NS	NS
cis -chlordane	ND[b]	NS	NS	0.750 (<0.05)
trans -nonachlor	0.752 (<0.001)	1.000 (<0.001)	0.964 (0.005)	0.967 (<0.001)
cis -nonachlor	NS	0.905 (0.005)	0.857 (<0.05)	0.883 (<0.005)
oxychlordane	0.697 (<0.001)	0.857 (<0.02)	0.964 (0.005)	0.767 (<0.05)
Total chlordanes	0.722 (<0.001)	0.905 (0.005)	0.929 (0.01)	0.867 (0.005)
4,4'-DDD	ND	NS	NS	0.833 (0.01)
4,4'-DDE	0.657 (<0.001)	0.857 (<0.02)	0.786 (0.05)	0.950 (<0.001)
Total DDTs	0.665 (<0.001)	0.905 (0.005)	0.857 (<0.05)	0.917 (0.002)

[a] NS = not significant, $p < 0.05$.
[b] ND = below the limit of detection in at least one tissue.

Table 3.4. Information on six recaptured loggerhead sea turtles.

	Date			Size			% lipid in blood	
Turtle ID	1st capture	2nd capture	Days between sampling	1st capture	2nd capture	Sex	1st capture	2nd capture
1	29-Jun-01	16-Jul-01	17	59.0	59.2	F	0.195	0.315
2	15-Jun-01	16-Jul-01	31	59.3	59.8	F	0.170	0.226
3	2-Jun-00	11-Aug-00	70	54.3	55.7	F	0.165	0.304
4	17-Jul-01	8-Oct-01	83	64.9	66.2	F	0.208	0.191
5	16-Jun-00	18-Jul-01	397	73.3	73.6	M	0.266	0.385
6	5-Jun-00	13-Jul-01	403	73.5	75.4	F	0.199	0.101

[a] Size = straight carapace length from nuchal notch to posterior marginal notch.

Table 3.5. Mean (SD) organochlorine contaminant concentrations (ng/g wet mass unless stated otherwise) in fat of sea turtles.

Species	Age Class/Sex[a]	Status	Tissue	Year	Location[a]	PCB 153	Total PCBs	4,4'-DDE	Total DDTs	N	Reference
Loggerhead	J	live	adipose	2000 & 2001	NC	80.9 (86.4)	256 (269)	64.4 (64.8)	67.0 (68.7)	44	this study
Loggerhead	J & A	fresh dead to decomposed	adipose	1991 & 1992	VA & NC	146(120)	551 (473)	195 (266)	206 (268)	20	Rybitski *et al*. 1995
Loggerhead	J & A	dead	adipose	1994 & 1995	Med. Sea	241 (17.7)	840 (60.0)	509 (173)	528 (185)	3	Mckenzie *et al*. 1999
Loggerhead	J	dead	adipose	1993	Med. Sea	87.9 (NA)	334 (179)			4	Corsolini *et al*. 2000
Loggerhead	NA[b]	dead	adipose	1986	NA		647	300		1	Lake *et al*. 1994
Kemp's ridley	J	fresh dead	yellow fat	1998 to 2000	MA & NC	161 (173)	701 (893)	99.6 (76.4)	101 (77.9)	9	this study
Kemp's ridley	J	fresh dead	brown fat	1998 to 2000	MA & NC	135 (127)	525 (545)	90.9 (71.9)	92.8 (73.6)	10	this study
Kemp's ridley	J	decomposed	adipose	1991	VA & NC	189 (96.4)	660 (333)	194 (98.2)	223 (106)	3	Rybitski *et al*. 1995
Kemp's ridley	J	dead	adipose	1989	NY	161 (95.6)	476 (273)	232 (157)	261 (176)	6	Lake *et al*. 1994
Kemp's ridley	J	dead	adipose	1985	NY	384 (289)	1250 (985)	386 (250)	454 (298)	7	Lake *et al*. 1994
Leatherback	AF	euthanized	adipose	1999	NC	41.0	129	13.2	13.2	1	this study
Leatherback	AF	euthanized	blubber	1999	NC	664	2330	288	292	1	this study
Leatherback	AM	dead	adipose	1993 & 1995	Scotland	26.9[c]	113[c]	33.5[c]	36[c]	2	Mckenzie *et al*. 1999
Leatherback	AM	drowned in fishing gear	adipose	1993 to 1996	Wales & Scotland	42.3 (32.7)	152 (94.3)	45.0 (30.8)		3	Godley *et al*. 1998
Leatherback	AM	dead	blubber	1988	Wales		1200[d]			1	Davenport *et al*. 1990
Green	JM	moderately decomposed	adipose	2000	NC	28.7	81.1	15.0	15.0	1	this study
Green	J	dead	adipose	1995	Med. Sea	15.3 (16.0)	136 (113)	9.13 (8.73)	12.4 (9.93)	3	Mckenzie *et al*. 1999
Green	M	dead	adipose	1998	NE Australia	70[d]	171[d]	45[d]	53[d]	1	Vetter *et al*. 2001
Green	JM, AF, AM	fresh dead	adipose	1992 & 1993	Hawaii		285 (330)[e]			3	Miao *et al*. 2001
Green	J	dead	adipose	NA	Hawaii		22.9 (21.3)	4.97 (8.82)		5	Rybitski 1993
Green	J	dead or euthanizied	adipose	NA	Hawaii		<1000[f]	<100[f]		12	Aguirre *et al*. 1994

[a] J = juvenile; A = adult; F = adult female; M = adult male; A = adult; NC = North Carolina; VA = Virginia; Med = Mediterranean; MA = Massachusetts; NY = New York.
[b] NA = not available.
[c] SD was not calculated on sample size of 2.
[d] Values were reported as ng/g lipid.
[e] Values were reported as ng/g dry weight.
[f] Analytical limit of detection for total PCBs was 1000 ng/g and for 4,4'-DDE was 100 ng/g.

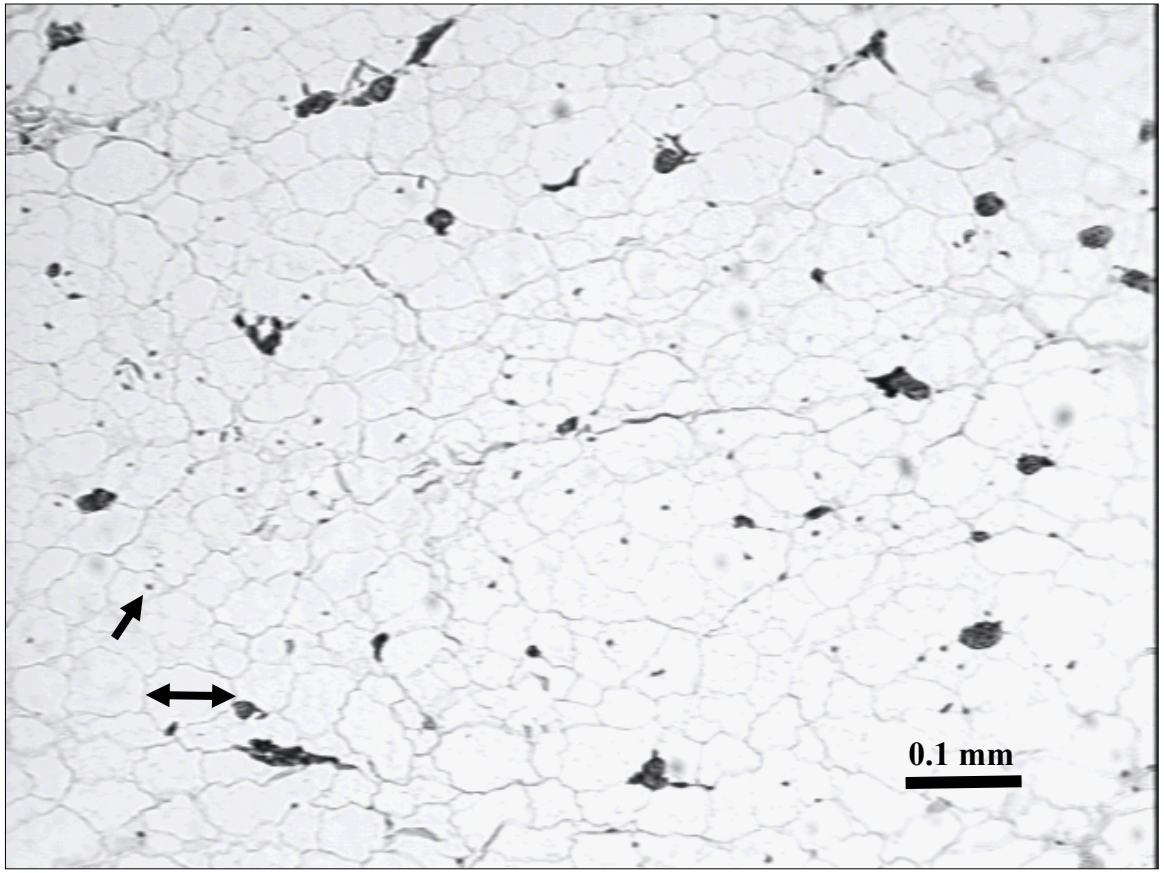


Figure 3.1. Histological section of subcutaneous fat from a loggerhead sea turtle, showing sheets of irregular adipocytes and sparse dark-stained fibrovascular stroma. The section was stained with hematoxylin and eosin. The double-ended arrow indicates the diameter of a single adipocyte; the regular arrow is pointing at a nucleus.

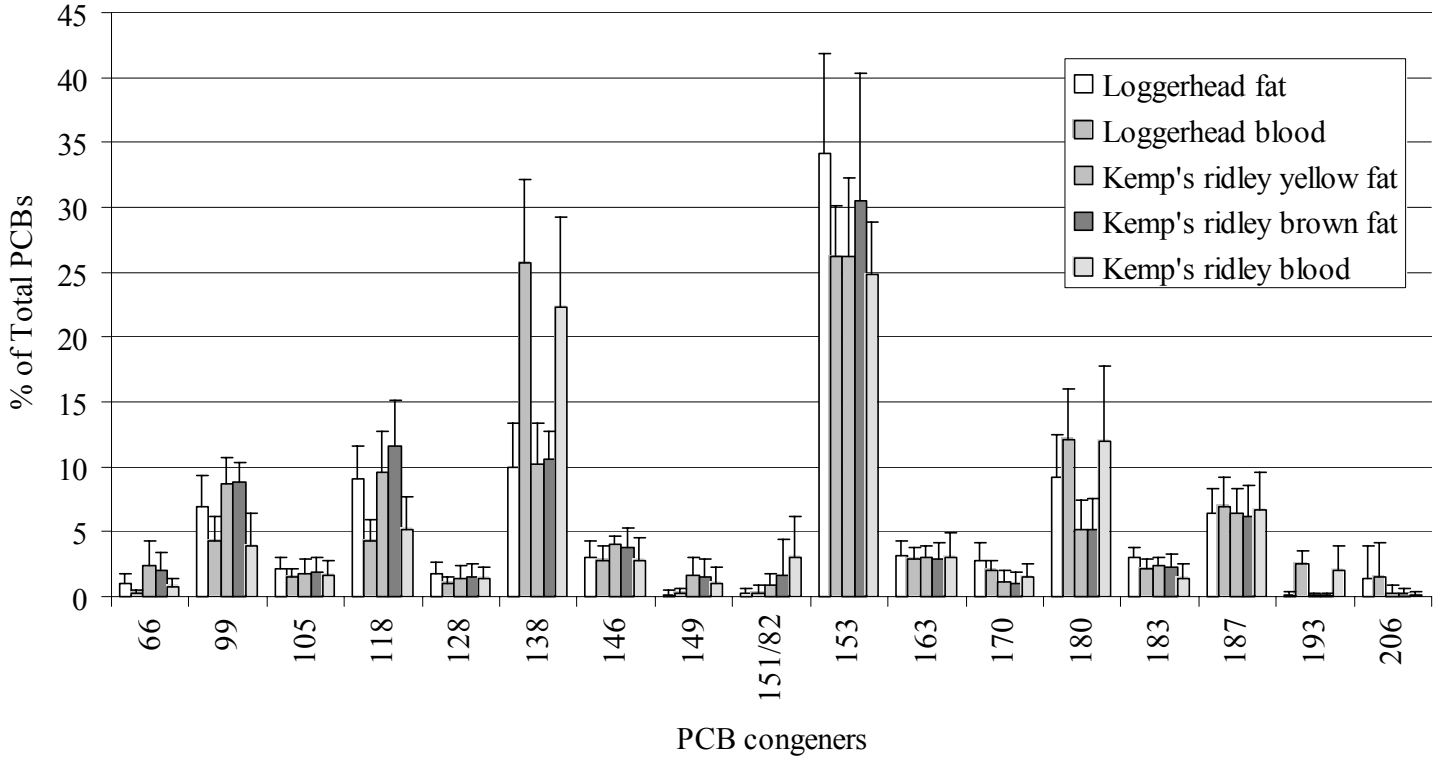
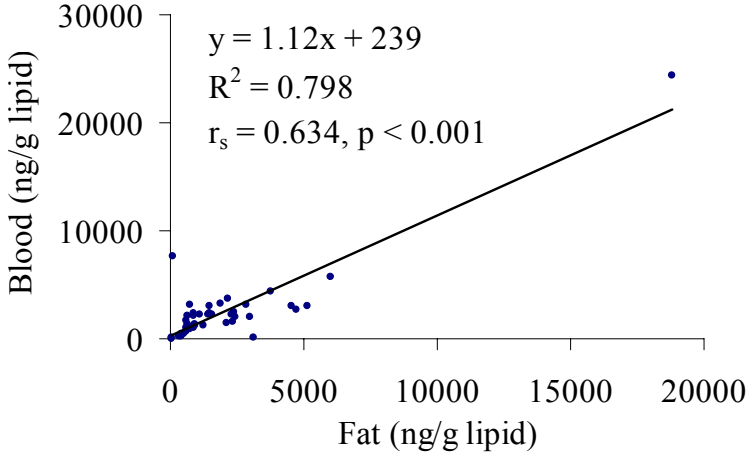


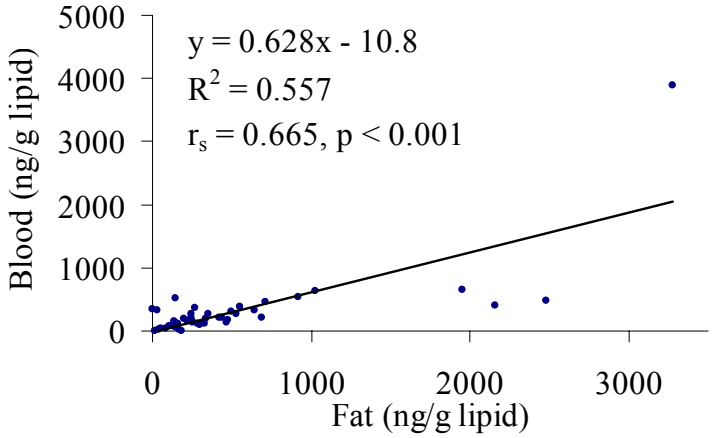
Figure 3.2. Pattern of PCB congeners (wet mass basis) in blood and fat of loggerhead and Kemp's ridley sea turtles (mean and standard deviation). Only congeners with greater than 2 % of the total PCB concentration in at least one of the tissues are included. Sample sizes are 44 for loggerhead blood and fat, 9 for yellow fat, 10 for brown fat, and 8 for blood of Kemp's ridley sea turtles.

Figure 3.3. Relationships between fat biopsy and whole blood concentrations of organochlorine contaminants in loggerhead sea turtles. Organochlorine concentrations are lipid-normalized. Linear regression statistics and the Spearman correlation coefficients (r_s) and p-values are given.

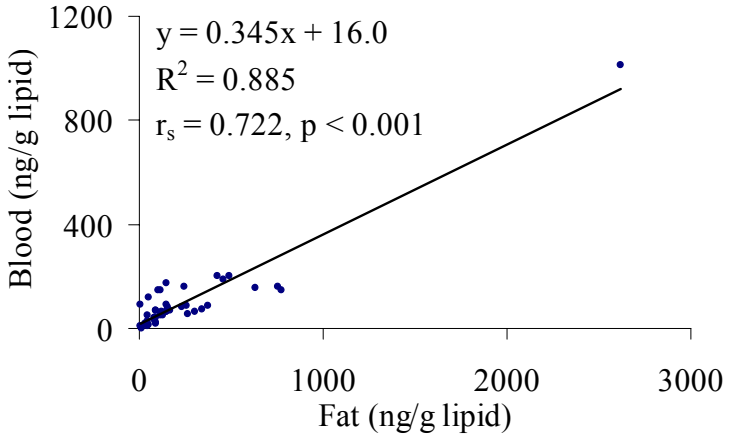
A) Total PCBs



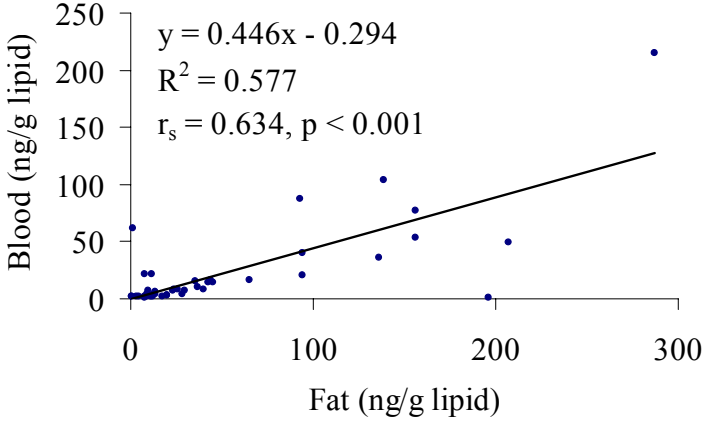
B) Total DDTs



C) Total Chlordanes



D) Mirex



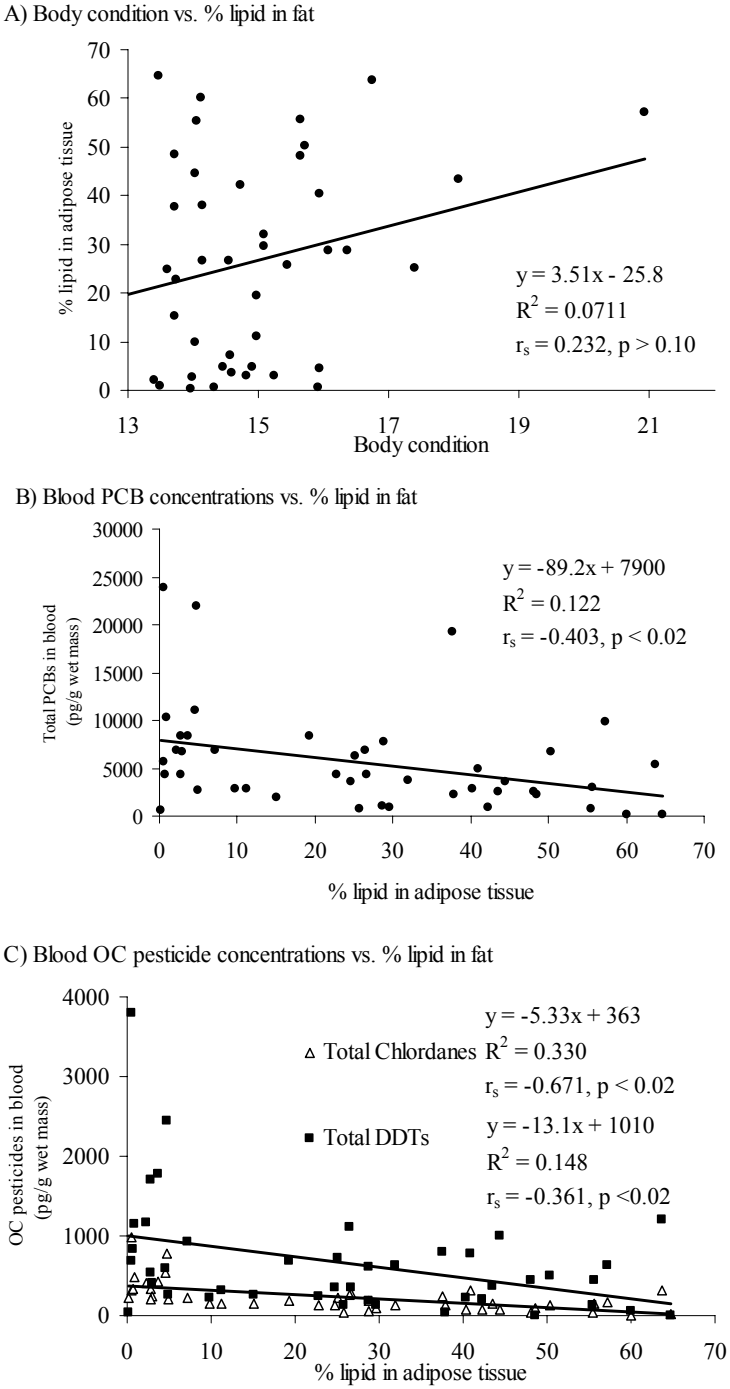


Figure 3.4. Relationships of body condition and blood OC concentrations (pg/g wet mass) to percent lipid content in the adipose tissue of loggerhead sea turtles. Percent lipid did not correlate to body condition (see Materials and Methods for calculation) (A). PCB (B) and OC pesticide (C) concentrations in the blood (pg/g wet mass) correlated to percent lipid in the fat biopsies. Linear regression statistics and the Spearman correlation coefficients (r_s) and p-values are given.

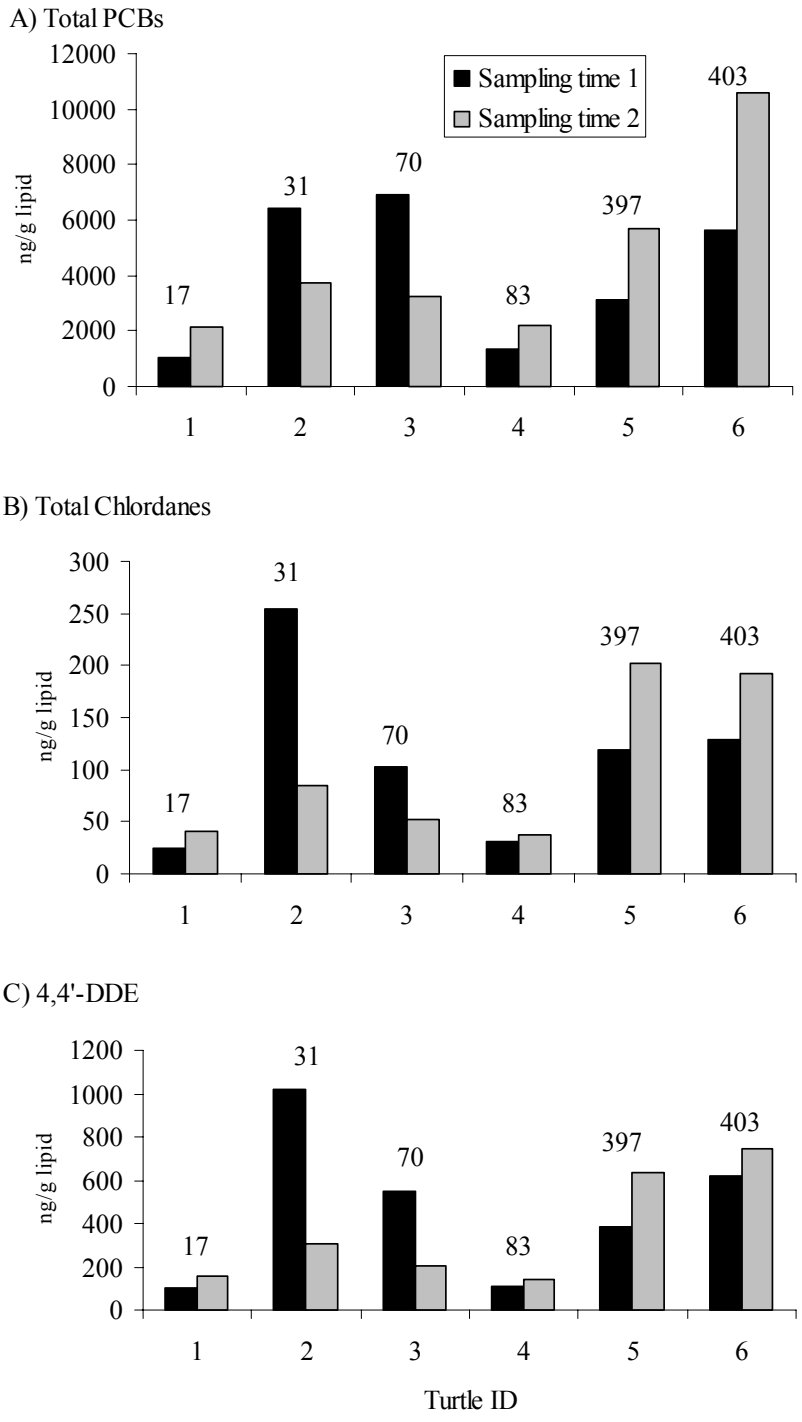


Figure 3.5. Organochlorine concentrations (ng/g lipid) measured in whole blood of six loggerhead sea turtles captured at two different times. Numbers above bars indicate duration of time (days) between blood sampling. Contaminants were analyzed in only a single sample per turtle per sampling event.

CHAPTER FOUR

Are organochlorine contaminants affecting the clinical health of loggerhead sea turtles?

ABSTRACT

Widespread and persistent organochlorine (OC) contaminants, such as polychlorinated biphenyls (PCBs) and pesticides are known to have a broad toxicity in wildlife. We investigated, for the first time, their possible health effects on loggerhead sea turtles (*Caretta caretta*). Using non-lethal sampling techniques of fat biopsies and blood collection, we compared the contaminant concentrations measured in live turtles to clinical health assessment data, including hematology, plasma chemistry, and body condition. PCBs and OC pesticides were determined in 44 fat biopsies and 50 blood samples using gas chromatography with electron capture detection and mass spectrometry. Several notable and statistically significant correlations were observed. Blood concentrations of certain OCs negatively correlated to red blood cell counts, hemoglobin, and hematocrit, indicative of anemia. Some OC contaminants positively correlated to white blood cell counts and to the heterophil:lymphocyte ratio, suggesting modulation of the immune system. Some OC concentrations were correlated positively to aspartate aminotransferase (AST), indicating possible tissue damage, and negatively to alkaline phosphatase (ALP). Significant correlations to levels of some contaminants also suggested possible alteration of protein (↑ blood urea nitrogen, ↑ total plasma protein, ↓ albumin:globulin ratio), carbohydrate (↓ glucose), and ion (↑ osmolality, ↑ sodium, ↓ magnesium) regulation. Moreover, three extremely emaciated and lethargic turtles exhibited signs of wasting and had blood OC concentrations that were two orders of magnitude higher than the average 'apparently healthy' turtle. These correlations suggest that OC contaminants may be affecting the

health of loggerhead sea turtles even though sea turtles accumulate lower concentrations of OCs compared to other wildlife.

INTRODUCTION

Organochlorine (OC) compounds, including polychlorinated biphenyls (PCBs) and OC pesticides have contaminated the global environment (Iwata *et al*. 1994; Safe 1993). OC contaminants bioaccumulate into animal tissues where they cause a plethora of toxic effects. Hepatotoxicity, wasting, immunotoxicity, developmental abnormalities, and reproductive toxicity have been observed in laboratory animals (Safe 1993). These responses, as well as endocrine disruption and neurobehavioral effects have been associated with OC contaminant exposure in wildlife (Fox 2001). Furthermore, these toxic responses have been linked to wildlife population declines. Declines of bald eagles (*Haliaeetus leucocephalus*) and double-crested cormorants (*Phalacrocorax auritus*) from the Great Lakes region and elsewhere were blamed on egg shell thinning by DDT (Grasman *et al*. 1998). A reduction of an alligator population from a contaminated lake in Florida was linked to OC pesticides which caused developmental and reproductive abnormalities (Guillette *et al*. 1995). In addition, massive die-offs of marine mammals during viral epizootics were thought to be linked to immunosuppression caused by PCBs and other OC compounds (Ross *et al*. 1996). Populations of all sea turtle species are already threatened with extinction because of numerous man-made pressures, but the effects of contaminants on sea turtles are undocumented.

OC compounds have been detected in tissues of threatened and endangered sea turtles (Pugh and Becker 2001). Recently, Keller and coauthors (in prep a) measured

OC concentrations in the blood and adipose samples of 44 live, juvenile loggerhead sea turtles (*Caretta caretta*) from North Carolina. PCBs were found at the highest concentrations (mean wet mass concentrations were 256 ng/g adipose and 5.56 ng/g blood), followed by 4,4'-DDE (64.4 ng/g adipose and 0.650 ng/g blood). Lower concentrations of chlordanes, mirex, dieldrin, and heptachlor epoxide were also detected in these turtles. The blood PCB concentrations were similar to those of American alligators (*Alligator mississippiensis*) from reference and contaminated lakes in Florida (Guillette *et al*. 1999) and to those found in human blood (Feeley 1995). However, the PCB concentrations found in the loggerhead samples were much lower than those found in plasma of snapping turtles (*Chelydra serpentina*) and birds from the Great Lakes (de Solla *et al*. 1998; Grasman and Fox 2001) and those measured in blood of bottlenose dolphins (*Tursiops truncatus*; Lahvis *et al*. 1995).

Although sea turtles accumulate lower concentrations of these contaminants, their sensitivity to the effects of OCs is unknown. Sensitivity to contaminants can vary profoundly from one species to another. For example, the dose of TCDD that causes 50 % lethality (LD_{50}) ranges over four orders of magnitude among six species of mammals commonly used in laboratory experiments (McConnell 1985). The difference in sensitivity to contaminants could be even greater between wildlife species (Smith and Hall 1994). Hematology and blood chemistry values may provide a good starting point for examining the sensitivity of sea turtles to the effects of OCs.

Clinical health assessments usually begin with a physical examination with adjunct tests including a review of hematology and clinical blood chemistry values. Common parameters include total and differential counts of blood cells, activities of

plasma enzymes, and concentrations of plasma protein, glucose, and electrolytes. Reductions in red blood cells (RBCs) indicate anemia, while altered white blood cell (WBC) counts can signal a modulation of the immune system. Elevated blood enzyme activities, such as AST, can suggest damage to tissues or a particular organ. Changes in protein, glucose, or electrolyte concentrations may indicate altered physiological regulation of these essential components. These parameters are used to assess the health of humans and wildlife alike, because they are non-invasive indicators of the effects of disease, malnutrition, or toxic insults.

OC contaminants have been shown to alter hematological and blood chemistry values in laboratory-exposed animals and environmentally-exposed humans and wildlife. For example, the liver is the primary target of the toxicity of PCBs in humans, and an elevation in blood AST activity is one of the most sensitive indicators of this liver damage (Feeley 1995). Likewise, increased blood AST, as well as increased incidence of liver necrosis, was observed in rats exposed to PCB 156 (Haag-Grönlund *et al*. 1997) or to Aroclor 1242 (Bruckner *et al*. 1973) and American kestrels (*Falco sparverius*) exposed to PCB 126 (Hoffman *et al*. 1996). These experimental studies establish AST as a non-invasive indicator of OC-induced liver damage. For most wildlife species, though, the distribution of AST and other enzymes among organs is not known; therefore, it is difficult to interpret which organ is damaged based on an increase of a particular enzyme in the blood.

Other biochemical effects of PCBs on the liver include changes in activities of gluconeogenic enzymes and lipogenic enzymes, which can be monitored by assessing changes in blood concentrations of glucose and lipids (Boll *et al*. 1998; Lorenzen *et al*.

1999). Similarly, the kidneys, along with the salt gland in marine turtles and birds, serve to regulate water and electrolyte balance. OC contaminants have been shown to alter blood electrolytes in laboratory-exposed pelicans (Greichus *et al*. 1975), environmentally-exposed birds (Fox personal communication), and occupationally-exposed humans (Lawton *et al*. 1985), indicating possible impaired function of these osmoregulating organs.

The immunotoxic effects of OCs have been illustrated by changes in WBC counts. This has been shown in laboratory-exposed animals, such as white-footed mice (*Peromyscus leucopus*; Segre *et al*. 2002) and harbor seals (*Phoca vitulina*; de Swart *et al*. 1995) as well as environmentally-exposed wildlife (Grasman *et al*. 1996) and humans (Lawton *et al*. 1985; Lu and Wu 1985). One particular indicator, an elevation in the ratio of heterophils to lymphocytes, is used as an indicator of stress on the immune system. An increase in this ratio was correlated to TEQs in herring gull (*Larus argentatus*) chicks from the Great Lakes (Grasman *et al*. 2000a). TEQs, PCBs, and DDE concentrations in Caspian tern (*Sterna caspia*) chicks also significantly correlated to an increase in this ratio (Grasman *et al*. 1996).

These indicators have been used widely to assess the health and physiologic status of reptiles (Campbell 1996). For example, increases in AST activity and the heterophil:lymphocyte ratio were seen in captive green iguanas (*Iguana iguana*) with renal disease (Knotek *et al*. 2002). Even so, changes in these parameters in nearly all reptile species are not well understood; therefore, most interpretation assumes what is known for mammals and birds (Ramsay and Dotson 1995). What is known in reptiles is that these values vary profoundly with seasonal changes and nutritional status.

Thus, these confounding factors should always be considered when examining toxicological effects on reptiles (Campbell 1996; Zapata *et al*. 1992).

General health assessments have been performed on some select populations of sea turtles (George 1997). Normal ranges for general health indicators have been reported for loggerhead sea turtles from Chesapeake Bay (George 1997) and from Cape Canaveral, Florida (Lutz and Dunbar-Cooper 1987; Bolten et al. 1992). One study followed the changes of these indicators in injured and sick loggerheads from North Carolina as they were rehabilitated back to health (Harms *et al*. 2002). Blood chemistry ranges have also been reported for Kemp's ridley sea turtles (*Lepidochelys kempii*) from New York Bight (Carminati *et al*. 1994) and for green sea turtles (*Chelonia mydas*) from the Bahamas (Bolten and Bjorndal 1992) and from the United Arab Emirates (Hasbun *et al*. 1998). Comparisons within and among these studies illustrate differences between health status, species, seasons, and locations. The general morphology and staining characteristics of blood leukocytes have also been examined in Kemp's ridley turtles (Cannon 1992), captive green sea turtles (Wood and Ebanks 1984), and green sea turtles from Hawaii (Work *et al*. 1998). However, there is great controversy over the criteria for identifying different leukocyte types (Work *et al*. 1998).

Hematology and blood chemistry values have been shown to be altered by hypothermia (cold-stunning), disease, and oil exposure in sea turtles. Cold-stunned Kemp's ridley turtles were shown to have electrolyte imbalances (increased Mg, Ca, K, and P concentrations) indicating possible salt gland failure (Carminati *et al*. 1994). These turtles also exhibited increased blood enzyme activities suggesting tissue

damage and decreased blood urea nitrogen (BUN) concentrations indicative of reduced feeding.

A number of research projects have shown altered hematology, plasma chemistries, and immune functions in green sea turtles with fibropapillomatosis (FP), a disease in which non-cancerous tumors grow on skin and eyes and impede foraging. Aguirre *et al*. (1995) first demonstrated that diseased turtles from Hawaii had lower hematocrit (HCT) and WBC counts, a higher ratio of heterophils to lymphocytes, reduced protein, glucose, BUN, and ion concentrations, and altered plasma enzyme profiles. Recent studies showed that these alterations were more pronounced in turtles with increasing severity of FP (Aguire and Balazs 2000; Work and Balazs 1999; Work *et al*. 2001). Furthermore, immune function, as measured by mitogen-induced lymphoproliferation (LP), was suppressed in the turtles with the greatest severity of disease (Work *et al*. 2001). A parallel study on green turtles from Florida corroborated the findings from Hawaii (Cray *et al*. 2001). Decreased LP, increased heterophils, reduced lymphocytes, and changes in protein concentrations were observed in the Florida diseased turtles compared to healthy turtles.

Only one class of contaminants, oil, has been studied for health effects on sea turtles (Lutcavage *et al*. 1995; Vargo *et al*. 1986; Fritts and McGehee 1981). Juvenile loggerhead turtles that were experimentally-exposed to a thin film of South Louisiana crude oil exhibited a significant increase in WBCs. RBCs decreased by almost 50% of the controls. Reductions in glucose and BUN concentrations seen in both the controls and oiled turtles were attributed to lack of feeding during the exposure period, but the oiled turtles recovered more slowly than the controls upon feeding. Turtles exposed to

a thicker film of oil exhibited the same responses described above, but in addition had complete failure of the salt gland to produce fluid (Vargo *et al*. 1986). In another study, Kemp's ridley eggs exposed to unweathered oil resulted in reduced hatching success and decreased hatchling survival (Fritts and McGehee 1981).

The effects of OC contaminants on reptilian hematological and blood chemistry values have been investigated in only one study. Albers *et al*. (1986) compared blood chemistries of snapping turtles from three locations with differing contamination of PCBs and OC pesticides. Site differences were observed in OC concentrations, but no differences in blood chemistry parameters were seen that would have indicated contaminant-induced physiological impairment. Although no effects were seen in that study, it is still important to examine the health effects of OC contaminants on sea turtles. Their sensitivity to the effects of OC contaminants is unknown. Furthermore, all species of sea turtles are threatened or endangered, and OC contaminants may have contributed to their past and current population declines. Therefore, this study sought to determine whether relationships exist between indicators of health and OC concentrations in the threatened juvenile loggerhead sea turtle.

MATERIALS AND METHODS

Turtles

Forty-eight free-ranging, juvenile loggerhead sea turtles with straight carapace lengths (SCL) between 46 cm and 77 cm were collected as bycatch from a pound net fishery located in Core Sound, North Carolina (between the northernmost site, 34°

52.71′ N, 76° 18.94′ W, and the southernmost site, 34° 49.68′ N, 76° 22.95′ W) during two summer sampling periods (July 31 to August 11, 2000 and July 13 to 20, 2001). All turtles were measured for SCL from nuchal notch to the posterior marginal notch using forestry calipers, and most were weighed to the nearest 0.1 kg. Laparoscopy was performed to determine sex of 42 of the turtles. For the few that could not undergo this procedure due to size limitations or illness, sex was determined by plasma testosterone concentrations (Owens 1997).

Three turtles captured in the pound net fishery had obvious signs of impaired health. One impaired turtle (turtle ID 1328) was captured on July 16, 2001 and was one of the 48 turtles described above. The other two impaired turtles were captured outside of this sample set. They were captured on Nov. 29, 1999 (turtle ID 2-24) and May 20, 2002 (turtle ID 5-9) when water temperatures were low (17 ^{o}C and 15.8 ^{o}C, respectively). All three impaired turtles were extremely emaciated and lethargic and were not actively swimming upon capture. The two turtles captured on cooler days (ID's 2-24 and 5-9) were not likely hypothermic, because the other turtles captured on the same days did not show the typical signs of hypothermia (cold-stunning) which are floating at the surface and lethargy. None of them had signs of recent external injuries that may have contributed to their condition; therefore, their illnesses were categorized as unknown internal infections. These turtles were sent for treatment to the Karen Beasley Sea Turtle Rescue and Rehabilitation Center in Topsail Island, NC, and were subsequently released.

Tissue Collection

Blood samples were collected from the dorsocervical sinus of each turtle. Biopsies of subcutaneous fat were collected surgically from the left inguinal region of 44 turtles captured in the summer sampling periods for contaminant analysis as described elsewhere (Keller *et al* in prep a). Following an observation period during which recovery of the animals was monitored, the turtles were released near their capture location. Fat biopsies were not taken from turtle ID's 2-24 and 5-9.

General Health Assessment

Turtles were examined for external injuries and obvious signs of illness (i.e.; emaciation, lethargy). Body condition was calculated as turtle mass (kg) divided by the cubed SCL (cm) and multiplied by 100,000 [$kg/SCL^3 \times 100{,}000$] as described by Bjorndal *et al*. (2000). Blood samples for hematology and plasma chemistry were collected within 15 min of capture, were kept cool on ice or in a refrigerator, and were processed within 6 hours of blood collection unless otherwise stated. To maximize consistency, hematology was performed by a single technician familiar with sea turtle hematology, and a single reference laboratory was used for plasma chemistries.

Hematology

Hematological examination was performed on 14 of the 21 turtles captured in the summer of 2000. Blood was stored in sodium heparin tubes (Monoject®, Sherwood Medical, St. Louis, MO) for a complete blood cell count. Natt-Herrick solution and Neubauer counting chambers (American Optical Corp., Scientific Instrument Div., Buffalo, NY) were used to obtain total WBC counts. Differential

counts were performed on 13 of the turtles using Wright-Geimsa-stained thin blood smears. Concentrations of heterophils, lymphocytes, monocytes, eosinophils, azurophils, basophils, and RBCs were determined (Table 4.1). The hetophil:lymphocyte ratio was calculated using the concentrations of these cell types. The granulocyte:lymphocyte ratio was calculated as the sum of the concentrations of heterophils, eosinophils, azurophils, and basophils divided by the concentration of lymphocytes. Total WBCs were estimated using blood smears for 20 of the turtles captured in the summer of 2001. HCT and hemoglobin (HGB) concentrations were determined on 14 of the turtles from year 2000. HCT was measured using hematocrit tubes and a hematocrit centrifuge. HGB was determined colorimetrically by the cyanmethemoglobin method following red cell lysis.

Plasma chemistry

Blood collected from 14 of the 21 turtles captured in 2000 was stored in sodium heparin tubes (Monoject®, Sherwood Medical, St. Louis, MO). Tubes were centrifuged, and 300 ul plasma was placed in polyethylene cryogenic vials (Nalgene® Cryoware™, Nalge Company, Rochester, NY) and stored at -70 °C until plasma chemistry analysis was completed within one week.

Blood from 26 of the 27 turtles captured in the summer of 2001, including one impaired turtle (ID 1328), was collected directly into blood collection tubes containing sodium heparin (Vacutainer®, Becton, Dickinson, and Co., Franklin Lakes, NJ). More than 1 mL of plasma was transferred to snap cap polyethylene tubes and stored at -80 ^{o}C until plasma chemistry analysis was completed within 10 days.

Plasma chemistries were analyzed from the other two impaired turtles (ID's 2-24 and 5-9). Blood was collected immediately upon capturing ID 2-24 and within one hour of capturing ID 5-9. Blood was stored in blood collection tubes containing sodium heparin (Vacutainer®, Becton, Dickinson, and Co., Franklin Lakes, NJ). More than 1 mL of plasma was transferred to snap cap polyethylene tubes and stored at -80 ^{o}C until plasma chemistry analysis was completed within 39 months for ID 2-29 and within 9 months for ID 5-9.

Plasma chemistry examinations were completed using an automated clinical analyzer (Roche Diagnostic Hitachi 912, Indianapolis, IN). This measured glucose, total protein, albumin, globulin, BUN, uric acid, creatinine, bilirubin, AST, ALP, lactate dehydrogenase (LDH), creatine phosphokinase (CPK), gamma glutamyltransferase (GGT), calcium (Ca), phosphorus (P), sodium (Na), potassium (K), chloride (Cl), and magnesium (Mg). Plasma osmolality was measured on a 5100B Vapor Pressure Osmometer (Wescor, Inc., Logan, UT) using plasma collected from blood that was drawn within 5 hours of capture from the turtles in 2000 and within 15 min of capture from turtles in 2001. See Table 4.2 for a list of these parameters.

Contaminant Analysis

PCB and OC pesticide concentrations and lipid content were previously determined in whole blood samples and fat biopsies of 44 turtles captured in the summers of 2000 and 2001, including turtle ID 1328 (Keller *et al*. in prep a). An additional 4 whole blood samples from healthy turtles captured in July 2001 and blood

from the other two impaired turtles (ID's 2-24 and 5-9) were analyzed using identical methods.

Statistics

All statistical analyses were performed using Systat 8.0 software (SPSS, Inc, Chicago, IL). The OC concentrations were not normally distributed even after log-transformation, therefore non-parametric tests were used. The health assessment data were compared to lipid-normalized contaminant concentrations (ng/g of lipid in tissue) using the Spearman Rank Correlation Test. Correlation analyses between OC levels and plasma chemistry values used all available data except data for the impaired turtles captured in the cooler months (ID's 2-24 and 5-9). Analyses between OC levels and body condition indices used all turtles, including ID's 2-24 and 5-9. When a compound was below the analytical limit of detection (LOD), the concentration for that sample was set at half the LOD. However, when classes of OCs were summed, values below the LOD were set at zero. Differences in lipid-normalized OC concentrations between apparently healthy and impaired turtles were determined using the Mann-Whitney test. The Mann-Whitney test was also used to compare morphometric and health data between years.

RESULTS

The morphometric and health assessment data, including body condition, hematology, and plasma chemistry values are presented in Tables 4.1 and 4.2. Most of the turtles in this study appeared healthy upon initial external exam. All, but the

three impaired turtles, were active and swimming normally. Only minor and common external wounds, such as bruising and scute erosions were observed with the exception of one animal with a major puncture wound in the throat. One apparently healthy turtle died following the laparoscopic procedure. Subsequent histopathological examination showed that this turtle had extensive parasitic spirorchid trematode egg mass granulomas in its brain, thyroid, and adrenals.

All three impaired turtles exhibited signs of wasting, including extreme emaciation and lethargy. Their neck, shoulder, and inguinal regions were profoundly depressed, and the plastron of turtle ID 5-9 was depressed. Their body condition indices showed that they were below the mean nutritional status of this population of turtles (Table 4.1). They were not actively swimming upon capture (nor were they floating) and only moved slightly to breathe once aboard the boat. Turtle ID 5-9 could not maintain equilibrium in the water upon capture. The HCT values for turtle ID's 2-24 and 1328 were low (Table 4.1), which is a reliable indicator of poor health in sea turtles (Harms *et al*. 2002). They also exhibited lower concentrations of glucose, protein, globulin, and BUN and lower LDH activity compared to the means of the healthy turtles (Table 4.2). Uric acid concentrations and ALP activity were lower in turtle ID's 2-24 and 1328 than the means of the healthy turtles. Two of the impaired turtles (ID's 2-24 and 5-9) exhibited lower electrolyte (Ca, Na, K, Cl, and Mg) concentrations and anion gap compared to the means of the healthy turtles (Table 4.2).

Few statistically significant differences were observed between years for health indicators. Turtle length, weight, and WBC counts were not significantly different between years (Table 4.1). Body condition was slightly higher in 2001 than in 2000,

and this difference was statistically significant (Mann-Whitney test; p-value = 0.042). Few significant differences were observed between years for the plasma chemistry values (Table 4.2). Glucose concentrations and osmolality were higher, while uric acid, ALP, LDH, and anion gap were lower in year 2001 compared to 2000.

Several significant correlations were observed between contaminant concentrations and indicators of poor or altered health. The Spearman rank correlation coefficients are presented for only those health indicators that significantly correlated with more than one contaminant (Tables 4.3 to 4.7; see Appendix Fig. A1 for plots). 4,4'-DDE, total DDTs, total OCs, and some other OC compounds in both blood and adipose positively correlated to total WBCs and eosinophils (Table 4.3). Increasing blood concentrations of total chlordanes, mirex, and penta-chlorinated PCBs were significantly correlated to fewer lymphocytes. Increasing adipose concentrations of mirex and some classes of PCBs correlated to an elevation in the ratio of heterophils to lymphocytes as well as the granulocyte:lymphocyte ratio. The granulocyte:lymphocyte ratio was also positively correlated with adipose concentrations of total chlordanes and additional PCB classes. Blood concentrations of certain OC compounds negatively correlated to RBC counts, HCT, and HGB (Table 4.4).

Indicators of nutritional status and homeostatisis of proteins and glucose were significantly correlated to certain contaminants (Table 4.5). Body condition correlated positively to adipose concentrations of minor PCB classes, and negatively to total chlordanes and dieldrin in the blood. Glucose concentrations were negatively correlated to adipose concentrations of dieldrin, 4,4'-DDE, and total DDTs. Total

protein concentrations were positively correlated to adipose concentrations of four classes of PCB congeners. The ratio of albumin to globulin was negatively correlated to levels of total chlordanes in blood and adipose and to blood levels of heptachlor epoxide and PCB classes. BUN concentrations were positively correlated to levels of most OC classes measured in blood and to tetra-ortho chlorinated PCBs in adipose.

Activities of two enzymes correlated to OC concentrations (Table 4.6). AST activity was positively correlated to most of the OC compounds in the blood and adipose. Significant negative correlations were observed between ALP activity and many OC compounds in blood and adipose.

Few significant correlations were noted between electrolyte levels and contaminant concentrations (Table 4.7). However, plasma osmolality and sodium concentrations were positively correlated to certain OCs in the blood. Magnesium concentrations were negatively correlated to most of the OC classes in blood.

Contaminant concentrations detected in the blood and fat biopsies from the 44 turtles were reported elsewhere, and the concentrations in the two tissues were correlated to each other (Keller *et al*. in prep a). It should also be noted that almost all of the contaminants measured in the turtle tissues were inter-correlated. For example, adipose concentrations of total PCBs were significantly correlated to total DDTs (R_s = 0.679), to oxychlordane (R_s = 0.720), to *trans*-nonachlor (R_s = 0.716), and to mirex (R_s = 0.710) concentrations (all p-values < 0.05; data not shown). These inter-correlations of complex mixtures makes it difficult to discern which compound may be responsible for possible health effects.

The blood OC concentrations in the healthy turtles (N = 47) were compared to the impaired turtles (N = 3) in Figure 4.1. The ill turtles had two orders of magnitude greater concentrations of all OC contaminants than the healthy turtles. The fat biopsy from turtle ID 1328 had the highest lipid-normalized concentrations of OCs (18,800 ng/g total PCBs; 3,280 ng/g total DDTs; 2,620 ng/g total chlordanes; 25,000 ng/g total OCs) among all 44 fat biopsies which had mean concentrations of 2,010 ng/g total PCBs, 452 ng/g DDT, 246 ng/g chlordanes, and 2,810 ng/g total OCs.

DISCUSSION

Even though sea turtles appear to accumulate relatively low OC concentrations compared to other wildlife, we observed significant correlations between OCs and indicators of health. Preliminary data from an ongoing and parallel study corroborate these findings (Peden-Adams *et al*. 2002). Blood samples from juvenile loggerhead sea turtles captured in offshore waters of South Carolina, Georgia, and Florida were analyzed for OC contaminants and plasma chemistries. In that study, significant correlations were observed between total PCB concentrations and increased AST activity, increased concentrations of BUN, total protein, and globulin levels, and decreased albumin:globulin ratio. Similar correlations were seen in the current study, although not necessarily to total PCB concentrations.

These findings, although only correlative, are supported by a large number of previous experimental studies that are explained in more detail below. Environmental studies have also observed similar correlations in other species. Therefore, it is plausible that these correlations suggest that OCs may modulate the immune system,

affect protein, glucose, and ion homeostatsis, and cause organ damage in loggerhead sea turtles.

Increasing OC concentrations correlated with changes in the composition of circulating immune cells, specifically with increases in WBCs and the ratio of heterophils to lymphocytes. An increase in this ratio is known to be an indicator of general stress in birds (Gross and Seigel 1983) and of disease in sea turtles (Aguirre *et al*. 1995). Green sea turtles with FP exhibited an 6-fold increase in this ratio (Work *et al*. 2001) as well as suppressed immune functions (Cray *et al*. 2001; Work *et al*. 2001).

Previous experimental and correlative studies have shown that OCs affect immune cells in birds, reptiles, and mammals. Correlations were seen between OC concentrations and an elevation in the heterophil to lymphocyte ratio in herring gulls and Caspian terns from the Great Lakes (Grasman *et al*. 1996; Grasman *et al*. 2000a). The terns also showed evidence of suppressed immune functions as measured by the PHA-skin test (Grasman *et al*. 1996; Grasman and Fox 2001). Male American kestrels (*Falco sparverius*) experimentally exposed to PCBs exhibited increased WBCs and lymphoproliferative (LP) responses (Smits *et al*. 2002). Alligators from Lake Apopka, which had higher concentrations of DDE in their blood compared to reference lakes (Guillette *et al*. 1999), showed decreased cell counts in bone marrow, thymus, and spleen (Gross *et al*. 1996), altered thymus and spleen histology (Rooney *et al*. in press), enhanced LP responses (Rooney 1998), and reduced antibody titers to sheep red blood cells (SRBCs; Gross *et al*. 1997). In addition to their disrupted endocrine functions, this immunosuppression may have reduced their disease

resistance and contributed to the alligator population declines seen in this lake (Gross *et al*. 1996).

Similar effects on WBCs and immune functions have been shown in mammals. Rats exposed to acute concentrations of Aroclor 1254 exhibited decreased lymphocytes and a large increase in neutrophils (Bruckner *et al*. 1973, 1974). White-footed mice that were experimentally exposed to a mixture of PCBs exhibited reduced numbers of thymocytes and modulated immune functions (Segre *et al* 2002; Wu *et al*. 1999). Aroclor 1254 exposure in monkeys resulted in alterations in the ratio of T-helper to T-suppressor cells and suppression of antibody production to SRBCs (Arnold *et al*. 1993). In addition, humans with higher blood PCB concentrations exhibited altered WBC counts as well as altered immune functions (Lu and Wu 1985; Lawton *et al*. 1985; Daniel *et al*. 2001). These previous studies link changes in WBC counts to altered immune function and clearly show that OCs can affect WBC counts. Therefore, it is plausible that the correlations seen in the current study indicate modulation of the loggerhead immune system by OC contaminants. Additional evidence of this is provided by significant and positive correlations between LP responses and OC concentrations in these same loggerhead turtles (Keller *et al*. in prep b).

Indicators of anemia, such as lower RBC counts, HCT, and HGB concentrations, correlated to some OC compounds in loggerhead blood. Previous studies have shown that OC contaminants can decrease these parameters. Rats and monkeys exposed to PCBs exhibited decreased RBC counts, HGB, and HCT (Rice 1999; Chu *et al*. 1994; Bruckner *et al*. 1973; Arnold *et al*. 1993). Blood

concentrations of PCBs in capacitor workers correlated to decreased RBC counts (Lawton *et al*. 1985). In adult herring gulls from the Great Lakes, TEQs and DDE concentrations were also correlated to decreased HCT (Grasman *et al*. 2000a).

The liver is a major site for protein, glucose, and lipid regulation. OC contaminants are known to alter the activity of hepatic enzymes that are responsible for these regulatory functions (Boll *et al*. 1998; Lorenzen *et al*. 1999), and result in changes in blood concentrations of protein and glucose (McConnell 1985). Increased total plasma protein concentrations, as seen in the turtles, have been observed in rats exposed to PCB 126 (Rice 1999), capacitor workers that were occupationally-exposed to PCBs (Lawton *et al*. 1985), and mice exposed to chlordane (Kasawinah and Grutsch 1989). PCBs can also alter albumin and globulin levels (McConnell 1985). The turtles that had higher concentrations of OCs exhibited a decreased ratio of albumin to globulin. This response was previously observed in fish exposed to Aroclor 1254 (Camp *et al*. 1974), and decreased albumin was observed in white pelicans experimentally exposed to Aroclor 1254 (Greichus *et al*. 1975). In addition, changes in these protein classes have been correlated to PCB and DDE concentrations in Caspian tern and herring gull chicks from the Great Lakes (Grasman *et al*. 2000b).

BUN is typically monitored in mammals as an indicator of kidney dysfunction. Increased BUN concentrations suggests that the kidneys are not properly removing this nitrogenous waste product from the blood. The kidneys are a well known target for the toxic effects of PCBs (McConnell 1985). Increased BUN concentrations has been observed in rats exposed to PCB 126 (Rice 1999), capacitor workers (Lawton *et al*. 1985), and cynomolgus monkeys exposed to Aroclor 1254 (Arnold *et al*. 1990). In

turtles, however, BUN is a poor indicator of renal disease (Campbell 1996) and probably better represents nutritional status and protein metabolism. For example, BUN concentrations increased from a median of 50 mg/dL to 122 mg/dL as injured or ill loggerhead sea turtles were rehabilitated (Harms *et al*. 2002). The positive correlation between BUN and OC concentrations in the loggerhead turtles may suggest that turtles with higher BUN concentrations have been feeding recently and likely have higher levels of blood lipids which can transport the lipophilic contaminants. The fact that BUN was correlated strongly with OCs primarily measured in blood rather than in adipose further supports this conclusion. Future studies are needed to investigate the relationships between BUN, protein metabolism, blood lipids, and OC contaminants in sea turtles.

The negative correlations between glucose and OC concentrations observed in the loggerhead turtles are supported by a large number of previous studies. PCB and mirex exposure in rats (Boll *et al*. 1998; Chu *et al*. 1994; Rogers *et al*. 1984), chlordane exposure in mice (Khasawinah and Grutsch 1989), and exposure of quails to a metabolite of DDT (Westlake *et al*. 1979) resulted in decreased blood glucose concentrations. Glucose concentrations are related to nutritional status as shown in loggerhead sea turtles during an oil exposure experiment. Both control and oil-exposed turtles exhibited decreased glucose during the experiment when they were not fed, but the concentrations increased upon feeding (Lutcavage *et al*. 1995). However, the turtles exposed to oil took longer to recover than the control turtles, suggesting that the oil altered their physiological regulation of glucose. Blood glucose concentrations are tightly regulated by the liver and its complex interactions with the hypothalamus,

pituitary, and adrenals. Factors released from the hypothalamus trigger the pituitary to release adrenocorticotropic hormone (ACTH). ACTH then activates the adrenals to produce glucocorticoids, such as corticosterone, which stimulate hepatic intermediary metabolic enzymes, such as the gluconeogenic enzymes (PEPCK and FdPase) that are responsible for the production of glucose. Glucose is then released from the liver into the blood. OCs may interfere with any step in this pathway. PCBs are known to decrease the activity of PEPCK and FdPase in the liver of rats, which may explain their lower blood glucose concentrations (Boll *et al*. 1998). In herring gull embryos, higher PCB concentrations were correlated to decreased levels of corticosterone (Lorenzen *et al*. 1999). This decrease may explain their lower hepatic PEPCK and malic enzyme activities. However, more research would be required to conclusively determine a causal relationship and to explain the mechanism of OC-altered glucose levels in sea turtles.

Enzymes that are distributed in particular organs, such as AST, ALP, CPK, LDH, and GGT, leak into the blood upon cellular damage of that organ. In this way, blood enzyme activities have been useful as early warning monitors of subacute effects of contaminants in birds and mammals (Dieter 1974; Arnold *et al*. 1990; Feeley 1995). AST activity was elevated and ALP activity was decreased in the loggerhead sea turtles with higher concentrations of certain OCs. Increased AST is commonly used as an indicator of liver damage in birds and mammals. AST activity was increased in PCB-exposed humans (Feeley 1995), monkeys (Arnold *et al*. 1990), rats (Haag-Grönlund *et al*. 1997; Bruckner *et al*. 1973), and birds, including starlings, and canvasback ducks (Dieter 1975; Dieter *et al*. 1976). Quail exposed to DDE or

another DDT metabolite also exhibited increased AST activity, as well as mice exposed to chlordane (Dieter 1974; Westlake *et al*. 1979; Khasawinah and Grutsch 1989). American kestrels exhibited an increase in AST activity and a decrease in ALP activity following PCB exposure (Hoffman *et al*. 1996), a response identical to the correlations seen in the loggerhead turtles.

It is difficult to interpret the observed correlations between OC levels and plasma enzyme activities, because no previous studies have determined the distribution of these enzymes among organs of sea turtles. Actually, this research has only been carried out using two reptile species, the yellow rat snake (*Elaphe obsolete quadrivitatta*; Ramsay and Dotson 1995) and the green iguana (Wagner and Wetzel 1999). AST and LDH were the major enzymes found in the liver of the snake, but AST was also found in the kidney and heart at concentrations higher than the liver. ALP was identified primarily in the kidney of the snake. Moderate AST activity was found in all tissues examined in the iguana; therefore, the authors concluded that an increase in blood AST in this species may not reflect damage to a specific tissue. Based on the preponderance of experimental evidence showing that OCs produce liver damage and subsequently increase AST in blood of mammals and birds, it is plausible that the strong correlations between AST and OCs are indicative of liver damage in sea turtles. This interpretation is further supported by the lack of correlations between OC concentrations and CPK activity, because liver damage is expected to result in an increase in AST but no increase in CPK activity (Campbell 1996). More research is needed before this interpretation can be considered conclusive.

A few correlations were seen in the current study between OC contaminants and blood electrolyte concentrations. OC concentrations were correlated to increased plasma osmolality, increased Na concentrations, and decreased Mg concentrations. In fish, chlordane exposure has been shown to cause an increase in Na and Mg concentrations (Bansel *et al*. 1979). Capacitor workers similarly exhibited blood osmolality values above the normal range (Lawton *et al*. 1985). Birds from the most contaminated sites along the Great Lakes exhibited decreases in Mg, P, and albumin concentrations, a combination that indicates possible kidney damage (Fox, personal communication). Two of these parameters, Mg and albumin, were negatively correlated with some OCs in the loggerhead. The kidney is responsible for ion regulation in most species, but the salt gland is also very important in sea turtles. This gland concentrates ions from the blood and excretes the resulting fluid through ducts near the eye.

The salt gland concentrates sodium 8-fold and magnesium 45-fold above the concentrations in plasma (Vargo *et al*. 1986). In this way, sea turtles can ingest saltwater and still maintain water and ion balance. Furthermore, this gland has been shown to be sensitive to the effects of oil (Vargo *et al*. 1986). In fact, exposure to a 0.5 cm layer of oil resulted in complete failure of the salt gland to produce fluid. If the kidneys or the salt gland of sea turtles are sensitive to OCs, as has been shown in kidneys of other species (McConnell 1985), then these correlations could suggest that OCs are affecting these organs thereby altering ion regulation in sea turtles.

Wasting syndrome, which can result from a combination of the endpoints discussed above, has been described as a loss of body weight and a decrease in food

intake, usually followed by death. This syndrome has been observed in experimental animals exposed to dioxins (Safe 1993) and in fish-eating birds and mink from the Great Lakes that were environmentally exposed to high concentrations of PCBs and DDT (Fox 2001). Three turtles captured in Core Sound, NC exhibited signs of severe wasting (i.e.; severe emaciation and lethargy). Turtles in this condition often do not recovery (MA Stamper; pers. observation).

Many plasma chemistry parameters and the HCT values of the impaired turtles fell outside the normal range defined by the apparently healthy turtles. The comparison between the healthy turtles and impaired turtle ID 1328 is straight forward, because they were all captured in the summer. Interpretation is more difficult for impaired turtle ID's 2-24 and 5-9, because they were captured during cooler months and seasonal changes are known to influence some blood chemistry parameters (Lutz and Dunbar-Cooper 1987). The authors showed that osmolality and concentrations of Na and K were shown to decrease during cooler months in loggerhead turtles from Florida. A normal seasonal change, rather than illness, may explain why turtle ID's 2-24 and 5-9 had Na concentrations below the normal summer range and low K concentrations compared to the summer means. Concentrations of Ca, Cl, Mg, and glucose and HCT were lower in the impaired turtles compared to the summer healthy turtles. These indicators were found to be seasonally constant by Lutz and Dunbar-Cooper (1987), therefore the illness, rather than seasonal influences, was likely the cause for the lower values observed in the impaired turtles.

The blood OC concentrations in these impaired turtles were two orders of magnitude higher than those in the healthy turtles. In studies on other species,

mobilization of lipid stores has been shown to increase blood concentrations of these contaminants (Lydersen *et al*. 2002). It is possible that the high concentrations observed in the emaciated turtles are due to recent lipid mobilization. However, our previous study offers two pieces of evidence that suggest that this is not the case for these turtles. Firstly, the OC concentrations in the blood of 44 loggerhead and 9 Kemp's ridley sea turtles significantly correlated to concentrations measured in adipose tissue suggesting that concentrations in the blood represent those in the fat (Keller *et al*. in prep a). Second and most convincing, turtle ID 1328, the only impaired turtle for which we had a fat biopsy, had the highest lipid-normalized adipose concentrations of all of the OC contaminants among the 44 biopsied loggerheads. In fact, the adipose concentration of total OCs in this turtle was nearly one order of magnitude higher than the mean for all 44 turtles.

It is not known whether the impaired turtles accumulated the high concentrations of OC contaminants prior to or after becoming ill. Illness may have forced them to feed on different prey items that contained higher OC concentrations and/or forced them into marginal feeding areas that were more contaminated. However, the high OC concentrations observed in the impaired turtles were greatly different from those in the healthy turtles, suggesting that OC accumulation occurred over a long time period prior to becoming ill. It is unlikely that a sick turtle with a presumably lower feeding rate could accumulate these concentrations during an illness. It is more likely that these three turtles were feeding in contaminated areas prior to their illness. Juvenile loggerhead turtles show strong site fidelity. They return to preferred habitat when experimentally displaced or after winter migrations (Avens

et al. in press). Future studies should track rehabilitated turtles that contain such high OC concentrations to determine their habitat preferences.

A comparison of the OC concentrations in these impaired loggerhead turtles to other wildlife reveals that the loggerhead sea turtle may be more sensitive to the effects of OC contaminants than previously thought. The fat biopsy of turtle ID 1328, an impaired turtle, had PCB concentrations of 18.8 ug/g lipid, which was lower than those measured in adipose tissue of apparently healthy snapping turtles (Pagano *et al*. 1999; Albers *et al*. 1986; Helwig and Hora 1983; Stone *et al*. 1980). The snapping turtles from New Jersey and Maryland accumulated mean total PCB concentrations of 23.55 to 291.13 ug/g lipid depending on the site (Albers *et al*. 1986). Although differences in OC concentrations were detected among the sites, blood chemistry values did not indicate any physiological impairment at any site. Furthermore, PCB concentrations in blubber of healthy marine mammals (mean for harbor seals = 32.7 ug/g lipid; for striped dolphins = ~300 ug/g lipid) were higher than those measured in this impaired loggerhead turtle (Hall *et al*. 1992; Aguilar and Borrell 1994).

In blood, the mean wet weight total PCB concentrations of the impaired loggerhead turtles (127 ng/g total PCBs; 14.9 ng/g total DDTs) were much lower than concentrations in apparently healthy bottlenose dolphins from Sarasota, Florida (402 ng/g total PCBs and 237 ng/g DDE; Lahvis *et al*. 1995). The impaired turtles had higher PCB levels than those reported for female alligator serum from Lake Apopka (2.4 ng/g total PCBs; 17.98 ng/g DDE; Guillette *et al*. 1999), but the turtles had lower DDE concentrations. The blood PCB concentrations of the impaired loggerheads were lower than in snapping turtle plasma from the most contaminated sites of the

Great Lakes (414.8 ng/g total PCBs; 10.1 ng/g DDE), but the loggerhead PCB and DDE concentrations were greater than snapping turtles from references sites (18.2 ng/g total PCBs; 0.2 ng/g DDE; de Solla *et al*. 1998). Endocrine related developmental effects have been observed in the alligators of Lake Apopka and in the snapping turtles from the most contaminated sites of the Great Lakes (Guillette *et al*. 1999; de Solla *et al*. 1998). However, neither of these studies report observations of severe wasting as was seen in the three juvenile loggerhead turtles. If OC contaminants contributed to the cause of this illness, then these comparisons suggest that other wildlife may be more resistant to the health effects of OCs, while sea turtles may be more sensitive at lower concentrations.

In conclusion, this study provides the first evidence, although strictly correlative at this point, that OC contaminants may be affecting sea turtle health. The concentrations of OCs are relatively low in sea turtles, yet we observed significant and plausible correlations between OC levels and health indicators for a wide variety of biological functions, including immunity and homeostasis of proteins, carbohydrates, and ions. The turtles exhibiting signs of wasting had two orders of magnitude higher blood OC concentrations than the apparently healthy turtles. However, these impaired turtles still had concentrations of OCs that were lower than healthy populations of other wildlife species. These comparisons indicate that sea turtles may be more sensitive to OC contaminants than previously suspected. Studies using experimentally- and environmentally-exposed animals support the correlative findings of this current study. Further work will be required to determine the precise causal relationships between OC contaminants and health effects in sea turtles. Additional

populations, such as those exposed to higher levels of OCs, and more sensitive life stages (i.e.; embryo) might also be investigated as they may face a greater risk than juvenile turtles foraging in North Carolina.

Table 4.1. Morphometrics and hematology for juvenile loggerhead sea turtles from Core Sound, NC.

	Summer 2000			Summer 2001[a]				Impaired turtle ID's		
	N	Mean (SD)	Range	N	Mean (SD)	Range	p-value[b]	2-24	1328	5-9
General										
Dates			Jul. 31 - Aug. 11			Jul. 13 - Jul. 20		Nov. 29, 1999	Jul. 16, 2001	May 20, 2002
Water temperature (^{o}C)			26.1 - 28.1			24 - 28.2		17.0	26.6	15.8
SCL (cm)[c]	21	60.9 (7.9)	45.7 - 74.0	26	62.8 (6.5)	49.0 - 77.3	0.716	61.3	64.7	82.6
Weight (kg)	21	34.1 (11.8)	14.4 - 56.6	23	36.4 (9.3)	16.0 - 52.6	0.664	32.8	32.4	65.0
Body Condition[c]	21	14.6 (1.8)	11.4 - 20.9	23	15.1 (1.1)	13.5 - 18.1	0.042	14.2	12.0	11.5
Sex ratio (F:M)	21	15:6		26	18:8			Unknown	F by testosterone	F by tail length
Male Testosterone (pg/ml)	6	111.0 (53.7)	37.2 - 232	8	78.7 (22.1)	44.5 - 96.9	nt	14.5		
Female Testosterone (pg/ml)	15	6.7 (4.0)	0.67 - 12.8	18	5.7 (2.2)	2.7 - 10.5	nt		3.3	
Hematology										
Red blood cells (RBC; 10^{6}/ul)	14	0.410 (0.098)	0.275 - 0.615							
Hemoglobin (HGB; g/dL)	14	9.82 (1.46)	7 - 12							
Hematocrit (HCT; %)	14	31.5 (4.3)	23 - 38					8	22	
White blood cells (WBC)[d]	14	14.8 (4.0)	5.8 - 20.72	19	13.4 (6.4)	0 - 25.5	0.489		13.6	
Heterophils[d]	13	4.3 (2.5)	1.3 - 8.2							
Lymphocytes[d]	13	9.5 (2.5)	4.6 - 15.0							
Monocytes[d]	13	nd[f]	nd							
Eosinophils[d]	13	1.1 (0.9)	0.14 - 2.7							
Azurophils[d]	13	0.8 (0.4)	0.17 - 1.5							
Basophils[d]	13	0.03 (0.11)	0 - 0.38							
Heterophils:Lymphocytes[d]	13	0.5 (0.4)	0.1 - 1.4							
Granulocytes:Lymphocytes[e]	13	0.74 (0.45)	0.25 - 1.71							

[a] Summer 2001 values do not include turtle ID 1328.
[b] Comparison between year 2000 and 2001 using Mann-Whitney t-tests; nt = not tested.
[c] SCL = straight carapace length; body condition = kg/cm^{3}*100,000.
[d] WBC counts in year 2000 were performed using Natt-Herrick solution, whereas counts in 2001 were estimated from blood smears. Differential counts were performed using blood smears, units = 10^{3}/ul of blood.
[e] Granulocytes:Lymphocytes = (heterophils + eosinophils + azurophils + basophils)/lymphocytes.
[f] nd = none detected.

Table 4.2. Plasma chemistries for juvenile loggerhead sea turtles from Core Sound, NC.

	Summer 2000			Summer 2001[a]				Impaired turtle ID's		
	N	Mean (SD)	Range	N	Mean (SD)	Range	p-value[b]	2-24	1328	5-9
Carbohydrate and protein homeostasis										
Glucose (mg/dL)	14	101 (17)	76 - 143	25	114 (18)	82 - 143	0.041	78	93	55
Protein (g/dL)	14	4.0 (0.7)	2.8 - 5.2	25	4.1 (0.7)	2.8 - 5.9	0.725	2.6	2.4	3.3
Albumin (g/dL)	14	1.1 (0.2)	< 1.0 - 1.4	25	1.1 (0.2)	< 1.0 - 1.5	0.753	< 1.0	< 1.0	< 1.0
Globulin (g/dL)	14	2.9 (0.6)	1.9 - 4.1	25	3.0 (0.6)	1.9 - 4.5	0.509	1.7	1.5	2.4
Albumin:Globulin	14	0.41 (0.08)	0.24 - 0.50	25	0.39 (0.07)	0.23 - 0.60	0.428	0.5	0.6	0.4
Urea nitrogen (BUN; mg/dL)	14	88 (38)	25 - 150	25	108 (40)	43 - 197	0.208	70	83	66
Uric acid (mg/dL)	14	1.1 (0.8)	0.4 - 3.4	25	0.7 (0.6)	0.3 - 2.7	0.045	0.4	0.4	1.4
Creatinine (mg/dL)	14	< 0.1	< 0.1 - 0.1	25	< 0.1	< 0.1 - 0.1	nt	< 0.1	< 0.1	< 0.1
Bilirubin (mg/dL)	14	< 0.1	0 - 0.2	25	< 0.1	< 0.1 - 0.2	nt	0.1	0.2	< 0.1
Enzymes[c]										
AST (U/L)	14	209 (69)	128 - 355	25	240 (52)	157 - 342	0.081	179	234	251
ALP (U/L)	14	31 (20)	12 - 74	25	18 (7)	9 - 36	0.037	3	14	27
LDH (U/L)	14	248 (132)	77 - 465	25	150 (59)	60 - 310	0.025	25	84	55
CPK (U/L)				25	1263 (1187)	281 - 5667	nt	263	737	5934
GGT (U/L)	14	< 3	< 3	25	< 3	< 3 - 4	nt	< 3	< 3	< 3
Ions										
Osmolality (mOsm)	21	334 (92)	234 - 696	25	350 (16)	320 - 380	0.003		335	
Calcium (mg/dL)	14	8.9 (1.2)	7.5 - 11.4	25	8.1 (1.1)	5.5 - 9.9	0.147	6.5	7.7	7.0
Phosphorous (mg/dL)	14	6.9 (0.9)	5.6 - 8.5	25	7.1 (0.9)	5.6 - 9.1	0.660	4.4	5.2	7.6
Ca:P	14	1.3 (0.3)	0.9 - 2.0	25	1.2 (0.2)	0.7 - 1.6	0.101	1.48	1.48	0.92
Sodium (mmol/L)	14	158 (3)	154 - 164	25	159 (2)	154 - 162	0.636	142	156	149
Potassium (mmol/L)	14	4.4 (0.5)	3.1 - 5.6	25	4.5 (0.4)	3.8 - 5.5	0.426	3.6	4.2	3.2
Na:K	14	36.3 (4.8)	28.2 - 50	25	35.2 (3.1)	28.5 - 42.1	0.482	39.7	37.1	46.6
Chloride (mmol/L)	14	118 (4)	111 - 125	25	116 (4)	110 - 122	0.283	104	115	109
Magnesium (mg/dL)	14	5.2 (0.7)	3.9 - 6.5	25	5.3 (0.7)	4.0 - 6.7	0.587	3.9	4.8	4.7
Anion gap	14	19.4 (5.4)	12.6 - 30.2	25	14.7 (4.6)	6.4 - 23.6	0.010	8.6	6.2	10.2

[a] Summer 2001 samples do not include impaired turtle ID 1328.

[b] Comparison between year 2000 and 2001 using Mann-Whitney t-tests; nt = not tested.

[c] AST aspartate aminotransferase; ALP alkaline phosphatase; LDH lactate dehydrogenase; CPK creatine phosphokinase; GGT gamma glutamyl transferase.

Table 4.3. Spearman Rank Correlation coefficients between OC concentrations and white blood cell counts in loggerhead sea turtles.

	Total WBC		Eosinophils		Lymphocytes		H:L Ratio[a]		G:L Ratio[a]	
	Adipose	Blood	Adipose	Blood	Adipose	Blood	Adipose	Blood	Adipose	Blood
N	31	34	13	13	13	13	13	13	13	13
heptachlor epoxide	0.109	0.083	0.391	0.071	-0.151	-0.132	0.348	-0.079	0.523	0.005
Total chlordanes[b]	0.338	0.216	0.308	0.511	-0.214	**-0.577***	0.421	0.269	**0.566***	0.407
dieldrin	0.242	0.135	0.423	0.088	-0.148	-0.192	0.269	0.382	0.451	0.418
mirex	0.225	0.152	0.247	0.324	-0.379	**-0.588***	**0.606***	0.112	**0.676***	0.159
4,4'-DDE	**0.413***	**0.359***	**0.626***	**0.714***	-0.088	-0.231	0.443	0.188	0.533	0.231
Total DDTs	**0.527***	**0.343***	**0.626***	**0.707***	-0.088	-0.237	0.443	0.160	0.533	-0.169
TCDD-like PCBs[c]	0.304	0.331	0.527	0.473	-0.302	-0.538	**0.615***	0.225	**0.676***	0.302
non-TCDD-like PCBs[c]	**0.369***	0.327	0.527	0.418	-0.220	-0.412	0.484	0.198	0.538	0.225
Mono-ortho Cl PCBs	0.315	0.325	0.484	0.500	-0.231	-0.473	0.555	0.187	**0.599***	0.258
Di-ortho Cl PCBs	**0.367***	0.333	0.549	0.440	-0.225	-0.489	0.516	0.231	**0.566***	0.280
Tri-ortho Cl PCBs	0.292	0.271	0.489	0.407	-0.077	-0.313	0.412	0.093	0.440	0.115
Tetra-ortho Cl PCBs	0.290	0.230	-0.147	0.490	0.025	-0.154	0.360	-0.302	0.257	-0.221
multi-ortho Cl PCBs	0.287	0.276	0.451	0.407	-0.088	-0.313	0.445	0.093	0.467	0.115
Tri-Cl PCBs	0.253	-0.033	-0.314	0.175	0.122	-0.245	-0.009	-0.253	-0.122	-0.245
Tetra-Cl PCBs	0.349	-0.042	-0.028	0.412	0.486	-0.251	0.147	-0.209	0.062	-0.119
Penta-Cl PCBs	0.274	0.313	0.473	0.407	-0.352	**-0.604***	**0.593***	0.291	**0.643***	0.357
Hexa-Cl PCBs	**0.359***	0.318	**0.566***	0.445	-0.231	-0.451	0.522	0.220	**0.582***	0.269
Hepta-Cl PCBs	**0.519***	**0.361***	0.533	0.522	-0.170	-0.412	0.484	0.148	0.527	0.203
Octa-Cl PCBs	0.336	0.041	0.404	**0.569***	-0.162	-0.365	0.525	-0.006	0.525	0.099
Nona-Cl PCBs	0.056	0.169	0.023	0.347	-0.090	-0.445	0.520	-0.003	0.435	0.084
Deca-Cl PCBs	0.325	0.217	-0.260	0.490	-0.006	-0.154	0.410	-0.302	0.276	-0.221
Total PCBs	**0.360***	0.332	**0.599***	0.397	-0.231	-0.418	0.511	0.250	0.535	0.211
Sum OCs	**0.381***	**0.348***	**0.610***	**0.599***	-0.181	-0.451	0.478	0.220	0.544	0.291

An askerisk indicates a significant correlation; p-value < 0.05.

[a] H:L Ratio = heterophil:lymphocyte ratio; G:L Ratio = granulocyte:lymphocyte ratio.

[b] Sum of *cis*-, *trans*-chlordane, *cis*-, *trans*-nonachlor, and oxychlordane.

[c] The 12 PCB congeners identified by the EPA that have TCDD-like activity are 77, 81, 105, 114, 118, 123, 126, 156, 157, 167, 169, and 189. Only PCB 105, 118, 156, and 157 were determined and summed in this study.

Table 4.4. Spearman Rank Correlation coefficients between OC concentrations and hematology data of loggerhead sea turtles.

	RBC[a]		Hematocrit		Hemoglobin	
	Adipose	Blood	Adipose	Blood	Adipose	Blood
N	14	14	14	14	14	14
heptachlor epoxide	-0.175	-0.393	-0.370	-0.533	-0.384	**-0.545***
Total chlordanes[b]	-0.262	**-0.611***	-0.348	**-0.706***	-0.317	**-0.760***
dieldrin	-0.027	-0.529	-0.192	**-0.577***	-0.210	**-0.630***
mirex	-0.289	-0.464	-0.387	-0.412	-0.373	-0.427
4,4'-DDE	0.016	-0.218	-0.015	-0.035	-0.156	-0.125
Total DDTs	-0.039	-0.165	0.014	-0.027	-0.125	-0.113
TCDD-like PCBs[c]	-0.158	**-0.594***	-0.181	-0.374	-0.286	-0.421
non-TCDD-like PCBs[c]	0.067	-0.447	0.018	-0.308	-0.121	-0.434
Mono-ortho Cl PCBs	-0.144	**-0.538***	-0.080	-0.339	-0.186	-0.403
Di-ortho Cl PCBs	0.016	-0.529	-0.035	-0.378	-0.188	-0.463
Tri-ortho Cl PCBs	0.164	-0.324	0.159	-0.179	-0.022	-0.351
Tetra-ortho Cl PCBs	-0.075	-0.079	0.229	-0.008	0.073	-0.130
multi-ortho Cl PCBs	0.153	-0.324	0.150	-0.179	-0.034	-0.351
Tri-Cl PCBs	0.241	-0.088	0.477	-0.094	0.545	-0.088
Tetra-Cl PCBs	0.030	-0.123	0.405	-0.047	0.318	-0.146
Penta-Cl PCBs	-0.096	**-0.565***	-0.208	-0.425	-0.282	-0.454
Hexa-Cl PCBs	-0.002	-0.513	-0.049	-0.374	-0.177	-0.474
Hepta-Cl PCBs	-0.071	-0.373	0.144	-0.208	0.065	-0.331
Octa-Cl PCBs	-0.063	-0.247	0.233	-0.012	0.070	-0.114
Nona-Cl PCBs	0.089	-0.256	0.095	-0.071	-0.121	-0.191
Deca-Cl PCBs	-0.122	-0.079	0.206	-0.008	0.091	-0.130
Total PCBs	-0.002	-0.484	-0.049	-0.332	-0.177	-0.434
Sum OCs	0.004	-0.491	-0.046	-0.321	-0.188	-0.451

An askerisk indicates a significant correlation; p-value < 0.05.

[a] RBC = red blood cell count.

[b] Sum of *cis*-, *trans*-chlordane, *cis*-, *trans*-nonachlor, and oxychlordane.

[c] The 12 PCB congeners identified by the EPA that have TCDD-like activity are 77, 81, 105, 114, 118, 123, 126, 156, 157, 167, 169, and 189. Only PCB 105, 118, 156, and 157 were determined and summed in this study.

Table 4.5. Spearman Rank Correlation coefficients between OC concentrations and health assessment data in loggerhead sea turtles.

	Body Condition[a]		Glucose		Protein		Albumin		Globulin		Albumin: Globulin		BUN[a]	
	Adipose	Blood	Adipose	Blood	Adipose	Blood	Adipose	Blood	Adipose	Blood	Adipose	Blood	Adipose	Blood
N	44	46	36	39	37	40	37	40	37	40	37	40	37	40
heptachlor epoxide	-0.108	-0.274	-0.221	-0.162	-0.097	0.097	**-0.377***	-0.303	-0.018	0.199	-0.197	**-0.394***	-0.076	0.028
Total chlordanes[b]	-0.084	**-0.291***	-0.277	-0.202	0.002	0.137	**-0.365***	-0.210	0.089	0.217	**-0.332***	**-0.339***	0.092	**0.338***
dieldrin	-0.066	**-0.359***	**-0.352***	-0.304	0.046	0.074	-0.208	-0.097	0.086	0.098	-0.179	-0.170	0.091	0.055
mirex	-0.151	-0.168	-0.246	-0.092	0.039	0.011	-0.313	**-0.315***	0.104	0.095	-0.280	-0.268	0.073	0.242
4,4'-DDE	-0.053	-0.101	**-0.445***	-0.244	-0.073	0.033	-0.271	-0.185	-0.021	0.102	-0.122	-0.197	0.130	**0.464***
Total DDTs	-0.025	-0.106	**-0.475***	-0.246	-0.133	0.022	-0.207	-0.183	-0.087	0.089	-0.071	-0.182	0.229	**0.463***
TCDD-like PCBs[c]	-0.105	-0.157	-0.281	-0.145	0.067	0.204	-0.283	-0.152	0.146	0.282	-0.318	**-0.370***	0.100	**0.387***
non-TCDD-like PCBs[c]	-0.035	-0.142	-0.293	-0.161	-0.050	0.199	-0.267	-0.065	0.016	0.251	-0.192	-0.293	0.140	**0.370***
Mono-ortho Cl PCBs	-0.055	-0.146	-0.231	-0.138	0.072	0.204	-0.236	-0.151	0.142	0.281	-0.282	**-0.365***	0.161	**0.402***
Di-ortho Cl PCBs	-0.045	-0.161	-0.303	-0.177	-0.038	0.193	-0.289	-0.092	0.031	0.250	-0.210	-0.308	0.117	**0.362***
Tri-ortho Cl PCBs	0.060	-0.033	-0.318	-0.056	-0.045	0.238	-0.057	0.036	-0.021	0.269	-0.038	-0.224	0.188	**0.508***
Tetra-ortho Cl PCBs	0.170	0.087	0.055	0.080	0.243	0.192	0.155	-0.036	0.252	0.236	-0.118	-0.205	**0.328***	**0.478***
multi-ortho Cl PCBs	0.063	-0.017	-0.313	-0.047	-0.030	0.243	-0.045	0.026	-0.007	0.277	-0.047	-0.233	0.194	**0.507***
Tri-Cl PCBs	**0.362***	0.066	0.257	-0.114	0.114	-0.109	0.190	0.100	0.083	-0.162	0.083	0.248	0.250	0.036
Tetra-Cl PCBs	0.296	-0.023	0.137	0.065	**0.377***	0.312	0.301	0.175	**0.373***	**0.318***	-0.264	-0.176	0.279	**0.434***
Penta-Cl PCBs	-0.041	-0.144	-0.226	-0.130	0.061	0.223	-0.243	-0.119	0.132	0.294	0-.278	**-0.362***	0.196	**0.403***
Hexa-Cl PCBs	-0.052	-0.188	-0.297	-0.182	-0.023	0.207	-0.286	-0.079	0.047	0.262	-0.224	**-0.316***	0.111	**0.356***
Hepta-Cl PCBs	0.021	-0.081	-0.305	-0.151	-0.035	0.139	-0.293	-0.096	0.037	0.193	-0.229	-0.248	0.212	**0.387***
Octa-Cl PCBs	0.204	0.052	-0.201	0.066	**0.357***	0.085	0.236	-0.102	**0.365***	0.141	-0.289	-0.158	0.196	**0.405***
Nona-Cl PCBs	0.212	0.076	-0.011	0.131	**0.461***	0.271	**0.422***	0.110	**0.447***	0.290	-0.234	-0.180	0.141	**0.536***
Deca-Cl PCBs	**0.334***	0.060	0.263	0.210	**0.492***	0.277	**0.386***	0.071	**0.483***	**0.314***	-0.205	-0.236	0.254	**0.482***
Total PCBs	-0.049	-0.139	-0.294	-0.161	-0.025	0.202	-0.269	-0.064	0.042	0.255	-0.217	-0.296	0.118	**0.377***
Sum OCs	-0.072	-0.152	-0.318	-0.177	-0.062	0.170	-0.295	-0.115	0.008	0.231	-0.204	-0.290	0.145	**0.409***

An askerisk indicates a significant correlation; p-value < 0.05.

[a] Body condition = kg/cm^{3}x100,000; BUN = blood urea nitrogen.

[b] Sum of *cis*-, *trans*-chlordane, *cis*-, *trans*-nonachlor, and oxychlordane.

[c] The 12 PCB congeners identified by the EPA that have TCDD-like activity are 77, 81, 105, 114, 118, 123, 126, 156, 157, 167, 169, and 189. Only PCB 105, 118, 156, and 157 were determined and summed in this study.

Table 4.6. Spearman Rank Correlation coefficients between OC concentrations and plasma enzyme activities in loggerhead sea turtles.

	AST[a]		ALP[a]	
	Adipose	Blood	Adipose	Blood
N	37	40	37	40
heptachlor epoxide	**0.372***	**0.493***	**-0.384***	-0.312
Total chlordanes[b]	**0.580***	**0.604***	-0.325	-0.291
dieldrin	**0.349***	0.087	-0.157	0.118
mirex	**0.477***	**0.613***	**-0.369***	**-0.435***
4,4'-DDE	0.280	**0.463***	-0.195	-0.164
Total DDTs	0.261	**0.456***	-0.059	-0.158
TCDD-like PCBs[c]	**0.438***	**0.597***	-0.318	**-0.342***
non-TCDD-like PCBs[c]	**0.400***	**0.582***	-0.271	-0.282
Mono-ortho Cl PCBs	**0.441***	**0.591***	-0.254	**-0.338***
Di-ortho Cl PCBs	**0.401***	**0.584***	**-0.328***	-0.293
Tri-ortho Cl PCBs	0.280	**0.606***	-0.157	-0.197
Tetra-ortho Cl PCBs	0.294	**0.548***	0.204	-0.273
multi-ortho Cl PCBs	0.297	**0.609***	-0.148	-0.211
Tri-Cl PCBs	0.073	0.009	0.233	0.003
Tetra-Cl PCBs	0.189	**0.384***	**0.434***	-0.090
Penta-Cl PCBs	**0.458***	**0.588***	-0.276	**-0.314***
Hexa-Cl PCBs	**0.406***	**0.571***	**-0.325***	-0.268
Hepta-Cl PCBs	**0.415***	**0.573***	-0.197	-0.312
Octa-Cl PCBs	0.086	**0.564***	0.248	-0.265
Nona-Cl PCBs	0.125	**0.619***	0.118	-0.176
Deca-Cl PCBs	0.206	**0.550***	0.179	-0.175
Total PCBs	**0.399***	**0.592***	-0.296	-0.287
Sum OCs	**0.398***	**0.581***	-0.279	-0.297

An askerisk indicates a significant correlation; p-value < 0.05.

[a] AST = aspartate aminotransferase; ALP = alkaline phosphatase.

[b] Sum of *cis*-, *trans*-chlordane, *cis*-, *trans*-nonachlor, and oxychlordane.

[c] The 12 PCB congeners identified by the EPA that have TCDD-like activity are 77, 81, 105, 114, 118, 123, 126, 156, 157, 167, 169, and 189. Only PCB 105, 118, 156, and 157 were determined and summed in this study.

Table 4.7. Spearman Rank Correlation coefficients between OC concentrations and plasma ion levels in loggerhead sea turtles.

	Osmolality		Sodium		Magnesium	
	Adipose	Blood	Adipose	Blood	Adipose	Blood
N	44	47	37	40	37	40
heptachlor epoxide	0.010	-0.095	0.122	**0.371***	0.180	-0.083
Total chlordanes[a]	-0.064	0.110	0.241	0.296	-0.022	-0.251
dieldrin	-0.087	-0.226	0.138	0.130	-0.096	-0.248
mirex	-0.007	0.076	0.183	**0.323***	-0.125	-0.311
4,4'-DDE	0.062	0.191	0.118	0.201	-0.165	**-0.346***
Total DDTs	-0.012	0.197	0.123	0.201	-0.257	**-0.344***
TCDD-like PCBs[b]	0.051	0.165	0.142	**0.348***	-0.176	**-0.398***
non-TCDD-like PCBs[b]	0.113	0.095	0.139	0.289	-0.242	**-0.482***
Mono-ortho Cl PCBs	0.114	0.179	0.108	**0.361***	-0.219	**-0.402***
Di-ortho Cl PCBs	0.075	0.074	0.142	0.306	-0.226	**-0.465***
Tri-ortho Cl PCBs	0.156	0.252	0.033	0.235	-0.309	**-0.550***
Tetra-ortho Cl PCBs	0.116	**0.475***	0.092	0.200	-0.193	**-0.331***
multi-ortho Cl PCBs	0.164	0.264	0.038	0.250	-0.305	**-0.537***
Tri-Cl PCBs	0.244	-0.033	-0.217	0.108	-0.047	-0.247
Tetra-Cl PCBs	0.066	**0.343***	0.076	0.195	-0.135	-0.342
Penta-Cl PCBs	0.173	0.137	0.161	**0.358***	-0.194	**-0.402***
Hexa-Cl PCBs	0.087	0.061	0.117	0.294	-0.221	**-0.465***
Hepta-Cl PCBs	-0.076	0.153	0.214	0.287	-0.317	**-0.501***
Octa-Cl PCBs	-0.096	**0.379***	0.135	0.195	-0.149	**-0.463***
Nona-Cl PCBs	0.028	**0.385***	-0.053	0.231	-0.236	**-0.522***
Deca-Cl PCBs	-0.067	**0.432***	0.057	0.145	-0.209	**-0.440***
Total PCBs	0.103	0.095	0.132	0.294	-0.230	**-0.476***
Sum OCs	0.080	0.130	0.135	0.287	-0.224	**-0.445***

An askerisk indicates a significant correlation; p-value < 0.05.

[a] Sum of *cis*-, *trans*-chlordane, *cis*-, *trans*-nonachlor, and oxychlordane.

[b] The 12 PCB congeners identified by the EPA that have TCDD-like activity are 77, 81, 105, 114, 118, 123, 126, 156, 157, 167, 169, and 189. Only PCB 105, 118, 156, and 157 were determined and summed in this study.

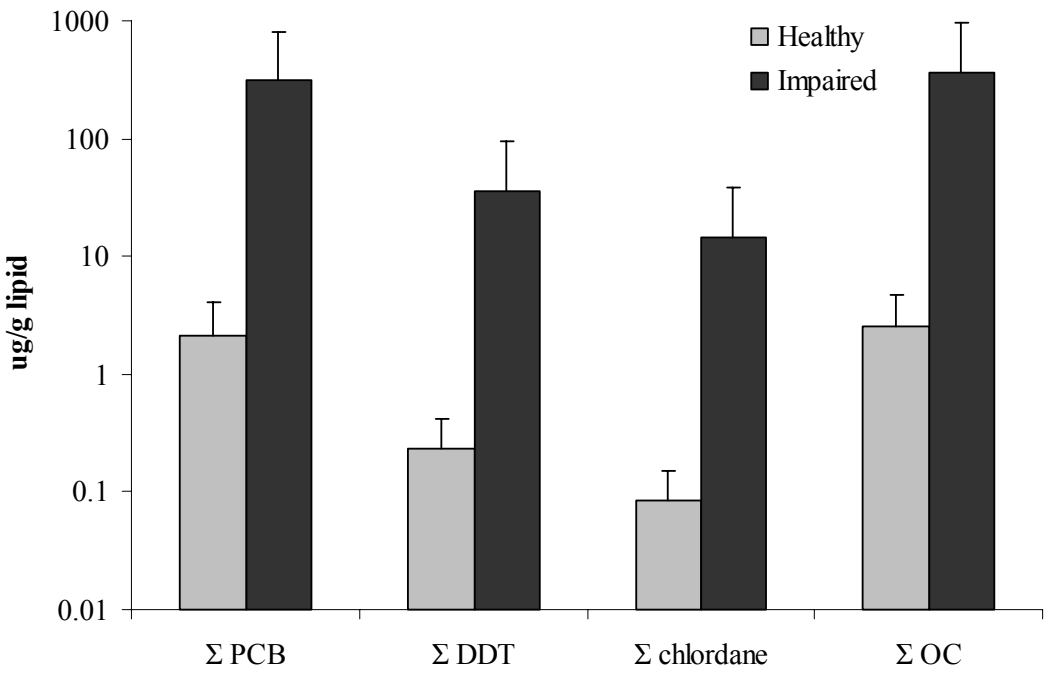


Figure 4.1. Blood concentrations of the major classes of organochlorine contaminants in 47 apparently healthy juvenile loggerhead sea turtles and 3 turtles exhibiting signs of wasting from Core Sound, North Carolina. The concentrations of all contaminants classes, including total organochlorines (Σ OC), in the sick turtles are significantly greater than those in the healthy turtles (p-value < 0.005; Mann-Whitney test).

CHAPTER FIVE

Influence of season, gender, and body condition on the loggerhead sea turtle immune system

ABSTRACT

A fully functioning immune system is vital to the survival and population recovery of threatened and endangered sea turtles. The effectiveness of the immune system to fight diseases can be reduced by a number of natural and anthropogenic factors, such as seasonal changes, malnutrition, disease states, and contaminant exposure. One common method of measuring immune function is the mitogen-induced lymphoproliferation (LP) assay. The goals of this study were to develop the LP method for juvenile loggerhead sea turtles (*Caretta caretta*) and to examine how season, gender, and nutritional status, as measured by body condition, influence the loggerhead immune system. Two different methods of measuring LP were compared 1) the traditional radioactive ^{3}H-thymidine assay and 2) a non-radioactive, colorimetric method utilizing 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyl tetrazolium (MTT). The ^{3}H-thymidine assay resulted in higher LP values and is recommended over the non-radioactive MTT method. The LP response and white blood cell (WBC) counts varied with seasonal changes. LP responses to phytohemagglutinin (PHA) and lipopolysaccharide (LPS) were higher in July than September, and responses to phorbol dibutyrate (PDB) were higher in July than September and November. A similar trend was seen with WBC counts, which were higher in the summer than during May and the fall. These seasonal immune fluctuations were associated with changes in water temperature and photoperiod and agree with previous reptile studies. The affect of gender and body condition on LP was also investigated, but they did not greatly influence LP in the juvenile turtles examined.

INTRODUCTION

The reptilian immune system shares many similarities to that of mammals (Zapata and Cooper 1990). Similar lymphoid organs and cell populations perform the necessary immune functions to protect reptiles against foreign pathogens and parasites. These organs and immune cells include thymus, spleen, bone marrow, gut-associated lymphoid tissue, macrophages, granulocytes, and lymphocytes. Reptiles possess all the major immune functions, including 1) non-specific immunity such as macrophage and natural killer cell activity; 2) cell-mediated immunity, such as the destruction of tissue grafts by T-lymphocytes; and 3) humoral immunity involving antibody production and memory cells that allow them to mount a stronger and faster immune response upon subsequent exposure to a particular antigen (Zapata and Cooper 1990).

It has been known for some time that the reptile lymphoid structures and immune functions fluctuate profoundly with seasonal changes (Zapata *et al*. 1992). In most ectotherms, immunity strengthens in the summer and weakens in the winter. These immune fluctuations are likely caused by seasonal changes in both environmental conditions such as temperature and photoperiod, and in concentrations of steroids such as corticosterone and testosterone (Zapata *et al*., 1992). For example, the lizard *Chalcides ocellatus* demonstrates peak immune functions in summer when corticosterone and testosterone concentrations are lower, and weaker immune responses in winter when these hormones are at higher concentrations (Saad and El Ridi 1988; Saad *et al*. 1990). Injection of hydrocortisone or testosterone in lizards during the summer, when their endogenous hormone levels are low, results in

decreased humoral and cell-mediated responses, including lymphoproliferative responses (Saad *et al*. 1986; Saad and El Ridi 1988; Saad *et al*. 1990). The tortoise *Mauremys caspica* exhibits a reduction in the mass of lymphoid organs in the summer which relates to increased levels of corticosterone (Leceta and Zapata 1985). Injection of testosterone into these tortoises causes thymic involution and lymphopenia (Saad *et al*. 1991).

The immunosuppressive effects of testosterone are thought to contribute to gender differences in immune functions in fish, birds, and mammals (see Zapata and Cooper 1990; Klein 2000; Schuurs and Verheul 1990; Zuk and McKean 1996). In birds and mammals, females typically have stronger immune functions, greater resistance to infections, and a higher incidence of autoimmune disease than males (Klein 2000; Schuurs and Verheul 1990). Physiologic levels of androgens generally suppress immune responses, whereas estrogens primarily enhance, but can occasionally suppress, cell-mediated and humoral immune functions (Klein 2000). Gender differences have been rarely studied in reptiles, but it is clear that testosterone can be immunosuppressive in the lizard and tortoise (Saad *et al*. 1991; Saad *et al*. 1990).

Malnutrition and disease may also weaken immune functions (Zapata and Cooper 1990). Nutritional deficiency in juvenile snapping turtles (*Chelydra serpentina*) results in thymic involution which is reversible when nutrition is improved (Borysenko and Lewis 1979). Fibropapillomatosis, a common disease that primarily afflicts green sea turtles (*Chelonia mydas*), produces non-cancerous tumors on the skin, near the eyes, mouth, and anus usually causing death from starvation. In both

Florida and Hawaii, green turtles with fibropapillomas exhibit compromised immune systems compared to disease-free turtles (Cray *et al*., 2001; Lutz *et al*., 2001; Work *et al*., 2001).

Environmental contaminants, including metals and halogenated aromatic hydrocarbons are also associated with suppressed immune function in wildlife. Fish from the Chesapeake Bay, birds near the Great Lakes, and marine mammals from various locations exposed to high levels of contaminants are reported to exhibit suppressed immune function (Faisal *et al*., 1991; Grasman *et al*., 1996; Ross *et al*., 1996). Toxicological effects have not, however, been thoroughly investigated in reptiles.

The basic fundamentals of the reptilian immune system are known, but the immune functions of endangered sea turtles have not been well characterized. As mentioned previously, some immune functions of the green sea turtle have been investigated in terms of disease status, but no studies have examined the basic seasonal, gender, or nutritional influences on the immune system of any sea turtle species.

Lymphocytes, one type of white blood cell (WBC), play several defensive roles in the immune system. They are categorized as either T-lymphocytes (T-cells) or B-lymphocytes (B-cells). T-cells perform a variety of immune functions and are further categorized as helper, cytotoxic, or regulatory T-cells. Helper T-cells proliferate following activation by antigen-presenting macrophages. These activated T-cells then signal B-cells to produce antibodies. Cytotoxic T-cells recognize and destroy virally-infected cells or tumor cells. Regulatory T-cells dampen immune

functions in order to prevent extensive damage to self cells. B-cells, once activated by helper T-cells, proliferate and secrete antibodies against foreign molecules. Some B-cells become memory cells that mount a stronger and quicker immune response upon subsequent exposure to a particular antigen. Together these cells form the basis for a properly functioning immune system. Without them, the body is extremely vulnerable to infections.

One way to test the responsiveness of lymphocytes is to measure their proliferation following exposure to a mitogen. This assay, termed mitogen-induced lymphoproliferation (LP), is a standard method employed to assess cell-mediated and humoral immune functions. Specific mitogens activate either T-cells or B-cells by binding to cell surface markers and result in the polyclonal expansion or proliferation of the cells. LP assay have been performed using WBCs from green sea turtles (McKinney and Bentley 1985; Cray *et al*. 2001; Lutz *et al*. 2001; Work *et al*. 2001), but LP has not been investigated in other sea turtle species.

The loggerhead sea turtle is considered a threatened species under U.S. law because of past and current population declines (TEWG 2000). It is important to assess sea turtle immune responses and understand how their immunity may be compromised by natural and anthropogenic factors, because the health of the immune system directly relates to the survival of individuals and the stability or recovery of populations. For this reason, we examined the LP response of juvenile loggerheads and investigated how it was influenced by natural factors including season, gender, and body condition.

MATERIALS AND METHODS

Sampling

Free-ranging juvenile loggerhead sea turtles with straight carapace lengths (SCL) between 49 cm and 77.3 cm were captured as by-catch from a pound net fishery located in Core Sound, North Carolina, USA in summer and fall of 2001. Blood was collected within 15 minutes of capture from the dorsocervical sinus using double-ended needles directly into blood collection tubes containing sodium heparin (Vacutainer®, Becton, Dickinson, and Co., Franklin Lakes, NJ). Blood was kept cool either on ice or in a refrigerator until processed for LP. Turtles were tagged, measured, weighed, and released near their capture location. Body condition was calculated as weight (kg) divided by the cube of SCL from the nuchal notch to the most posterior marginal notch (cm) and multiplied by 100,000 [Body condition = $kg/SCL^3 \times 100{,}000$] as described in Bjorndal *et al*. (2000). Sex of the turtles captured in July was determined by plasma testosterone concentration, and most were verified by laparoscopic examination (Owens 1997).

Mitogen-Induced Lymphoproliferation

Various cell plating conditions, mitogen types and concentrations, and days of mitogen concentration were tested. Cell proliferation was measured using two different methods, employing either ^{3}H-thymidine or an alternative colorimentric assay (MTT). For ease of comparison, details of both procedures are shown in Table 5.1.

^{3}H-thymidine assay

Blood was sent overnight with frozen ice packs to the Medical University of South Carolina and processed on the day following collection. WBCs were collected from the buffy coat (the layer of WBCs between the plasma and RBCs) using a slow spin technique. The tubes were centrifuged at 500 rpm (42 x g) for 20 min at 8 ^{o}C. The WBCs were collected by gently swirling the buffy coat into the plasma and transferring them into a new tube. Following centrifugation at 1500 rpm (377 x g) for 8 min, the cells were rinsed once with MPA media which consisted of RPMI 1640 media (Mediatech, Inc., Herndon, VA) that was supplemented with 5% FBS (Hyclone, Logan, UT), and final concentrations of 1% (v/v) of 100x solution of non-essential amino acids (Gibco, Grand Island, NY), 1 mM sodium pyruvate (Gibco, Grand Island, NY), 10 mM HEPES (Mediatech, Inc., Herndon, VA), 50 IU/ml penicillin, and 50 µg/ml streptomycin (Mediatech, Inc., Herndon, VA). The cells were centrifuged again at 1500 rpm (377 x g) for 8 min, resuspended in 1 ml of MPA media, and counted via trypan blue exclusion using a hemacytometer. All counts were performed by one person to maximize consistency. Cells were then split into two tubes per turtle sample and diluted with different media types, either MPA media as described above or JMKA media. JMKA media differed from MPA media by only the FBS type (BioWhittaker, Walkersville, MD lot # 8S006F). Cells were plated at a density of 1.8 x 10^{5} cells/well into 96 well plates.

Mitogens chosen to stimulate T-cells were phytohemagglutinin P (PHA; Amersham Pharmacia Biotech Inc., Pascataway, NJ) and concanavalin A (ConA) from Jack Bean (*Canavalia ensiformis*) (Sigma, St. Louis, MO). Mitogens that stimulate B-

cells were lipopolysaccharide (LPS) from *Escherichia coli* of either serotype 0111:B4 used with MPA media or serotype 0127:B8 used with JMKA media (Sigma, St. Louis, MO) and phorbol 12,13-dibutyrate (PDB; Sigma, St. Louis, MO). PDB was shown to stimulate avian B-cells previously (Scott and Savage 1996). Mitogens were diluted into either MPA media or JMKA media and added to wells containing the same media type (see Table 5.1). Each control (MPA or JMKA media only) and mitogen concentration was tested in triplicate wells with a final volume of 100 µl/well with JMKA media and 200 µl/well with MPA media. Cells were incubated at 30 ^{o}C with 5% CO_2. Mitogen concentrations and media types are listed in each figure legend. Following either a 4-day incubation (96 hr) or a 5-day incubation (120 hr), 0.5 µCi/well of ^{3}H-thymidine (ICN Biomedicals, Inc., Irvine, CA) was added in a volume of 100 µl to each well. Plates were reincubated for 16 hr and then harvested onto Unifilter plates (Packard, Meridian, CT) using a Packard Filtermate™ 96-well plate harvestor, and the plates were allowed to dry. Once dry, 25 µL of Microscint™ 20 (Packard, Meridian, CT) was added to each well, and the samples were analyzed using a Packard Top Count™-NXT scintillation counter. Stimulation index (SI) was calculated as the cpm of mitogen-stimulated cells divided by the cpm of unstimulated control (MPA or JMKA media only, respectively) cells.

MTT assay

In order to compare LP measured by the ^{3}H-thymidine assay to a non-radioactive, colorimetric MTT assay, 23 paired loggerhead blood samples from July 2001 were measured for LP using both techniques. See Table 5.1 for a comparison of both methods. Sample collection, cell preparation, and culture conditions were

identical to those used for the ^{3}H-thymidine assay except for the following minor differences. These changes in the procedure were necessary due to time restrictions, material availability, and a need to remain consistent with past and planned experiments. WBCs were collected using the slow spin technique described above, but the cells were isolated, counted, and plated on the same day as blood collection. Media used for all mitogen exposures for the MTT assay will be referred to as JMKB media. JMKB media consisted of RPMI 1640 media (Mediatech, Inc., Herndon, VA) supplemented with 5% FBS (BioWhittaker, Walkersville, MD lot # 8S006F), and final concentrations of 1% (v/v) of 100x solution of non-essential amino acids (Mediatech, Inc., Herndon, VA), 1 mM sodium pyruvate (BioWhittaker, Walkersville, MD), 10 mM HEPES (BioWhittaker, Walkersville, MD), 100 IU/ml penicillin, and 100 ug/ml streptomycin (Mediatech, Inc., Herndon, VA). Triplicate wells in 96 well plates for controls (JMKB media only) and mitogen exposures were seeded with 1.8 x 10^{5} cells/well and contained a total volume of 100 ul/well. The same mitogen types, lots, and concentrations were used in both methods. On day 5 of mitogen exposure (116 hr), cell proliferation was measured using CellTiter 96, a non-radioactive cell proliferation assay employing 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyl tetrazolium (MTT) (Promega, Inc., Madison, WI). The dye solution was added to each well (15 ul/well) and incubated for 4 hours. Solubilization solution was then added (100 ul/well) to stop the cellular conversion of the MTT dye. Plates were placed at 30 ^{o}C for 15 hr to allow the formazan product to solubilize completely. Absorbance was read at 570 nm and 650 nm on a spectrophotometric plate reader (Molecular Devices Spectramax 190, Sunnyville, CA). Absorbance at 570 nm was subtracted from the

absorbance at 650 nm ($Abs_{570-650}$) for each well. Furthermore, $Abs_{570-650}$ was corrected by subtracting $Abs_{570-650}$ of wells that did not contain cells but contained JMKB media and the MTT kit reagents. Stimulation index (SI) was calculated as the corrected absorbance of mitogen-stimulated cells divided by the absorbance of unstimulated, control (JMKB media only) cells.

Seasonal Comparison of WBC Counts

Seasonal changes in WBC counts were examined using two methods of cell isolation. WBCs were isolated from the buffy coat using the slow spin technique as described above from blood of turtles captured from July to November, 2001. A two-step Percoll (Sigma, St. Louis, MO) gradient was used to isolate WBCs from blood samples of turtles captured in Core Sound, NC from July 1998 to June 2001 as described by Harms *et al*. (2000). WBCs isolated using both techniques were counted via trypan blue exclusion.

Statistics

All statistical analyses were performed using Systat 8.0 (SPSS Inc., Chicago, IL). Non-parametric tests were used when the data did not fit a normal distribution. Most of the SI responses were not normally distributed. Differences between the ^{3}H-thymidine and MTT methods were tested using paired samples that were measured for LP in JMKA media with ^{3}H-thymidine and JMKB media with MTT (Table 5.1). The Wilcoxon signed rank test was used for this comparison as a non-parametric equivalent to a paired t-test. Differences in LP response among the three months (July, September, and November) were tested using data collected with the ^{3}H-thymidine assay. Because the distributions of LP responses for each month were not

normally distributed, the non-parametric Kruskal-Wallis test was used to determine if the months differed from each other. For more detailed comparisons between months, the LP responses were first transformed using log (x+1). Using this log-transformed data, analysis of variance (ANOVA) and the Least Significant Difference (LSD) tests were used to determine differences between months. All other tests chosen and p-values are listed in the figure legends or in the results section.

RESULTS

LP Method Comparison

Mean stimulation indices (SI) of all turtles are presented for all of the methods, media types, mitogen types, mitogen concentrations, and months in Table 5.2. SI measured using MTT were consistently 1.65 or lower regardless of the mitogen type or concentration. These SI are very similar to an index of 1.0 which would represent no stimulation. For turtles captured in July, the ^{3}H-thymidine assay resulted in mean SI that ranged from 1.40 to 7.12 for ConA, LPS, and PDB and higher (29 to 114) for PHA. The different media types (MPA vs. JMKA) or duration of mitogen exposure (4 days vs. 5 days) did little to change the SI. MPA media and mitogens on day 4 produced slightly higher SI than MPA media and mitogens on day 5, but this pattern was only observed in July and November. Using the ^{3}H-thymidine assay and JMKA media in July, PHA stimulated T-cells more than ConA, but the opposite was seen in the MTT assay. PDB stimulated B-cells more than LPS in July using JMKA media and ^{3}H-thymidine or using the MTT assay, but LPS generally resulted in higher SI than PDB using MPA media in all months.

Using paired samples collected from turtles captured in July, the traditional radioactive ^{3}H-thymidine assay resulted in significantly higher SI than the non-radioactive MTT assay (Fig. 5.1). Significant differences were detected between ^{3}H-thymidine assay and MTT for the T-cell mitogen PHA (Fig. 5.1A) and for B-cell mitogens LPS and PDB (Fig. 5.1B). Both assays gave similar results for ConA. These data suggest that loggerhead lymphocytes are responsive to mitogens (primarily PHA, LPS, and PDB), and that the ^{3}H-thymidine assay is preferable over the MTT method.

Influence of Natural Factors

Using only the ^{3}H-thymidine assay, the LP responses were compared among loggerhead turtles that were captured at three different times of the year (July, September, and November) in order to examine seasonal changes (Table 5.2). Generally, SI were higher in July and dropped in the fall months. Significant differences were observed between months for LP stimulated by 10 ug/ml LPS in MPA media tested on day 5, by all concentrations of PDB with both media types, and by 5 ug/ml PHA in JMKA media. A seasonal difference was not seen by using ConA. More detailed comparisons between months are shown for selected mitogen concentrations in Figure 5.2. LP stimulated by PHA and LPS was significantly higher in July than in September (Fig. 5.2). However, the November responses to these mitogens were not statistically different from the July responses, likely due to the large variation associated with the July mean. LP stimulated by PDB was significantly higher in July than in September and November.

WBC counts also followed this seasonal pattern with a peak in the summer months and reductions in the fall (Fig. 5.3). Turtles displayed significantly lower WBC counts in May than the summer months using the Percoll isolation technique. WBC counts isolated from the buffy coat using the slow spin technique were significantly lower in November than July and August.

Gender did not influence LP in these juvenile loggerheads. In order to examine these influences separate from confounding seasonal changes, only turtles sampled in July were used. July was chosen because LP responses were the strongest and the largest sample size was available. Although there appeared to be a stronger response of females to PHA, there were no statistically significant differences between sexes for any of the mitogens tested (Mann-Whitney t-test; all p-values > 0.05; Fig. 5.4).

July LP responses were also compared to the nutritional status of the turtles as measured by body condition. Correlations between body condition and LP responses to selected mitogens and media types are shown in Figure 5.5. Only one significant correlation was observed between SI and body condition. The LP response to 0.2 μg/ml PDB was positively correlated to body condition (Spearman Rank Correlation; $R_s = 0.528$, $p \leq 0.02$; Fig. 5.5D). LP measured with all of the other mitogens and other concentrations of PDB did not correlate to body condition (Fig. 5.5A, B, and C; not all data are shown).

DISCUSSION

In comparing LP methods for loggerhead sea turtles, higher SI were measured using the ^{3}H-thymidine assay than the MTT assay. The loggerhead mean SI to PHA was 114 using the ^{3}H-thymidine assay, while it was 1.45 using the MTT assay. These results suggest that the radioactive method may be more sensitive, thus more appropriate for measuring LP in sea turtles. A comparison of previously reported LP values for green sea turtles supports this conclusion. Using the MTT assay, Lutz *et al*. (2001) reported consistently low SI values at 1.0 to 1.5 for five mitogens (ConA, LPS, PHA, pokeweed mitogen, and a combination of ionomycin and PMA) tested on healthy green turtles from Florida. These SI values were nearly identical to the low SI observed in the loggerhead turtles from this study using the MTT assay. In contrast, LP responses of green sea turtles measured by the ^{3}H-thymidine assay to PHA were much higher; mean SI was approximately 30 in Florida (Cray *et al*., 2001) and 130 in Hawaii (Work *et al*., 2001). By producing higher SI values, the ^{3}H-thymidine method may be more sensitive, thereby allowing the detection of changes on a finer scale than the MTT assay. The comparison between the two techniques suggests that the ^{3}H-thymidine method is preferred for analyzing LP in sea turtles.

Interestingly, the loggerhead response to ConA was much lower than that seen in the green turtle. Using ^{3}H-thymidine, the loggerhead mean SI to ConA was approximately 3.6, while the green turtle mean SI was roughly 30 in Florida (Cray *et al*., 2001) and 125 in Hawaii (Work *et al*., 2001). These apparent differences may reflect species-specific differences in immune response to ConA. However,

differences in culture conditions (i.e.; mitogen concentrations, stimulation time, temperature, CO_2, and media types) may also account for the dissimilarity in response.

This study is the first to investigate and document a seasonal variation in immune functions of any sea turtle species. As expected, the loggerhead LP response fluctuated with seasonal changes and followed the normal trend seen in ectothermic vertebrates with a peak in summer and lower values in winter (Zapata *et al*. 1992). From the current results, we cannot determine whether temperature or photoperiod, singly or in combination, is the causal agent of this seasonal fluctuation. Future experimental manipulations may answer this question.

Compromised immune functions in the winter may have serious consequences on host resistance and survival of sea turtles. Many sea turtles each year become "cold-stunned" when they become entrained in cold water masses before migrating to warmer waters for the winter. Cold-stunned turtles float because they lose control of buoyancy and are not able to swim. Many of these turtles strand on beaches, and without proper care most do not survive (Spotila *et al*. 1997). Weakened immune systems may explain why many cold-stunned turtles are susceptible to infections, such as bacterial pneumonia, splenitis, pancreatitis, and fungal infections (Campell 1996; Turnbull *et al*. 2000; Manire *et al*. 2002).

Gender did not greatly influence loggerhead LP. The PHA response was higher in females, but not to a statistically significant extent. A larger sample size may eventually reveal differences between males and females, especially in their T-cell stimulation by PHA. A parallel study using juvenile loggerhead turtles from the offshore waters of South Carolina, Georgia, and Florida did not identify any sex

differences even with a larger sample size (n = 58; Peden-Adams *et al*. 2001). This was unexpected because gender differences are often seen in other animals (Klein 2000; Zapata *et al*. 1992; Schuurs and Verheul 1990).

Gender differences in immune function are thought to be caused by different levels of sex steroids (Klein 2000; Zapata *et al*. 1992; Schuurs and Verheul 1990). Evidence of this phenomenon has been documented in reptiles. Immune parameters such as lymphoid organ mass in a tortoise and immune function tests in a lizard decreased during particular seasons when testosterone concentrations were high (Leceta and Zapata 1985; Saad *et al*. 1990). When injected with testosterone the lizards exhibited reduced lymphoid organ masses, suppressed antibody production, and increased skin allograft survival (Saad *et al*. 1990) and intense lymphopenia was demonstrated in tortoises (Saad *et al*. 1991). In contrast, results of our current study on juvenile sea turtles suggest that testosterone did not affect LP. Stronger LP responses were observed in the summer when sea turtle plasma testosterone concentrations are presumably the highest (Owens and Morris 1985). Secondly, plasma testosterone levels of juvenile males (greater than 40 pg/ml) do not overlap those of juvenile females (below 30 pg/ml) as shown in many sea turtle species (Owens 1997) as well as the turtles in this study (Joanne Braun-McNeill personal communication). Still no statistically significant difference was seen in their LP responses. This gender difference of a few pg/ml of testosterone in this age class may not be sufficient to produce differential LP responses. What may be a more interesting comparison for future research is between juvenile and adult females. Adult females have higher circulating testosterone (up to 300 pg/ml) than juveniles, and steroid

levels fluctuate profoundly in adult females. Testosterone dramatically increases in adult females just before they migrate to the mating areas, and then it drops stepwise with each successive clutch (Owens 1997). Future studies may find significant steroid-induced changes in immune function between juvenile and adult females or between adults at different stages of the reproductive season.

The lack of correlations between body condition and the LP response could be caused by a number of reasons. Firstly, the body condition index that was chosen in this study may not reliably predict nutritional status in loggerhead sea turtles. However, Bjorndal *et al*. (2000) found that this condition index for green sea turtles was sensitive to changes in growth rates and population density. During times when more turtles were feeding on the same amount of resources, turtle growth rates slowed and body condition decreased. Secondly, nutritional status of sea turtles may not affect the response of their immune cells, however snapping turtles that were intentionally malnourished have shown signs of immunosuppression (Borysenko and Lewis 1979). Third, and most likely, nutritional status of the turtles sampled in this study may have fallen within the normal, healthy range. Since body condition indices for loggerhead turtles are not available in the literature, a comparison could not be made between the nutritional status of these turtles and past investigations. Only one turtle in the current study appeared emaciated and lethargic, suggesting that overall the turtles were of good nutritional status. A final consideration is the small sample size. The number of animals examined was limited to only 16 to 22 per mitogen. More samples may be needed to provide sufficient statistical power to detect significant correlations, especially if more turtles with poorer condition were included.

In conclusion, this study investigated, for the first time, how the sea turtle immune system may be influenced by natural factors. To accomplish this, we optimized the LP assay for the loggerhead sea turtle and found that the ^{3}H-thymidine assay was superior to the non-radioactive MTT method. As expected, we observed a seasonal change in immune function in juvenile sea turtles. This finding should be considered when comparing different studies or between samples collected at different times of the year. Gender and nutritional status did not greatly influence the immune system in the juvenile turtles examined, but adults and turtles with a wider range of body conditions should be tested. These results provide the framework for future research into how the immune system of the loggerhead sea turtle may be compromised by disease and contaminants.

Table 5.1. Comparison of methods used in this study to measure lymphoproliferation in loggerhead sea turtles.

Proliferation assay	Plating conditions	Mitogens (type)[a]	ug/ml of culture	mitogen exposure (days)
^{3}H-thymidine	MPA Media	ConA (C-5275)	20	4
	Mediatech RPMI-1640			5
	Hyclone FBS	LPS (L-2630)	10	4
	Gibco/Mediatech supplements			5
	200 ul/well	PDB (P-1269)	0.2	4
	1.8 x 10^5 cells/well			5
	day after blood collection			
	JMKA Media	PHA (APB)	5, 10	5
	Mediatech RPMI-1640	ConA (C-2010)	10, 20	5
	BioWhittaker FBS (lot # 8S006F)	LPS (L-3129)	2.5, 5	5
	Gibco/Mediatech supplements	PDB (P-1269)	0.2, 0.4, 0.8	5
	100 ul/well			
	1.8 x 10^5 cells/well			
	day after blood collection			
MTT	JMKB Media	PHA (APB)	5, 10	5
	Mediatech RPMI-1640	ConA (C-2010)	10, 20	5
	BioWhittaker FBS (lot # 8S006F)	LPS (L-3129)	2.5, 5	5
	BioWhittaker/Mediatech supplements	PDB (P-1269)	0.2, 0.4, 0.8	5
	100 ul/well			
	1.8 x 10^5 cells/well			
	same day as blood collection			

[a] APB= Amersham Pharmacia Biotech; all other mitogen types were purchased from Sigma and their catalog numbers are shown in parentheses.

Table 5.2. Comparison of stimulation indices of loggerhead sea turtle lymphoproliferation using various methods in July and across different seasons.

				Stimulation Indices (SI)						
				July		September		November		
Method	Media	Mitogen (ug/ml culture)	Day	Mean (SEM)	N	Mean (SEM)	N	Mean (SEM)	N	K-W p-value[a]
^{3}H-thymidine	MPA	ConA (20)	4	3.94 (0.95)	19	3.01 (0.83)	5	2.35 (0.65)	6	0.603
		ConA (20)	5	2.47 (0.52)	24	4.80 (2.91)	6	2.19 (0.45)	6	0.981
		LPS (10)	4	3.41 (0.52)	19	2.34 (0.60)	5	1.76 (0.33)	6	0.189
		LPS (10)	5	3.01 (0.47)	24	4.18 (1.10)	6	1.35 (0.11)	6	**0.042***
		PDB (0.2)	4	4.52 (1.23)	19	1.85 (0.89)	5	1.18 (0.18)	6	**0.043***
		PDB (0.2)	5	2.84 (0.55)	24	0.91 (0.17)	6	0.96 (0.19)	6	**0.019***
	JMKA	PHA (5)	5	114 (55)	17	4.16 (2.15)	6	5.87 (1.46)	6	**0.048***
		PHA (10)	5	29.1 (13.5)	17	2.48 (0.83)	6	19.9 (12.1)	6	0.166
		ConA (10)	5	1.89 (0.37)	10	2.61 (1.26)	6	1.46 (0.17)	6	0.987
		ConA (20)	5	3.56 (0.70)	24	1.94 (0.47)	6	1.54 (0.22)	6	0.125
		LPS (2.5)	5	2.01 (0.39)	17	0.76 (0.12)	6	1.26 (0.23)	6	0.063
		LPS (5)	5	1.40 (0.25)	17	1.20 (0.37)	6	1.01 (0.13)	5	0.762
		PDB (0.2)	5	7.12 (2.88)	10	0.95 (0.36)	6	1.78 (0.25)	6	**0.033***
		PDB (0.4)	5	6.08 (1.73)	17	0.77 (0.18)	6	1.23 (0.08)	5	**0.003***
		PDB (0.8)	5	4.10 (1.28)	17	NT		NT		NT
MTT	JMKB	PHA (5)	5	1.45 (0.16)	24	NT		NT		NT
		PHA (10)	5	1.35 (0.20)	24	NT		NT		NT
		ConA (10)	5	1.65 (0.10)	24	NT		NT		NT
		ConA (20)	5	1.51 (0.10)	24	NT		NT		NT
		LPS (2.5)	5	0.71 (0.08)	24	NT		NT		NT
		LPS (5)	5	0.67 (0.07)	24	NT		NT		NT
		PDB (0.2)	5	1.38 (0.08)	24	NT		NT		NT
		PDB (0.4)	5	1.46 (0.09)	24	NT		NT		NT
		PDB (0.8)	5	1.55 (0.11)	24	NT		NT		NT

[a] NT = not tested; K-W = Kruskal-Wallis test, a non-parametric equivalent to an ANOVA. Asterisks indicate a significant difference among months. SI = proliferation of stimulated cells/proliferation of unstimulated cells. N = sample size.

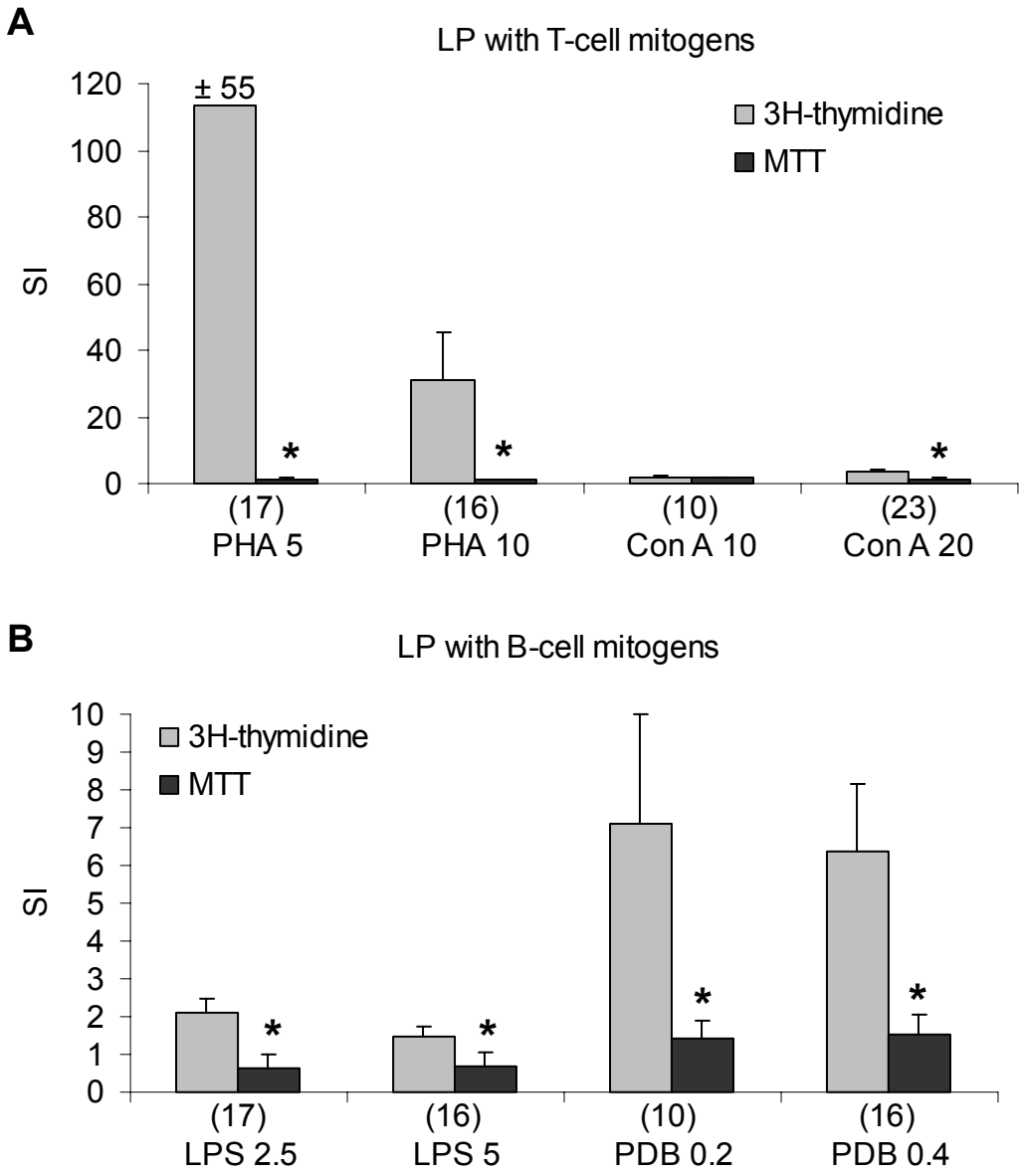
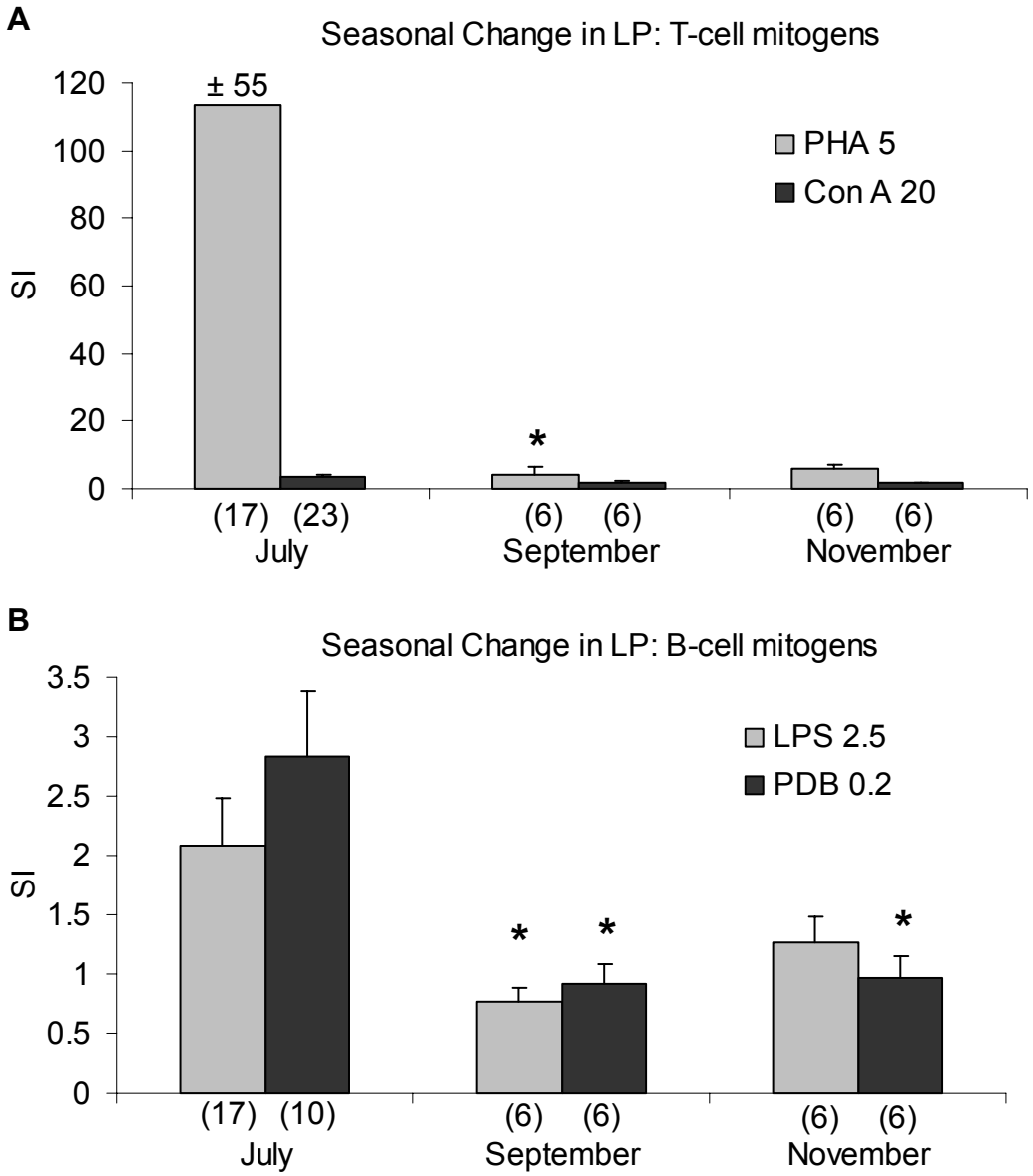


Figure 5.1. Juvenile loggerhead mitogen-induced LP measured in July by the traditional ^{3}H-thymidine assay and the non-radioactive MTT assay. A. T-cell mitogens; B. B-cell mitogens. Data are reported as mean ± SEM of only samples in which both methods were performed. Sample sizes are in parentheses. Numbers next to mitogen names represent the concentration of mitogen used (μg/ml of culture). SI = stimulation index (stimulated value/unstimulated value). JMKA media was used for the ^{3}H-thymidine assay and JMKB media was used for the MTT. *An asterisk denotes a difference between the two methods; Wilcoxon signed rank test ($p \leq 0.05$).



Water temperature (°C)	26.1	24.8	17.7
Photoperiod (hr daylight)	14.25	12.0	10.75

Figure 5.2. Seasonal change in juvenile loggerhead mitogen-induced LP using the ^{3}H-thymidine method. A. T-cell mitogens; B. B-cell mitogens. Data are reported as mean ± SEM. Sample sizes are in parentheses. Numbers next to mitogen names represent the concentration of mitogen used (μg/ml of culture). SI = stimulation index (cpm stimulated/cpm unstimulated). JMKA media was used for PHA, ConA, and LPS, while MPA media and 5 days of exposure were used for PDB. *An asterisk denotes a difference from July; ANOVA and Least Significant Difference test ($p \leq 0.05$) with log-transformed data.

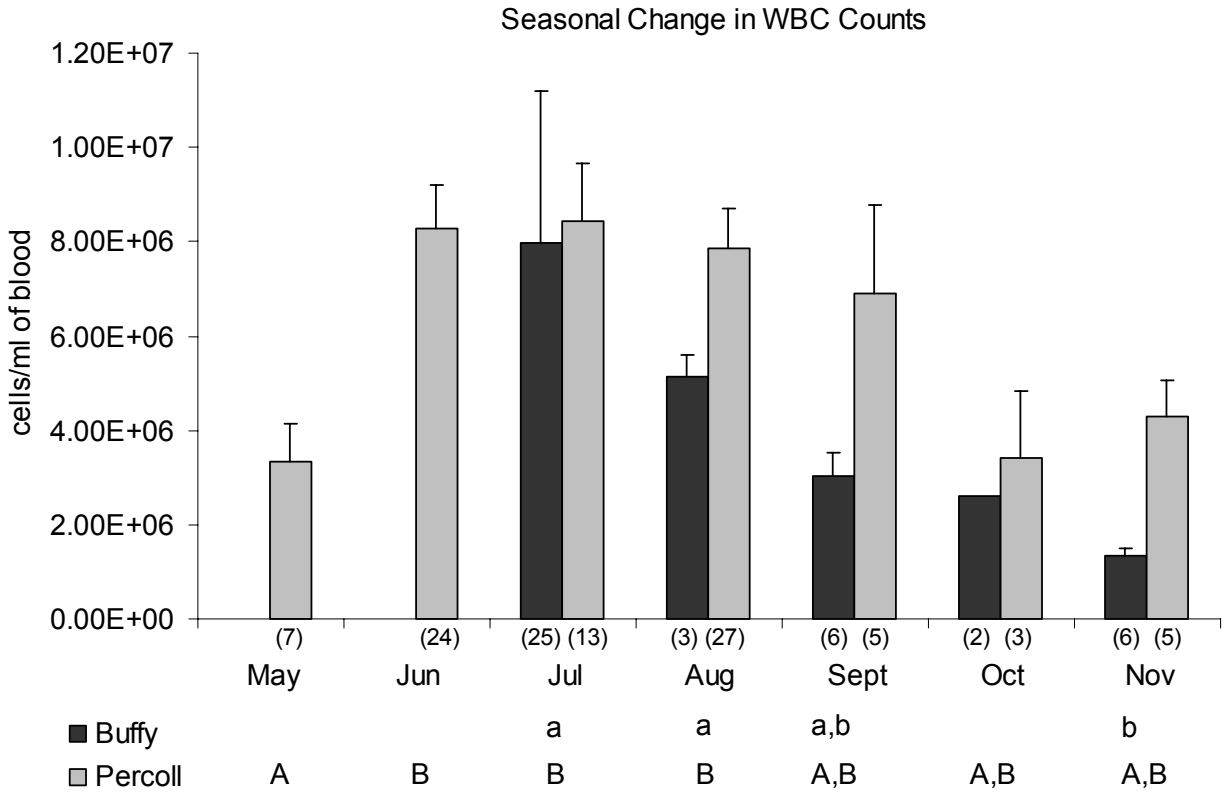


Figure 5.3. Seasonal change in juvenile loggerhead WBC counts measured by two methods. 'Buffy' signifies a count of total WBC removed from the buffy coat using the slow spin technique described herein. Turtles used for the buffy counts were captured in 2001. 'Percoll' signifies a count of isolated lymphocytes using the method described by Harms et al. (2000). Turtles used for the Percoll counts were captured from 1998 to 2001. Data are reported as mean ± SD. Sample sizes are in parentheses. Months with different letters (small case a or b for Buffy counts, upper case A or B for Percoll counts) within a row at the bottom of the figure are significantly different (ANOVA and Least Significant Difference test $p < 0.05$).

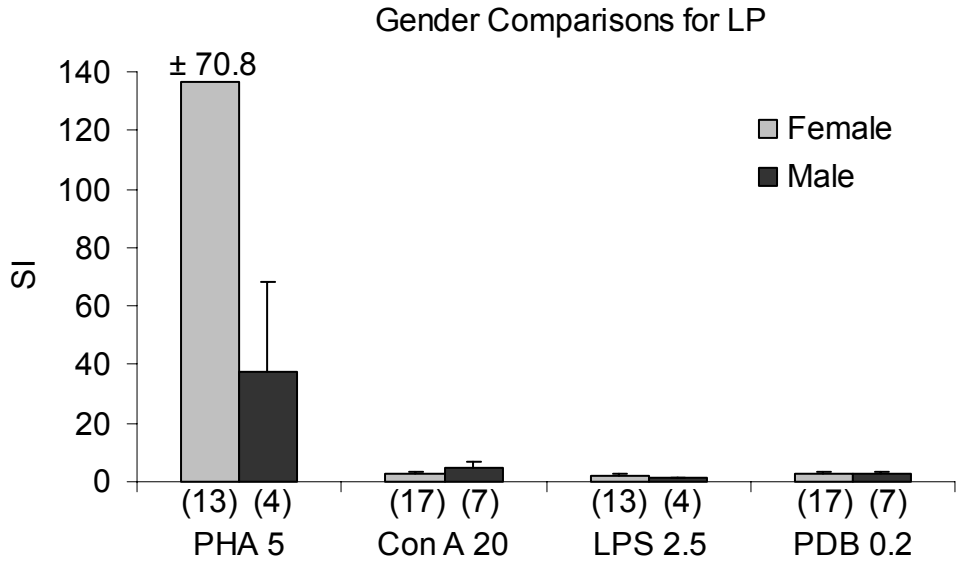


Figure 5.4. Gender comparison of juvenile loggerhead sea turtle LP captured in July, 2001 using the ^{3}H-thymidine method. Data are reported as mean ± SEM. Sample sizes are in parentheses. Numbers next to mitogen names represent the concentration of mitogen used (μg/ml of culture). SI = stimulation index (cpm stimulated/cpm unstimulated). JMKA media was used for PHA, ConA, and LPS, while MPA media and 5 days of exposure were used for PDB. No significant differences between males and females were detected using the Mann-Whitney test (all p-values were > 0.05).

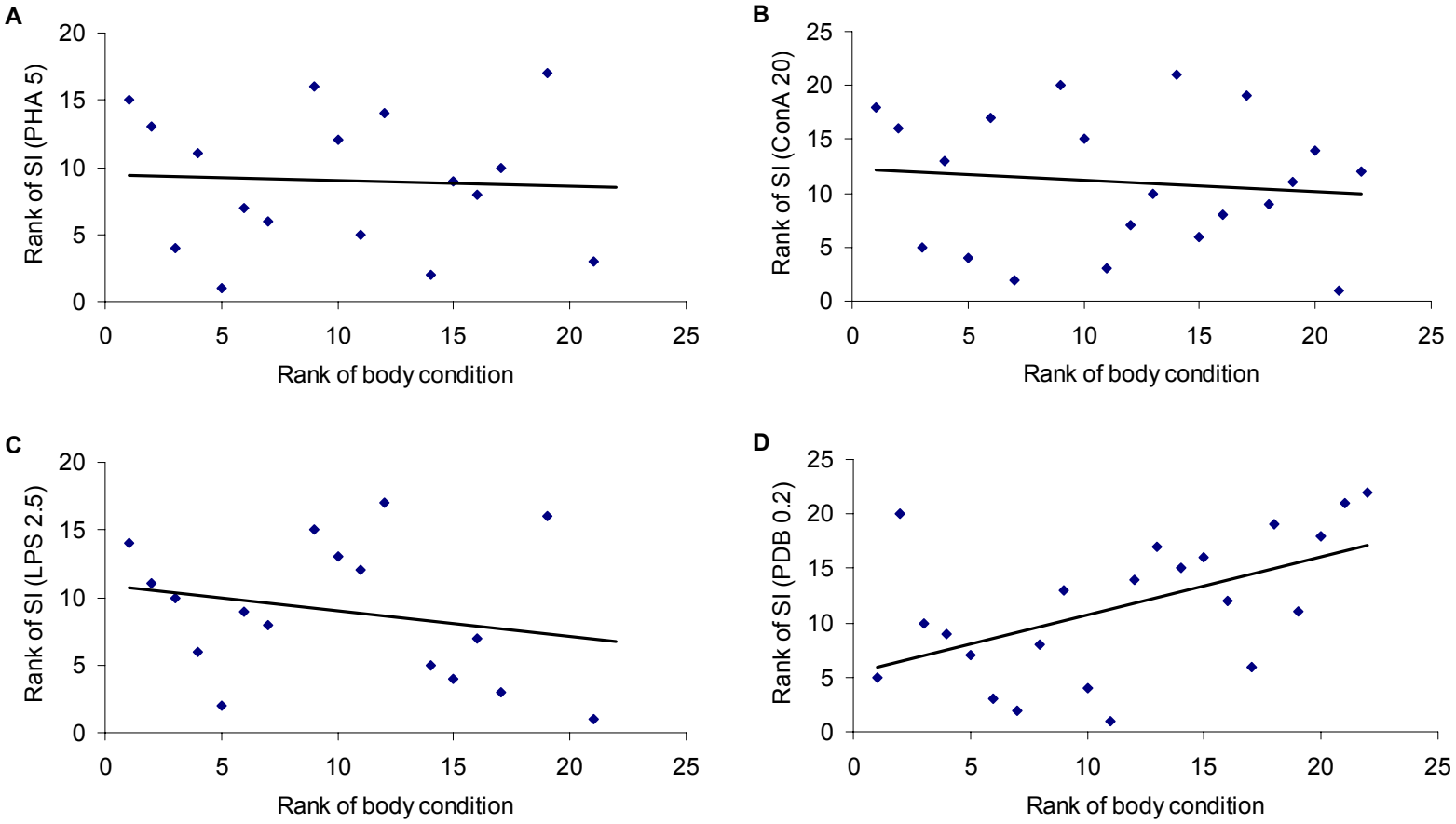


Figure 5.5. Spearman rank correlations between body condition indices and LP responses to PHA (A), ConA (B), and LPS (C) in JMKA media and to PDB (D) in MPA media with 5 days of exposure. Juvenile loggerhead sea turtles were captured in July 2001. Numbers next to the mitogen names represent the concentration of mitogen used (μg/ml of culture). Body condition = turtle mass (kg)/ turtle length $(cm)^3$ x 100,000. SI = stimulation index (cpm stimulated/cpm unstimulated) measured by the ^{3}H-thymidine method. PDB was the only mitogen that significantly correlated to body condition ($R_s = 0.528$; $p < 0.02$).

CHAPTER SIX

Effect of environmental organochlorine exposure on the lymphoproliferative immune response of loggerhead sea turtles

ABSTRACT

Correlative studies have shown associations between environmental contaminant exposure and altered immune responses in humans and many wildlife species. Laboratory studies support these correlative findings by providing ample evidence that organochlorine (OC) contaminants, such as polychlorinated biphenyls (PCBs) and metabolites of DDT, modulate immune responses. The goal of the current study was to examine the effect of organochlorine (OC) contaminants on the immune system of the threatened loggerhead sea turtle (*Caretta caretta*). Mitogen-induced lymphoproliferation (LP) was assessed using ^{3}H-thymidine in juvenile loggerhead sea turtles that were captured in July 2001 from North Carolina. Mitogens used included phytohemagglutinin (PHA), concanavalin A (ConA), lipopolysaccharide (LPS), and phorbol 12,13-dibutyrate (PDB). Lymphoproliferative responses to PHA, LPS, and PDB were significantly and positively correlated to several OC contaminant concentrations (including sum PCBs, sum DDTs, sum chlordanes, dieldrin, mirex, and sum OCs) in adipose tissue or whole blood. Additionally, loggerhead white blood cells (WBCs) were exposed *in vitro* to OC compounds, singly and as a mixture. LP responses were significantly decreased by *in vitro* exposure to Aroclor 1254 (a mixture of PCBs), 4,4'-DDE, and mirex, but only at concentrations that were orders of magnitude higher than concentrations detected in sea turtles. A mixture of OCs at the average concentrations measured in loggerhead sea turtle adipose tissue did not alter the LP response. The *in vivo* results, although only correlative, suggest that current, chronic exposure to OC compounds may enhance the immune system of loggerhead

sea turtles, whereas the *in vitro* results indicate that much higher concentrations can result in immunosuppression.

INTRODUCTION

All species of sea turtle are protected by national or international agencies because they have suffered dramatic population declines. Specifically, the loggerhead sea turtle is protected as a threatened species by the U.S. Endangered Species Act, which mandates that conservation measures be taken to promote the recovery of this species. Certain conservation policies, such as requiring shrimpers to use turtle excluder devices, have helped some loggerhead populations, but others are still declining (TEWG 2000). The subpopulation that nests from North Carolina to northern Florida has been declining by 2 to 3 % each year for unknown reasons (TEWG 2000). Recovery of populations depends directly on survival of individuals which is influenced by the health of the immune system. For these reasons, it is important to assess sea turtle immune responses and understand whether their immunity is compromised by environmental contamination.

Organochlorine (OC) contaminants, such as polychlorinated biphenyls (PCBs), DDT, and other pesticides, have been measured in tissues of the loggerhead sea turtle (reviewed by Pugh and Becker 2001 and Keller and McClellan-Green in prep). Blood concentrations of OCs detected in 44 juvenile loggerhead sea turtles from North Carolina (5.56 ng/g wet weight (ww) total PCBs, 0.650 ng/g ww 4,4'-DDE; Keller *et al*. in prep a) were similar to levels observed in alligators (A*lligator mississippiensis*) from reference lakes in Florida but were lower than those measured in alligators from

a contaminated lake (Guillette *et al*. 1999). Loggerhead blood OC concentrations were also lower than snapping turtles (*Chelydra serpentina*) from the Great Lakes (de Solla *et al*. 1998). Adipose concentrations of the same loggerhead turtles (0.256 ug/g wm PCBs and 0.0644 ug/g wm 4,4'-DDE; Keller *et al*. in prep a) were 2 to 4 orders of magnitude lower than those found in snapping turtles from the Great Lakes or the Hudson River (Olafsson *et al*. 1983; Pagano *et al*. 1999).

Although OC concentrations were found to be relatively low in sea turtles, these compounds have been associated with modulation of immune function in many wildlife species (reviewed by Keller *et al*. 2000). For example, immunosuppression has been reported in fish from the Chesapeake Bay, in birds near the Great Lakes, and in marine mammals that inhabit environments that contain high levels of contaminants (Faisal *et al*. 1991; Grasman *et al*. 1996; Ross *et al*. 1996a). Caspian terns (*Sterna caspia*) from a contaminated site in Lake Huron exhibited elevated plasma PCB and 4,4'-DDE concentrations as well as suppressed PHA-skin responses compared to terns from a reference site (Grasman and Fox 2001). In bottlenose dolphins (*Tursiops truncatus*) from Sarasota, Florida, higher blood concentrations of PCBs and 4,4'-DDE correlated to suppressed LP responses (Lahvis *et al*. 1995). Even humans possessing higher blood PCB concentrations exhibited decreased interleukin-4 and IFNγ levels as well as lower counts of MHC class II positive T and B lymphocytes and monocytes (Daniel *et al*. 2001).

Mass mortality events involving tens of thousands of marine mammals are thought to be caused partly by exposure to high concentrations of OCs. It is thought that the increased mortality was due to an increased susceptibility to viral infections

(Ross *et al*. 1996a). The seals and dolphins that died during these viral epizootics were shown to have higher levels of OC contaminants than animals that survived, suggesting that OC-related immunosuppression increased their susceptibility to viral diseases (Hall *et al*. 1992; Aguilar and Borrell 1994). These circumstantial claims of immunosuppression in wildlife can be supported by laboratory studies. For example, harbor seals (*Phoca vitulina*) fed a diet of fish from the contaminated Baltic Sea demonstrated numerous signs of immunosuppression compared to a second group of seals fed fish from the less contaminated Atlantic Ocean (Ross *et al*. 1996a). Moreover, rats used in a follow-up study exhibited immunosuppression when they were fed the Baltic Sea fish compared to the Atlantic fish (Ross *et al*. 1996b; Ross *et al*. 1997). These studies show that OC contaminants may lead to population declines by suppressing immune functions and decreasing host resistance.

Although most of these studies have focused on immunosuppression, enhanced immune responses have also been noted in mammals and birds. Offspring of adult female white-footed mice (*Peromyscus leucopus*) that were exposed to an environmentally-relevant dose of Aroclor 1254 exhibited increased mitogen-induced proliferation of thymocytes and splenocytes (Segre *et al*. 2002; Wu *et al*. 1999). Significant, positive correlations were observed between TEQ values determined by the CALUX assay and LP responses to three mitogens (ConA, PHA, and LPS) in seals along the U.S. Atlantic coast (Shaw *et al*. 2002). Adult male American kestrels (*Falco sparverius*) that were fed a mixture of Aroclor 1248, 1254, and 1260 demonstrated an increased PHA-skin response (Smits *et al*. 2002). Furthermore, increased LP responses were observed in juvenile herring gulls (*Larus argentatus*) captured at

heavily contaminated sites in the Great Lakes compared to birds from reference sites (Croisant and Grasman 2002). Increasing total DDT concentrations in the brain of warbler chicks (*Prothonotaria citrea*) significantly correlated to stronger T-cell proliferation (Peden-Adams *et al*. 1997). Polycyclic aromatic hydrocarbon concentrations in fish environmentally exposed in the Chesapeake Bay have also been significantly correlated to elevated B-cell proliferation (Faisal *et al*. 1991). These studies reveal that OC contaminants can enhance immune functions in some wildlife species.

Few studies have assessed immune function in reptiles following exposure to contaminants. Juvenile American alligators exposed in the laboratory to TCDD exhibited suppressed B-cell proliferation (Peden-Adams 1999). *In ovo* exposure to a mixture of 2,4'-DDE and coumestrol resulted in suppressed T-cell proliferation, while a mixture of 2,4'-DDE and 4,4'-DDE increased T-cell proliferation in American alligators (Peden-Adams 1999). Alligators from Lake Apopka, which had higher concentrations of DDE in their blood compared to reference lakes (Guillette *et al*. 1999), had altered histology of the thymus and spleen (Rooney *et al*. in press), decreased cell counts in bone marrow, thymus, and spleen (Gross *et al*. 1996), enhanced mitogen-induced LP responses (Rooney 1998), and reduced antibody titers to sheep red blood cells (Gross *et al*. 1997). These potential contaminant-induced changes in immune functions could play a significant role in their disease resistance and may have contributed to the population declines seen in this lake (Gross *et al*. 1996). In addition, veterinarians have suspected OC contaminants as a contributing risk factor for increased infections seen in wild box turtles (*Terapene carolina*).

Chlordane and heptachlor epoxide concentrations in the liver of two diseased box turtles were 2 to 5 fold higher than the mean concentrations from four healthy, control turtles (Tangredi *et al*. 1997). Similarly, Holladay *et al*. (2001) reported that total OC concentrations in livers of box turtles with aural abscesses were 5 times greater than those without abscesses.

No studies to date have determined the effects of environmental contaminants on sea turtle immune functions. Other than weights of lymphoid organs, composition of blood leukocyte populations (Work *et al*. 1998), and measuring antibody titers (Herbst and Klein 1995), only one assay that measures immune function (mitogen-induced lymphoproliferation; LP) has been optimized for sea turtles (Work *et al*. 2000; Keller *et al*. in prep b). Using this assay, we previously showed that the loggerhead sea turtle immune system varies with seasons (Keller *et al*. in prep b). This was expected because the reptilian immune system typically fluctuates seasonally, depending upon changes in temperature, photoperiod, and/or endogenous steroid concentrations (Zapata *et al*. 1992). In addition to seasonal changes, disease states also influenced the strength of the LP response in green sea turtles (*Chelonia mydas*) that are afflicted with fibropapillomatosis (FP) (Cray *et al*. 2001; Work *et al*. 2001). Green turtles with increasing severity of FP in Hawaii demonstrated a 3- to 6-fold decrease in T-cell proliferation compared to turtles that were disease-free or had minor severity of the disease (Work *et al*. 2001). These turtles also exhibited reduced total protein and globulin concentrations. Green turtles in Florida with FP also had profoundly reduced T-cell and B-cell proliferation, a reduced percentage of

lymphocytes, an increased percentage of heterophils, and decreased plasma protein compared to turtles that were disease-free (Cray *et al*. 2001).

The effects of OCs on sea turtle immune functions have never been assessed, but should be for many reasons. These compounds are present in significant concentrations in loggerhead tissues. They alter immune functions in many species as shown by other wildlife and laboratory studies. Declines are still evident for some loggerhead sea turtle populations and the causes are unknown. In addition, survival of individuals and populations of sea turtles depends on fully functioning immune responses. For all of these reasons, this study examined how the LP immune response of juvenile loggerhead turtles was influenced by 1) OC contaminant concentrations measured in their tissues and 2) *in vitro* exposure to those OC contaminants.

MATERIALS AND METHODS

Sampling

Free-ranging juvenile loggerhead sea turtles with straight carapace lengths (SCL) between 49 cm and 77.3 cm were captured as by-catch from a pound net fishery located in Core Sound, North Carolina, USA in summer of 2001. Blood was collected within 10 minutes of capture from the dorsocervical sinus using double-ended Vacutainer needles directly into Vacutainer blood collection tubes containing sodium heparin (Becton, Dickinson, and Co., Franklin Lakes, NJ). Blood was kept cool either on ice or in a refrigerator until processed for LP. Turtles were tagged, measured, weighed, and released near their capture location. Twenty-four turtles captured in July 2001 were used for the correlations between OCs and LP. Blood samples were

shipped overnight to the Medical University of South Carolina for LP. Four to five turtles used in the *in vitro* exposures were captured in August 2001 and were tested for LP at the Duke University Marine Laboratory.

Contaminant Analysis

OC contaminants including PCBs and pesticides were determined and reported elsewhere (Keller *et al*. in prep a). Briefly, whole blood and fat biopsies were spiked with internal standards and extracted with organic solvents. Biological molecules of large molecular weight were removed from the extracts using either gel permeation chromatography or alumina columns. Compounds were separated by polarity using an aminopropylsilane column and quantified using gas chromatography with electron capture detection and mass spectrometry.

Mitogen-induced Lymphoproliferation

The LP response was measured as described elsewhere (Keller *et al*., in prep b). Briefly, WBCs were removed on the day following blood collection by a slow spin technique. Cells were rinsed once with MPA media which consisted of RPMI 1640 media (Mediatech, Inc., Herndon, VA) that was supplemented with 5% FBS (Hyclone, Logan, UT), and final concentrations of 1% (v/v) of 100x solution of non-essential amino acids (Gibco, Grand Island, NY), 1 mM sodium pyruvate (Gibco, Grand Island, NY), 10 mM HEPES (Mediatech, Inc., Herndon, VA), 50 IU/ml penicillin, and 50 µg/ml streptomycin (Mediatech, Inc., Herndon, VA). WBCs were counted by one person to maximize consistency. Cells were split into two tubes and diluted into two different media types, MPA media as described above or JMKA media which differed

from MPA media by only the FBS type (BioWhittaker, Walkersville, MD, lot #8S006F). Cells were plated at 1.8 x 10^5 cells/well into 96 well plates.

Phytohemagglutinin P (PHA; Amersham Pharmacia Biotech Inc., Pascataway, NJ) and concanavalin A (ConA) from Jack bean (*Canavalia ensiformis*) (Sigma, St. Louis, MO) were chosen as mitogens that stimulate T-cell proliferation. Lipopolysaccharide (LPS) from *Escherichia coli* of either serotype 0127:B8 or 0111:B4 (Sigma, St. Louis, MO) and phorbol 12,13-dibutyrate (PDB; Sigma, St. Louis, MO) were chosen as B-cell mitogens. PDB was shown to stimulate avian B-cells previously (Scott and Savage 1996). Mitogens were diluted in either MPA media or JMKA media and added to wells containing cells in the same media type. Triplicate wells were tested for each control (MPA or JMKA media only) and mitogen concentration. Final volumes were 100 ul/well for mitogens in JMKA media and 200 ul/well for mitogens in MPA media. Cells were incubated at 30 ^oC with 5% CO_2.

Following a 5-day incubation (120 hr) or a 4-day incubation (96 hr) with mitogens, 0.5 uCi of ^3H-thymidine (ICN Biomedicals, Inc., Irvine, CA) was added in a volume of 100ul to each well. After a 16 hr pulse of ^3H-thymidine, the cells were harvested onto Unifilter plates (Packard, Meridian, CT) using a Packard Filtermate™ 96-well plate harvestor, and the plates were allowed to dry. Once dry, 25 µL of Microscint™ 20 (Packard, Meridian, CT) was added to each well, and the samples were analyzed using a Packard Top Count™-NXT scintillation counter. Data were reported as the stimulation index (SI = cpm of stimulated cells/cpm of unstimulated cells).

In vitro exposures

The procedures for preparing and culturing cells and testing proliferation for the *in vitro* exposures were identical to those explained previously for an assay that utilizes 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyl tetrazolium (MTT), the MTT assay (Keller *et al*. in prep b). Cells were plated on the same day of blood collection at 1.8 x 10^5 cells/well and incubated at 30 ^oC in 5% CO_2 in JMKB media which consisted of RPMI 1640 media (Mediatech, Inc., Herndon, VA) supplemented with 5% FBS (BioWhittaker, Walkersville, MD lot # 8S006F), and final concentrations of 1% (v/v) of 100x solution of non-essential amino acids (Mediatech, Inc., Herndon, VA), 1 mM sodium pyruvate (BioWhittaker, Walkersville, MD), 10 mM HEPES (BioWhittaker, Walkersville, MD), 100 IU/ml penicillin, and 100 ug/ml streptomycin (Mediatech, Inc., Herndon, VA). Triplicate wells received JMKB media containing either no additives (control); vehicle concentrations matching those of the contaminant exposures (less than 0.71% methanol or 0.25% DMSO); individual contaminants; a mixture of contaminants; mitogens alone; mitogens plus single contaminants; mitogens plus the mixture of contaminants; and mitogens plus vehicles. The mitogen types and concentrations that were chosen for this segment of the study (ConA at 10 ug/ml of culture and PDB at 0.8 ug/ml of culture) were previously shown to result in the highest LP responses when the colorimetric MTT assay was employed (Keller *et al*. in prep b). The contaminants detected most frequently in loggerhead sea turtle tissues were chosen. Aroclor 1254 (a technical mixture of PCBs), heptachlor epoxide, mirex, and oxychlordane (Chem Service, West Chester, PA) were dissolved in

methanol, and 4,4'-DDE was in DMSO. These were tested singly and in combination at concentrations shown in the figures and legends.

Proliferation was measured on day 6 (140 hr) of incubation using a non-radioactive cell proliferation assay employing MTT (CellTiter 96, Promega, Inc., Madison, WI) using techniques described previously (Keller *et al*. in prep b). Stimulation index (SI) for the control cells were calculated as the corrected absorbance of stimulated cells divided by corrected absorbance of unstimulated cells. For the contaminant-exposed cells, SI was calculated as the absorbance of stimulated cells exposed to both the contaminant and mitogen divided by the absorbance of unstimulated cells that were exposed to the contaminant alone.

Statistics

All statistical analyses were performed using Systat 8.0 (SPSS Inc., Chicago, IL). Non-parametric correlations (Spearman Rank Correlations) were used because the contaminant data did not fit a normal distribution before or after transformation. Correlations were used to examine the relationship between LP measured using ^{3}H-thymidine and OC concentrations (ng/g lipid of tissue) determined in the adipose or blood. When a compound was below the analytical limit of detection (LOD), the concentration for that sample was set at half the LOD. However, when classes of OCs were summed, values below the LOD were set at zero. Analysis of variance (ANOVA) and the Least Significant Difference test were used to determine differences between control LP and LP after *in vitro* contaminant exposure. Paired t-tests were used to determine differences between control LP and LP after exposure to the mixture of contaminants.

RESULTS

Correlation between OC concentrations and LP

Some LP responses were significantly correlated to OC concentrations in the blood and adipose of loggerhead turtles (Table 6.1; Appendix Fig. A2 and A3). LP responses to PHA at 5 ug/ml correlated to adipose concentrations of sum PCBs, sum DDTs, dieldrin, and sum OCs. Likewise, LP stimulated by 4 days of exposure to 10 ug/ml of LPS was significantly correlated to sum DDTs and dieldrin concentrations in the turtle adipose samples. PDB-stimulated LP at 0.8 ug/ml was also positively correlated to sum PCBs, sum DDTs, and dieldrin adipose concentrations. In addition, these same mitogen types and concentrations were significantly correlated to concentrations of OC contaminants measured in the blood. ConA stimulation, however, did not correlate with the concentration of any contaminant in either tissue. The significant relationships between OC concentrations and LP were all positive, indicating that turtles with higher levels of contaminants exhibited an elevation in LP responses to some mitogens.

In vitro exposure to OCs

Exposure of loggerhead sea turtle WBCs to OC contaminants *in vitro* suppressed LP responses to ConA, but not to PDB (Figs. 6.1 and 6.2). The technical PCB mixture Aroclor 1254 reduced the LP response to ConA at 30 ug/ml (ppm) and 50 ug/ml. 4,4'-DDE reduced LP at 50 ug/ml, and mirex decreased ConA-stimulated LP at 50 ug/ml. No test concentration of heptachlor epoxide nor oxychlordane significantly altered the LP responses to either ConA or PDB. When cells were

exposed to a mixture of these OCs at concentrations similar to those found in loggerhead adipose tissue, LP responses were not altered (Fig. 6.3).

DISCUSSION

Correlation between OC concentrations and LP

All significant correlations between OC concentrations and LP responses had positive slopes, suggesting a possible immunoenhancing effect. Enhanced immune responses have been seen in laboratory exposed animals. For example, white-footed mice exposed *in utero* to environmentally relevant concentrations of PCBs exhibited increased mitogen-induced proliferation of thymocytes and splenocytes compared to control mice (Segre *et al*. 2002; Wu *et al*. 1999). Likewise, adult male American kestrels exposed to mixtures of PCBs showed an increased PHA-skin response (Smits *et al*. 2002). American alligators exposed *in ovo* to a mixture of 2,4'-DDE and 4,4'-DDE demonstrated significantly stronger LP responses than controls (Peden-Adams 1999).

Correlative studies with wildlife and humans also show significant, positive correlations between enhanced immune responses and OC contaminant exposure. In warbler chicks, higher DDT brain concentrations significantly correlated to stronger T-cell proliferation (Peden-Adams *et al*. 1997). Enhanced LP responses were seen in the alligators from Lake Apopka compared to alligators from reference lakes (Rooney 1998). Increased LP responses were observed in juvenile herring gulls captured at more heavily contaminated sites in the Great Lakes than at reference sites (Croisant and Grasman 2002). Moreover, Shaw *et al*. (2002) observed significant, positive

correlations between TEQ values measured by the CALUX assay and LP values using 3 mitogens (ConA, PHA, and LPS) in seals along the U.S. Atlantic coast. Yu-Cheng patients that were accidentally exposed to PCBs also demonstrated enhanced LP with PHA but not ConA one year after the incident (Lü and Wu 1985). The immunoenhancement seen in these previous laboratory and environmental studies support the conclusion that OCs were enhancing loggerhead immune responses.

In a previous study with the same loggerhead sea turtles, it was observed that the ratio of heterophils to lymphocytes was significantly and positively correlated with adipose concentrations of particular groups of PCB congeners and mirex (Keller *et al*. in prep c). An elevation in this ratio of WBCs is known as a common response to most stressors in birds and mammals as well as sea turtles (Maxwell and Robertson 1998; Aguirre *et al*. 1995). For example, this ratio increased in chickens when they were socially stressed or fed corticosterone (Gross and Seigel 1983). Likewise, diseased green sea turtles with fibropapillomatosis from Hawaii and Florida also exhibited an increase in this ratio compared to disease-free turtles (Aguirre *et al*. 1995; Work *et al*. 2001; Cray *et al*. 2001). In addition, OC concentrations were correlated with an elevation in the heterophil to lymphocyte ratio in herring gulls and Caspian terns from the Great Lakes (Grasman *et al*. 1996; Grasman *et al*. 2000) in the same way they did in the loggerhead turtles. The fact that OC concentrations in the loggerhead sea turtles were significantly correlated to two immune parameters (increased LP and an increase in the heterophil to lymphocyte ratio) provides additional evidence that the turtles with elevated OC exposure may be exhibiting signs of immunomodulation.

An interesting, but purely speculative, mechanism that links endocrine disruption with immunotoxicity may explain the immunoenhancing effect of OCs in these animals. Many of the OC contaminants measured in the sea turtle tissues possess estrogenic activity. Aroclor 1242, chlordane, *cis*-nonachlor, *trans*-nonachlor, heptachlor epoxide, heptachlor, oxychlordane, dieldrin, 2,4'-DDD, 2,4'-DDT, 2,4'-DDE, and 4,4'-DDD (singly or in combination) have been shown to bind directly to the alligator estrogen receptor (Vonier *et al*. 1996; Arnold *et al*. 1997; Guillette *et al*. 2002). Aroclor 1242, 4,4'-DDE, *trans*-nonachlor, *cis*-nonachlor, and chlordane exposure has also caused a reversal of the sex of red-eared slider turtles (*Trachemys scripta*) that were incubated at male-producing temperatures (Willingham and Crews 1999). Aroclor 1242 was thought to elicit this estrogenic response by inducing cytochrome P450 aromatase, the key enzyme responsible for the conversion of testosterone to estradiol (Willingham and Crews 2000). Thus, the OC contaminants detected in the loggerhead turtles have estrogenic activity in other reptiles.

It has been known for some time that females of many species generally have stronger immune responses and a lower rate of infection than males (Schuurs and Verheul 1990). This sex-specific difference may be partly due to the differential concentrations of sex steroids. Low concentrations of estrogen, such as the endogenous levels measured in normal females, increases immune functions, while higher concentrations suppress them (Hall and Goldstein 1984). This dose response relationship is described as hormetic and is commonly encountered in diverse toxicological studies (Calabrese and Baldwin 2003). It is possible that low-level, chronic exposure to estrogenic OCs, as seen in the alligators from Lake Apopka

(Rooney 1998) and in the sea turtles of the current study, may produce similar enhancing effects on the immune system.

In contrast, harbor seals and Caspian terns which exhibited immunosuppression (Grasman *et al*. 1996; Ross *et al*. 1996) have tissue OC concentrations above those measured in the loggerhead sea turtle (Keller and McClellan-Green in prep). These higher concentrations may fall within the suppressed portion of the hormetic curve and may explain why their immune functions were suppressed. The complex interactions within the neuro-endocrine-immune axis are only now beginning to be studied in laboratory model species. They are not well understood in wildlife. Further studies are needed to examine the mechanistic links between endocrine disrupting and immunotoxic compounds.

Clearly, a limitation of the current study is the fact that correlations are largely circumstantial, no causal relationship can be identified with certainty. Since intentional, experimental exposures of protected sea turtles to contaminants are not feasible, a more comprehensive suite of non-invasive immune assays is required. These tests may provide additional evidence relating contaminant exposure with altered immune parameters. Other non-invasive tests that could be developed in sea turtles include assays that more broadly assess innate, cell-mediated, and humoral immune functions, such as natural killer cell activity, mixed leukocyte reaction, antibody titers, oxidative burst, and an *in vitro* plaque forming cell assay.

In vitro exposure to OCs

The *in vitro* exposure concentrations of OCs required to suppress LP responses of loggerhead sea turtles were at least 2 orders of magnitude higher than the

concentrations observed in blood of both healthy loggerhead sea turtles and those that had impaired health (see arrows in Figs. 6.1 and 6.2; Keller *et al*. in prep c). PCBs suppressed T-cell responses at 30 ppm, whereas total PCB concentrations in the blood of healthy loggerhead sea turtles were 5.17 ppb and 127 ppb in the impaired loggerheads (Keller *et al*. in prep c). Similarly, 4,4'-DDE decreased the T-cell response at 50 ppm, but blood of healthy loggerhead turtles contained on average 0.568 ppb 4,4'-DDE, and impaired turtles had 14.9 ppb (Keller *et al*. in prep c). It is important to note that proliferation in the *in vitro* exposure experiments was measured using a less sensitive technique (Keller *et al*., in prep b). Therefore, these data serve only as a preliminary assessment of *in vitro* effects of OC compounds on loggerhead sea turtle LP. If the more sensitive radioactive ^{3}H-thymidine method had been used, it is possible that significant decreases may have been detected at lower OC concentrations. However, based on the preliminary data, sea turtles would have to accumulate much higher OC concentrations before this particular immune function would be suppressed.

In vitro exposures cannot replicate chronic exposure to these compounds at the whole individual level, and the lymphocytes used in these experiments are isolated from animals that are already exposed to environmental contaminants. Despite these unavoidable limitations, this technique is a useful tool for examining the sensitivity of species that cannot be exposed to harmful chemicals in captivity either for ethical, legal, or logistical reasons. This technique has been used with beluga whale (*Delphinapterus leucas*) splenocytes (De Guise *et al*. 1998) and lymphocytes of several marine mammal species and humans (Nakata *et al*. 2002). Some interesting

species differences can be discerned between the loggerhead sea turtle and mammals, but it should be noted that these differences may be attributed to differing choices of methods rather than species sensitivity. T-cell proliferation in the beluga whale was not affected by any 4,4'-DDE concentrations tested (10 to 100 ug/ml), whereas the sea turtle T-cell response was significantly reduced at 50 ug/ml. A mixture of PCB congeners 138, 153, and 180 at 5 ug/ml each (15 ug/ml total PCBs) significantly reduced beluga whale T-cell responses, whereas it took 30 ug/ml of a technical mixture of PCBs to reduce T-cell proliferation in loggerhead sea turtles. Based on these interlaboratory comparisons, sea turtles may be more sensitive to 4,4'-DDE and less sensitive to PCBs than marine mammals.

In conclusion, using LP, the only assay that has been developed for examining immune function in loggerhead sea turtles, we observed positive correlations between LP and OC concentrations in adipose and blood of loggerhead sea turtles. These results, although only correlative, are similar to the findings of many other wildlife studies and suggest that the sea turtle immune system may be modulated by environmentally-relevant concentrations of OC contaminants. Since these compounds are also known to possess estrogenic activity and low levels of estrogen can enhance immune functions, a potential mechanism should be investigated that links endocrine disruption and immunoenhancement. Future studies should develop other immune function assays in order to provide supporting evidence that OC compounds are affecting the sea turtle immune system.

Table 6.1. Mitogen-induced lymphoproliferation (LP) of loggerhead sea turtles and Spearman rank correlation coefficients (R_S) comparing LP to OC contaminants in fat biopsies and whole blood.

					LP vs. adipose OCs (R_s)					
Media	Mitogen (ug/ml culture	Day	Mean SI (SEM)[a]	N	Sum PCBs	Sum DDTs	Sum Chlordanes	dieldrin	mirex	Sum OCs
MPA	ConA (20)	4	3.94 (0.95)	17	-0.020	-0.066	-0.203	-0.017	-0.191	-0.066
	ConA (20)	5	2.47 (0.52)	21	-0.095	-0.112	-0.125	-0.101	-0.173	-0.113
	LPS (10)	4	3.41 (0.52)	17	0.473	0.515*	0.346	0.627*	0.444	0.414
	LPS (10)	5	3.01 (0.47)	21	0.257	0.316	0.303	0.313	0.255	0.234
	PDB (0.2)	4	4.52 (1.23)	17	0.100	0.051	0.054	0.228	0.169	0.061
	PDB (0.2)	5	2.84 (0.55)	21	0.053	0.078	0.095	0.213	0.097	0.042
JMKA	PHA (5)	5	114 (55)	16	0.541*	0.562*	0.394	0.544*	0.482	0.515*
	PHA (10)	5	29.1 (13.5)	13	-0.082	0.027	-0.044	0.209	0.033	-0.06
	ConA (10)	5	1.89 (0.37)	9	0.217	0.233	0.283	0.167	0.233	0.217
	ConA (20)	5	3.56 (0.70)	20	-0.125	-0.099	-0.123	-0.024	-0.119	-0.116
	LPS (2.5)	5	2.01 (0.39)	16	0.476	0.500	0.421	0.426	0.426	0.441
	LPS (5)	5	1.40 (0.25)	13	0.187	0.148	0.335	0.236	0.341	0.203
	PDB (0.2)	5	7.12 (2.88)	9	-0.033	0.067	0.183	-0.133	-0.067	-0.033
	PDB (0.4)	5	6.08 (1.73)	13	-0.209	-0.187	-0.027	-0.242	-0.126	-0.203
	PDB (0.8)	5	4.10 (1.28)	16	0.518*	0.594*	0.494	0.603*	0.462	0.488

					LP vs. blood OCs (R_s)					
Media	Mitogen (ug/ml culture	Day	Mean SI (SEM)[a]	N	Sum PCBs	Sum DDTs	Sum Chlordanes	dieldrin	mirex	Sum OCs
MPA	ConA (20)	4	3.94 (0.95)	19	-0.091	0.042	-0.096	0.223	-0.047	-0.086
	ConA (20)	5	2.47 (0.52)	24	-0.008	0.037	-0.062	0.116	0.073	-0.023
	LPS (10)	4	3.41 (0.52)	19	0.425	0.382	0.411	0.619*	0.244	0.426
	LPS (10)	5	3.01 (0.47)	24	0.274	0.190	0.217	0.335	0.174	0.256
	PDB (0.2)	4	4.52 (1.23)	19	0.049	-0.063	0.095	0.319	-0.019	0.053
	PDB (0.2)	5	2.84 (0.55)	24	0.073	0.032	0.150	0.059	-0.049	0.072
JMKA	PHA (5)	5	114 (55)	17	0.578*	0.434	0.449	0.297	0.456	0.566*
	PHA (10)	5	29.1 (13.5)	17	0.069	-0.012	-0.159	-0.032	-0.201	0.039
	ConA (10)	5	1.89 (0.37)	10	0.358	0.236	0.273	-0.091	0.139	0.345
	ConA (20)	5	3.56 (0.70)	24	-0.109	-0.207	-0.254	0.006	-0.158	-0.155
	LPS (2.5)	5	2.01 (0.39)	17	0.395	0.306	0.336	0.395	0.338	0.414
	LPS (5)	5	1.40 (0.25)	17	0.245	0.147	0.152	0.252	0.176	0.238
	PDB (0.2)	5	7.12 (2.88)	10	-0.055	-0.115	-0.091	-0.382	-0.176	-0.030
	PDB (0.4)	5	6.08 (1.73)	17	-0.140	-0.211	-0.208	-0.233	-0.289	-0.142
	PDB (0.8)	5	4.10 (1.28)	17	0.532*	0.576*	0.515*	0.203	0.505*	0.569*

[a] SI = Stimulation index (cpm stimulated cells/cpm unstimulated cells).

* denotes a significant correlation (p-value < 0.05).

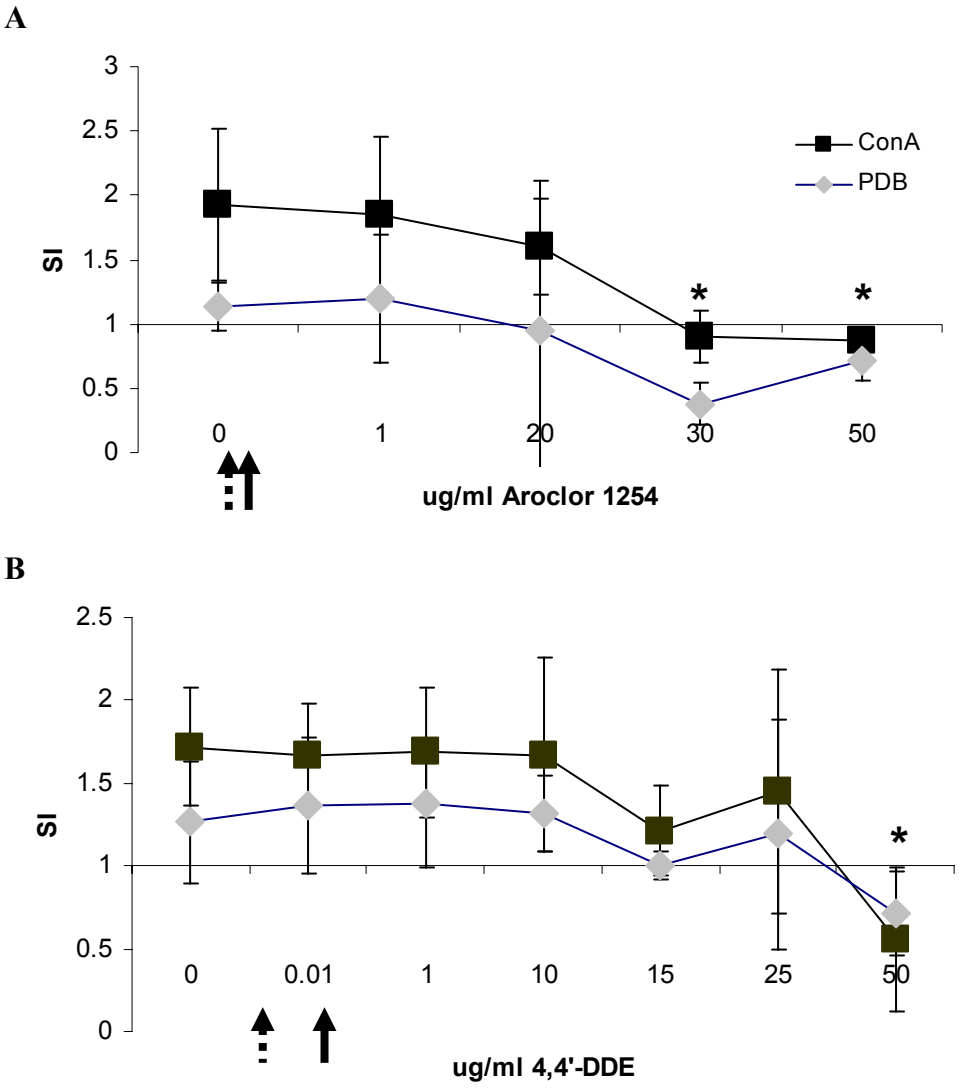


Figure 6.1. Effect of *in vitro* exposure to A.) Aroclor 1254 (a technical mixture of PCBs) and B.) 4,4'-DDE on loggerhead sea turtle mitogen-induced LP using the MTT assay. LP responses to ConA at 10 ug/ml of culture and to PDB at 0.8 ug/ml of culture were measured in 4 turtles captured in August using a non-radioactive MTT assay. Data are reported as mean ± SD; SI = stimulation index (absorbance of stimulated cells/absorbance of unstimulated cells); * denotes a difference from the control in ConA stimulated cells (ANOVA and Least Significant Difference test; $p < 0.05$). Arrows indicate average blood concentration (ug/g wet weight) measured in 47 healthy (broken arrows; 0.00517 ug/g total PCBs; 0.000568 ug/g 4,4'-DDE) and in 3 chronically ill (solid arrows; 0.127 ug/g total PCBs; 0.0149 ug/g 4,4'-DDE) juvenile loggerhead sea turtles from North Carolina (Keller *et al.*, in prep c).

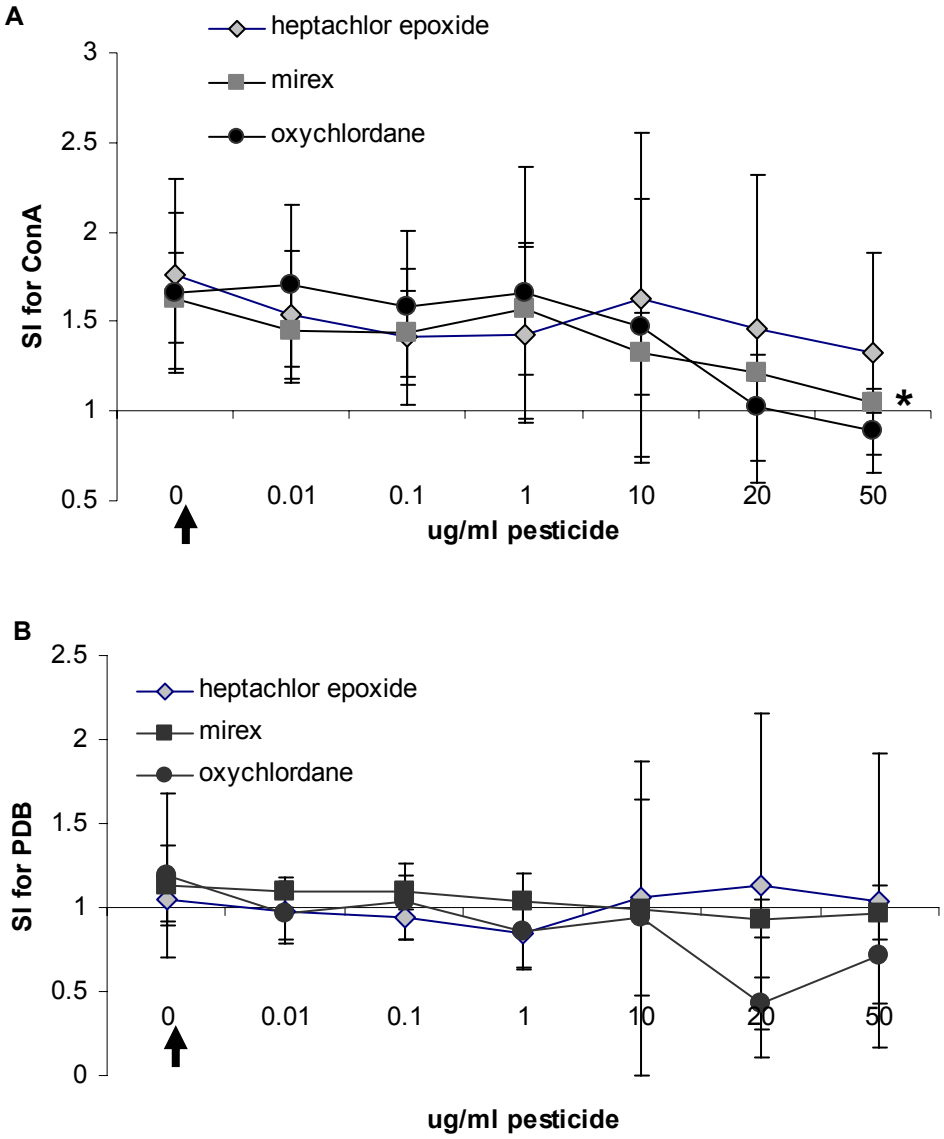


Figure 6.2. Effect of *in vitro* exposure to individual OC pesticides (heptachlor epoxide, mirex, and oxychlordane) on loggerhead sea turtle LP responses using the MTT assay with A.) ConA at 10 ug/ml of culture and B.) PDB at 0.8 ng/ml of culture. LP was measured in 4 turtles captured in August using a non-radioactive assay. Mean ± SD; SI = stimulation index (absorbance of stimulated cells/absorbance of unstimulated cells); * denotes a difference between mirex and the control (ANOVA and Least Significant Difference test; $p < 0.05$). For reference, average blood concentrations (wet weight basis) measured in 3 chronically ill juvenile loggerhead sea turtles from North Carolina were 0.000540 ug/g mirex, 0.000196 ug/g heptachlor epoxide, and 0.00118 ug/g oxychlordane (solid arrows, Keller *et al*., in prep c).

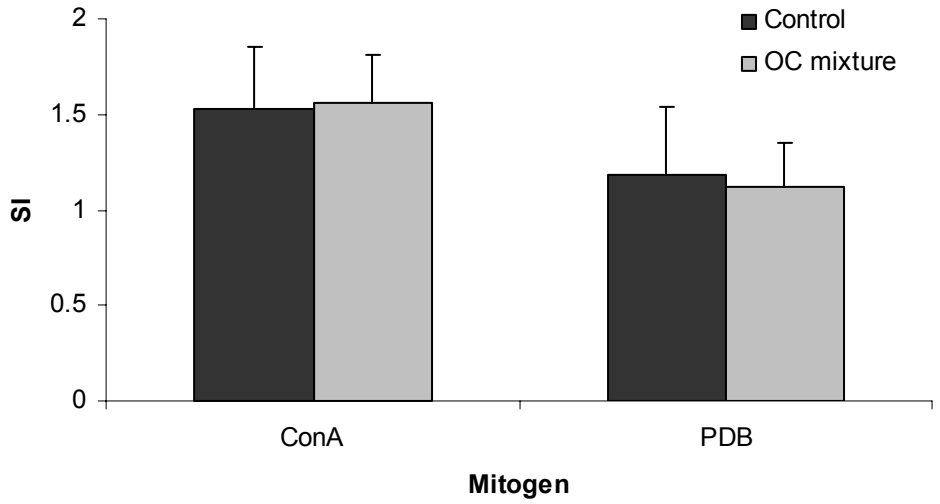


Figure 6.3. Effect of *in vitro* exposure to a mixture of OC contaminants at environmentally relevant concentrations on loggerhead sea turtle mitogen-induced LP using the MTT assay. Cells were exposed to a mixture of 0.1 ug/ml 4,4'-DDE, 0.93 ug/ml Aroclor 1254, 0.01 ug/ml oxychlordane, 0.01 ug/ml heptachlor epoxide, and 0.01 ug/ml mirex. LP responses to ConA at 10 ug/ml of culture and to PDB at 0.8 ug/ml of culture were measured in 5 turtles captured in August using a non-radioactive assay. Mean ± SD; SI = stimulation index (absorbance of stimulated cells/absorbance of unstimulated cells). Differences were not detected from the controls (paired t-test; $p < 0.05$).

CHAPTER SEVEN

Plasma vitellogenin in loggerhead sea turtles: description of normal reproductive physiology and a biomarker of estrogenic contaminant exposure.

ABSTRACT

Vitellogenin (VTG), a protein precursor to egg yolk, is expressed normally only in adult females in response to estrogen; however, males and juveniles can produce VTG when exposed to estrogenic compounds, including environmental contaminants. This study sought to measure plasma VTG in threatened loggerhead sea turtles (*Caretta caretta*) to 1) further our understanding of their reproductive maturation, and 2) to investigate VTG as a non-invasive biomarker of estrogenic contaminant exposure. Using a polyclonal antibody produced against VTG from the red-eared slider turtle (*Trachemys scripta*), we screened for the presence or absence of VTG in the plasma of 320 loggerhead sea turtles captured along the southeast coast of the U.S. which represented a wide range of size classes. We detected VTG in all 5 nesting females, in 90 % of females captured offshore that were greater than 87 cm straight carapace length (SCL), and in 73 % of large juvenile females between 77 cm and 87 cm SCL. VTG was present in the plasma of 2 % of smaller females (3 out of 155 females < 77 cm SCL) and in one turtle of unknown sex that was 68 cm SCL. This precocious VTG expression may be considered abnormal. As expected, none of the males were producing detectable VTG. Concentrations of polychlorinated biphenyls (PCBs) and organochlorine (OC) pesticides were measured in blood samples of two of the precocious females. On average, they had approximately double the OC concentrations than 36 other small juvenile females that were not producing VTG. A fat biopsy taken from one of the small precocious females had the second highest total PCB and 4,4'-DDE concentrations on a wet mass basis among 44 other juvenile turtles. These data suggest that loggerhead sea turtles inhabiting this region

of the Atlantic Ocean begin reproductive maturation around 77 cm SCL and that environmentally relevant concentrations of contaminants may be disrupting their endocrine system.

INTRODUCTION

Environmental contaminants have been shown to disrupt the endocrine and reproductive systems of a variety of wildlife, including marine and estuarine species (for reviews see Colborn *et al*. 1993; Oberdörster and Cheek 2000). In reptiles, endocrine disruption by organochlorine (OC) contaminants have been observed in snapping turtles (*Chelydra serpentina*) from the Great Lakes region and American alligators (*Alligator mississippiensis*) from lakes in Florida (de Solla *et al*. 1998; Guillette *et al*. 2000). These endocrine effects were linked to developmental abnormalities and possibly even population declines. These studies, however, did not investigate abnormal expression of vitellogenin, an egg yolk protein precursor that has been used as a biomarker of exposure to estrogenic compounds in other animals.

Vitellogenin (VTG) is a protein produced in the liver of egg-laying species and is deposited into developing oocytes. It is normally produced only by females of breeding age in response to increased levels of estradiol. However, males and juvenile females can produce VTG when they are exposed to an estrogen or to a contaminant that either mimics estrogen or increases the production of estrogen (Fig. 7.1). There have been numerous examples of abnormal VTG production in both laboratory and field studies. In fish, VTG expression was increased after exposure to effluents from sewage treatment plants or effluents from pulp and paper mills (Jobling *et al*. 2002; Mellanen *et al*. 1999). Fish (*Rutilus rutilus*) from rivers receiving sewage effluent that

were phenotypically male were shown to exhibit intersex and an increase in VTG production (Jobling *et al*. 2002). Frogs (*Xenopus laevis*) immersed in water containing 1 ppm OC pesticides (dieldrin and toxaphene) for 11 days showed elevated production of VTG (Palmer *et al*. 1998). Similar results have been seen in freshwater turtles. Male red-eared slider turtles (*Trachemys scripta*) injected with 1 ug/g estradiol or 250 ug/g 2,4'-DDT produced VTG, whereas the control males did not produce detectable levels of this protein (Palmer and Palmer 1995). Increased VTG production was observed in field-collected female painted turtles (*Chrysemys picta*) from two cattle ponds receiving high estrogen loads compared to females from a reference pond (Irwin *et al*. 2001). Males from these ponds were not producing higher VTG levels than males from the reference pond, which supported the hypothesis that the livers of male turtles need to be 'primed' with estrogenic signals before they will produce VTG.

Previous studies have shown that VTG can be induced and detected in sea turtles. Juvenile Kemp's ridley sea turtles (*Lepidochelys kempii*; 2 females and one male) injected with 1 mg/kg estradiol produced high levels of VTG from 1 to 31 weeks after the injection (Heck *et al.* 1997). VTG production peaked in these turtles at day 50 (Vargus 2000), and their blood continued to appear opaque and viscous until at least 11 weeks after the injection, even after serum estrogen had returned to undetectable levels (Heck *et al.* 1997). This strong and sustained response suggests that these turtles are highly sensitive to estradiol and that they either continue to produce VTG after estrogen levels have dropped or that VTG is stable in their blood. In captive breeding female Kemp's ridley sea turtles, plasma VTG levels peaked in

December prior to the mating season and dropped to low, but still detectable levels in summer months after nesting (Vargus 2000). Plasma VTG was also detectable in nesting females of olive ridley (*Lepidochelys olivacea*), loggerhead (*Caretta caretta*), leatherback (*Dermochelys coriacea*), green (*Chelonia mydas*), and black sea turtles (*Chelonia agassizii*), but was not detectable in juvenile hawksbill (*Eretmochelys imbricata*) sea turtles collected from Puerto Rico (Vargus 2000).

Sea turtles accumulate detectable concentrations of endocrine-disrupting compounds such as polychlorinated biphenyls (PCBs), DDT, chlordane, and metabolites (Keller *et al.* in prep). The levels of these contaminants are relatively low compared to concentrations in tissues of fish-eating wildlife, such as marine mammals and shorebirds, but the sensitivity of sea turtles to these compounds is completely unknown. Recent studies have shown that environmental contaminants, such as industrial compounds, herbicides, pesticides, and metals, can alter hormone production and reproductive structures in organisms at very low concentrations (i.e.; Sheehan *et al.* 1999, Hayes *et al.* 2002, Akingbemi and Hardy 2001). These previous studies and the fact that sea turtles have low levels of circulating estradiol (Owens and Morris 1985) as well as a marked VTG response to a single injection of estradiol (Heck *et al.* 1997) suggest that sea turtles may be highly sensitive to estrogenic compounds.

The overall goal of this study was to investigate the use of VTG as a biomarker of exposure to estrogenic contaminants in loggerhead sea turtles. To reach this goal, it was necessary to determine the threshold size at which female turtles begin vitellogenesis. Secondly, we identified precocious turtles as those that were smaller

than this threshold size and were expressing VTG, then compared their OC contaminant concentrations to normal juvenile turtles that were not expressing VTG.

MATERIALS AND METHODS

Sample collection

Blood was collected from 5 nesting female loggerhead sea turtles on Bald Head Island, North Carolina, U.S.A. (July 1998; June to July 1999). Additional blood samples were collected from 290 juvenile to adult loggerhead turtles that were captured from inshore waters of Core Sound, North Carolina (May to Nov. of 1998 through 2002; Appendix Table A3) and from offshore waters of South Carolina, Georgia, and Florida (summers of 2000 and 2002; Appendix Table A4). Blood samples were drawn immediately after capture from the dorsocervical sinus using double-ended needles and heparinized vacuum blood collection tubes (Becton, Dickinson, and Co., Franklin Lakes, NJ). A mixture of protease inhibitors was added to one tube of blood at 1.5 μg/ml leupeptin and 1.5 μg/ml aprotinin (final concentrations). Plasma was frozen at -80 ^{o}C for VTG analysis. A whole blood sample from each turtle was frozen at -20 ^{o}C for OC contaminant analysis. Fat biopsies for contaminant analysis were surgically removed from 44 juvenile loggerhead turtles from Core Sound, NC as described in Keller *et al*. (in prep). The turtles were tagged, measured, weighed, and released near the capture location.

Sex determination

A laparoscopy was performed on 42 juvenile turtles from Core Sound to determine their sex. The sex of the turtles that did not undergo this procedure was

determined based on plasma testosterone concentrations (Owens 1997) and tail lengths. Turtles with plasma testosterone concentrations less than 20 pg/ml were classified female, while those with concentrations above 30 pg/ml were categorized as males. Turtles exhibiting testosterone concentrations between 20 and 30 pg/ml were categorized as unknown sex. Large females often have testosterone concentrations above 30 pg/ml, so tail lengths were used to help determine the sex of these animals. As turtles reach reproductive maturity around 90 cm straight carapace length (SCL) measured from the nuchal notch to the posterior marginal notch, sexual dimorphisms become more prominent, especially tail length. Males that were greater than 88 cm SCL from this study displayed elongated tails of approximately 51 cm from the posterior tip of the plastron to the tip of the tail ($Tail_{P\text{-}T}$) and 9 cm from the cloaca to the tip of the tail ($Tail_{C\text{-}T}$). Females greater than 88 cm SCL had tail lengths of 23 cm and 6 cm, respectively.

Vitellogenin Analysis

We obtained a polyclonal antibody produced against VTG from a freshwater turtle, *T. scripta* (a gift from Dr. Kyle Selcer). The antibody was used to detect the presence or absence of VTG in loggerhead plasma. Plasma protein concentrations were determined according to Bradford (1976) using bovine serum albumin (BSA) as a standard.

The immunoblotting procedure was modified from Selcer and Palmer (1995). Plasma samples were diluted in sample buffer (5 % 2-mercaptoethanol, 2.3 % SDS, 62.4 mM Tris-HCl, pH 6.8, 0.02 % bromophenol blue, 10 % glycerol) and boiled for 4 min. Samples (50 ug protein) were separated on 5 % polyacrylamide gels (SDS-

PAGE). The proteins were transferred onto PVDF membranes at 70 V for 3 hours at 4 ^{o}C. The membranes were soaked in methanol, dried, and blocked overnight in phosphate-buffered saline (PBS) containing 3 % BSA. The membranes were washed in PBS ++ (PBS containing 0.1 % Tween 20 and 0.1 % BSA) and incubated in the *T. scripta* antibody (1:5000 dilution in PBS containing 0.1 % Tween 20 and 5 % BSA) for 2 hours. The membranes were washed in PBS ++ then incubated in donkey anti-rabbit secondary antibody (1:10,000) in PBS containing 0.1 % Tween 20 and 5 % BSA for 1 hour. The membranes were washed 3 times in PBS ++ and developed using ECL+plus Western Blotting Detection System (Amersham Biosciences, Buckinghamshire, England). Detection was performed by autoradiography. If there was uncertainty as to whether VTG was present or absent, the samples were re-screened using 60-75 ug of sample protein.

Organochlorine Contaminant Analysis

OC compounds including PCBs and pesticides were previously determined in fat biopsies and whole blood samples of 44 juvenile turtles captured from Core Sound (Keller *et al.* in prep). Blood samples were analyzed from additional turtles from Core Sound and turtles caught offshore of SC, GA, and FL using identical techniques. OC concentrations from a subset of these turtles are reported in this study.

RESULTS

The *T. scripta* antibody detected a large protein, approximately 218 kDa, in all five nesting female loggerhead turtles sampled (Fig. 7.2). This protein was of similar size to VTG in a positive control, which was plasma from an estrogen-treated *T.*

scripta. The size and immunoreactivity of this loggerhead protein suggested that it was VTG. A similar size protein was not recognized in the negative control sample from a male *T. scripta*. The antibody cross reacted with other lower molecular weight proteins in the loggerhead plasma suggesting that, in its present form, the antibody cannot be used in an ELISA (data not shown).

In order to use VTG as a biomarker, it was necessary to determine at what age (or size) female loggerhead turtles begin to normally produce VTG. Immunoblots of plasma from female turtles that ranged from 49 cm to 95.9 cm SCL are shown in Figure 7.3. Most females greater than 77 cm SCL were expressing VTG.

A total of 315 loggerhead plasma samples were screened for the presence of VTG (excluding the 5 nesting females). These included 84 males, 155 females smaller than 77 cm SCL, 25 females greater than 77 cm SCL, and 51 turtles of unknown sex. In Figure 7.4, we show the percentage of turtles within each sex and size category that tested positive for the presence of plasma VTG. Capture information, testosterone concentrations, and morphometrics for turtles that were expressing VTG are given in Table 7.1. Ninety percent of the females greater than 87 cm SCL, 73 % of the females ranging in size from 77 cm to 86.9 cm SCL, and very few of the smaller females were expressing VTG. These data suggest that the majority of female turtles begin vitellogenesis around 77 cm SCL. Interestingly, one large female was not expressing VTG. This turtle (ID 2057; 95.5 cm SCL) was captured in waters offshore of Florida on July 31, 2000, with a female plasma testosterone concentration (15 pg/ml) and a short tail length (23.0 cm $Tail_{P\text{-}T}$ and 5.5 cm $Tail_{C\text{-}T}$).

As expected, none of the males were expressing VTG (Fig. 7.4). One turtle, that was initially classified as a male based on testosterone levels, was expressing VTG (turtle ID 2037; Table 7.1). This turtle was 76.9 cm SCL, which is very close to the 77 cm threshold for female VTG production. The tail length of this turtle was shorter than any other male in this size class and similar to observed tail lengths of females. Because of the short tail length and positive VTG expression, this turtle was re-classified as a female and included it in the female data in Figure 7.4.

The presence of VTG helped to verify the sex of 5 other turtles (Table 7.1). The sex of these turtles was initially classified as unknown, because either their plasma testosterone concentrations were not measured (ID 0016) or they fell within the range of 20 to 30 pg/ml (ID's 2289 and 4026). Other adult-sized turtles were predicted to be males based on testosterone titers, but they were expressing VTG and had female tail lengths (ID's 0130 and 0139). These large turtles (> 77 cm SCL) were re-classified as females based on their expression of VTG and/or their short tail lengths.

VTG was detected in 3 of the 155 (2 %) female turtles smaller than 77 cm SCL and in one of the 15 turtles of unknown sex between 67 cm and 77 cm SCL (Fig. 7.4). Figure 7.5 depicts the immunoblots that contain these animals. Two of these turtles were captured twice (ID's 3-43 and 5-37) and were expressing VTG at both sampling times. The size of these turtles (Table 7.1) categorized them well within the juvenile size range and not of breeding size (Miller 1997). Based on their small size and the 77 cm threshold, the presence of VTG in these turtles may be considered abnormal. Turtle ID 2037 (described above as being initially classified as male) was not

considered abnormal, because its length (76.9 cm) was virtually at the threshold. The 4 small turtles were captured in geographically distinct areas from each other and over three years, therefore they were not clustered together at the time of sampling (Table 7.1).

Blood OC concentrations were determined for 2 of the 4 precocious turtles (ID's 3-43, which was sampled twice, and 0024) that were producing VTG (Table 7.2). Their mean blood concentrations were approximately twice the concentrations measured in blood from 36 size-matched females (< 77 cm) that were not producing VTG. We also analyzed a fat biopsy from the smallest female (turtle ID 3-43). She had the second highest adipose concentrations of 4,4'-DDE and total PCBs on a wet mass basis among the 44 turtles that were analyzed (Table 7.3; Keller *et al.* in prep).

DISCUSSION

The assessment of plasma VTG in this study provides a better understanding of the reproductive physiology of female loggerhead sea turtles. All nesting loggerhead females screened in the current study were expressing VTG. Female loggerheads are known to finish yolking all clutches of eggs prior to migrating to the mating and nesting areas (Miller 1997). The circulating VTG observed in these nesting turtles may be remnant from this previous vitellogenesis or constitutively expressed at low levels. The presence of VTG is not surprising as previous studies demonstrated that VTG was present in plasma of nesting loggerheads and 4 other sea turtle species (Vargus 2000), and that Kemp's ridley sea turtles sustain VTG levels above baseline 7 months after a single estrogen injection (Heck *et al.* 1997).

Not all breeding-size females caught offshore were expressing VTG. The absence of VTG in one female (turtle ID 2057; 95.5 cm SCL) could be the result of several factors. Loggerhead turtles take at least a year off between nesting seasons (Miller 1997), thus there may be times when VTG drops so low that it is undetectable by our methods. Vargus (2000) observed a cyclical pattern of VTG expression in nesting Kemp's ridley sea turtles with the lowest VTG concentrations occurring during the summer months of the nesting season. It would be reasonable to assume that this female may have laid her final clutch of eggs for the season just prior to our sampling. This turtle was captured on the last day of July in waters offshore of Florida. The peak nesting for loggerhead turtles in Florida is June to July, so it is plausible that this turtle had finished laying her final clutch for the year (Hanson *et al.* 1998). In addition, testosterone levels drop step-wise in females with each clutch laid. Concentrations as high as 300 pg/ml at the beginning of the nesting season decrease to less than 20 pg/ml after the last clutch is laid (Owens 1997). The testosterone concentration in this turtle was low (15 pg/ml), which suggests that she had either finished laying eggs for the season or that she did not nest that summer. Secondly, it is possible that this large (95.5 cm SCL), presumably old, female is past her reproductive age. The size or age at which sea turtles stop nesting and begin senescence is unknown, although females of this size or greater have been seen on nesting beaches (Parham and Zug 1997). A third possible explanation for the lack of VTG may be that this turtle was not capable of vitellogenesis due to a genetic or environmental cause. Her blood concentrations of OC contaminants were higher than any other turtle examined in this study (Table 7.2). If she had never produced eggs, it

is likely that over a lifetime she could have accumulated OC levels greater than other turtles. Adult females of many species have lower concentrations than juveniles or adult males due to the maternal transfer of contaminants to eggs, milk, or tissues of offspring (Beckman *et al.* 1999; Pagano *et al.* 1999; Bargar *et al.* 2001). It is unknown whether these levels could cause reproductive failure in sea turtles, but this should be the focus of future studies.

The ability to determine the sex of turtles is critical for sea turtle biology and conservation, but it has been a difficult challenge. The sex of juvenile turtles cannot be distinguished using external morphology, therefore studies have been forced to rely solely on plasma testosterone to predict sex ratios (Owens 1997). The findings of this study suggest that using VTG analysis in conjunction with testosterone and tail length measurements can help identify the sex of certain turtles that would otherwise be classified incorrectly or as unknown. VTG expression helped to categorize 6 large turtles as female. The presence of VTG in 3 turtles that could not be classified by testosterone levels strongly suggested that these animals were female. Two large turtles had testosterone concentrations greater than 30 pg/ml, which is typical for adult females (Owens 1997). Their tail lengths suggested that they were females, and their VTG expression further supported this determination. One smaller turtle (turtle ID 2037) that was expressing VTG was initially classified as a male based on testosterone concentrations (36.0 pg/ml). Due to its size (76.9 cm) being smaller than adults, tail length was not taken into account during the initial sexual classification of this animal. Typically tail length does not become sexually dimorphic until approximately 88 cm SCL. However, its expression of VTG prompted us to more thoroughly examine the

sex of this animal. During closer examination of all data, we found that the tail was shorter than other males of this size. Because of its expression of VTG, shorter tail, and fairly low testosterone for a male, we determined that this turtle was incorrectly classified. The use of VTG and tail length data in conjunction with plasma testosterone may increase the predictive ability of sexing these animals.

VTG analysis may help to determine the sex of some animals but not without limitations. It is only useful for large turtles that are expected to be producing VTG. In loggerhead turtles from the southeast coast of the U.S., 77 cm SCL appears to be that threshold. It is likely, though, that this size threshold may differ with species, season, or geographic location. In addition, VTG can help to rule out a male classification but not a female one. In other words, a large turtle that is not expressing VTG is not necessarily a male, because not all of the large females were producing VTG. Another limitation of VTG as a sexing tool is the influence of contaminants on the expression of this protein. Although the current study did not observe VTG expression in males, it is not entirely improbable.

These data also help to describe reproductive maturation in the loggerhead sea turtle. A better understanding of their basic biology will help researchers predict when individual turtles will join the reproductive age class. Based on the samples screened in this study, female loggerhead sea turtles along the southeast coast of the U.S. may enter reproductive maturation around 77 cm SCL. From observations taken during necropsies, 77 cm SCL coincides with the timing that follicles begin to grow (David Owens, pers. observation). Although one nesting loggerhead was recorded at 74 cm SCL (Frazer and Ehrhart 1985), the average length of nesting females reported along

the U.S. Atlantic coast is 87 to 92 cm (Miller 1997; Frazer and Ehrhart 1985). This disconnect indicates that juvenile females begin to produce VTG prior to active reproduction.

These findings not only further the understanding of basic reproductive biology of sea turtles, but also support the hypothesis that OC contaminants may disrupt their endocrine system. Approximately 2 % to 3 % of the juvenile females (smaller than 77 cm SCL) were 'abnormally' producing VTG. The cause for this precocious expression was unknown but could be explained by a number of reasons. These turtles may be experiencing precocious puberty whereby they begin to produce higher levels of endogenous estradiol at an earlier age (or smaller size). This, in turn, could signal the production of VTG. Exposure to exogenous estrogens may also explain the early VTG production in these small turtles. It is possible that these animals had recently consumed prey items that had high concentrations of estrogens. However, as observed in fish, uptake of estrogens through the digestive tract may not be sufficient to induce VTG (Frederick *et al.* 2002). Exposure to estrogenic contaminants may be another possible cause for the production of VTG in these small turtles. The average blood concentrations of OC contaminants were higher in the 2 VTG-expressing juveniles than in 36 size-matched normal females. This finding, in addition to the high fat concentrations of total PCBs and 4,4'-DDE in the smallest precocious female, suggests that OC contaminants may induce VTG and cause other endocrine-related effects.

Environmental OC contaminants have been shown to disrupt the endocrine and reproductive systems of other reptile species. A sexually dimorphic tail measurement

was shown to be feminized in male snapping turtles collected from sites in the Great Lakes region that were more heavily contaminated with OC compounds (de Solla *et al*. 1998). Additionally, a population of American alligators inhabiting Lake Apopka, Florida, declined drastically following a pesticide spill which included DDT (Guillette *et al*. 2000). The juvenile alligators from this lake show signs of endocrine disruption, including decreased plasma testosterone levels and smaller penis sizes (Guillette *et al*. 2000). However, these previous studies did not examine VTG.

The data reported in the current study support the hypotheses that VTG may be used as a biomarker of exposure to estrogenic contaminants in sea turtles, and that OC contaminants may be disrupting their endocrine system. However, the findings in the current study are solely circumstantial and are based on only a small number of precocious animals. Future studies should screen more juveniles for VTG expression and determine OC concentrations in tissues of all of the precocious animals. Captive exposure studies could definitively test these hypotheses, but legal, ethical, and logistical reasons may prevent these experiments. Therefore, a weight-of-evidence approach, incorporating additional field studies that examine other sea turtle species or populations, are needed to conclusively determine the influence of OC contaminants on the sea turtle endocrine system.

On a final note, only 2 % to 3 % of the juvenile females were precociously expressing VTG. This may initially seem insignificant, but the loggerhead sea turtle is a threatened species, and the northern nesting subpopulation has been declining by 2 % to 3 % per year (TEWG 2000). In light of this decline, a disruption of the endocrine system of 2 % to 3 % of the juvenile females may be significant at the

population level. It is currently not known if precocious VTG expression directly leads to future reproductive problems in sea turtles, but this has been noted in fish (Cheek *et al*. 2001). Fish exposed to an estrogenic contaminant (2,4'-DDT) for 8 weeks exhibited VTG induction and reduced fertility and hatching success. The question of whether contaminants are contributing to the decline of this sea turtle population is important and still remains to be answered.

In conclusion, this is the first study to describe VTG expression in any sea turtle species in relation to size (as a proxy for age) of juveniles and in relation to contaminant concentrations. We have shown that most juvenile females from the southeast coast of the U.S. enter vitellogenesis at 77 cm SCL. The detection of VTG in these larger females helps to describe the transition from juveniles to adults. Determining this threshold size is important in order to use VTG as a biomarker of contaminant exposure. Four turtles smaller than the threshold were producing VTG, and their blood and fat concentrations of OC contaminants were higher than normal turtles. These findings suggest that the endocrine system of loggerhead sea turtles may be sensitive to environmentally relevant concentrations of OC contaminants.

Table 7.1. Information on female loggerhead sea turtles that were expressing vitellogenin (VTG).

Turtle ID	Capture date	Capture location	T (pg/ml)[b]	SCL[c]	$Tail_{P-T}$ (cm)[d]	$Tail_{C-T}$ (cm)[d]
3-43[a]	6/2/2000	Core Sound, NC	4.8	52.3	NT[e]	NT
3-43[a]	8/11/2000	Core Sound, NC	8.3	53.7	NT	NT
0024	6/13/2000	offshore SC	8.0	61.1	13.4	3.8
4105	6/24/2002	offshore GA	7.5	66.0	11.1	3.1
5-37[a]	10/21/2002	Core Sound, NC	NT	68.1	NT	NT
5-37[a]	11/1/2002	Core Sound, NC	NT	68.0	NT	NT
2037	7/19/2000	offshore GA	36.0	76.9	14.9	2.9
1385	7/13/2001	Core Sound, NC	9.1	77.3	NT	NT
0029	6/15/2000	offshore SC	15.0	77.8	15.7	3.6
2289	7/18/2002	offshore GA	24.2	79.4	17.6	4.9
2225	6/19/2002	offshore GA	18.2	79.5	18.1	3.9
4080	6/6/2002	offshore SC	4.7	80.0	20.0	6.3
2302	7/23/2002	offshore GA	15.7	80.9	22.0	5.6
2080	8/4/2000	offshore GA	16.0	81.0	19.4	4.0
4026	7/24/2000	offshore SC	28.0	81.5	18.8	4.3
4091	6/13/2002	offshore SC	3.9	83.9	18.5	4.6
0026	6/13/2000	offshore SC	9.0	85.2	17.0	3.7
0126	6/18/2002	offshore SC	11.9	86.7	24.0	5.5
2062	8/1/2000	offshore FL	0.0	87.5	17.6	4.5
0042	7/6/2000	offshore SC	6.0	87.9	18.8	5.1
0016	6/7/2000	offshore SC	NT	88.6	23.7	5.4
2018	7/12/2000	offshore GA	2.0	89.2	24.0	5.1
0030	6/20/2000	offshore SC	18.0	91.9	23.5	5.0
0130	6/19/2002	offshore SC	86.0	92.3	24.1	7.8
4090	6/13/2002	offshore SC	8.1	93.0	19.3	5.7
2216	6/17/2002	offshore GA	7.4	97.9	31.0	7.0
0139	6/27/2002	offshore SC	101.3	103.5	19.2	4.5

[a] These turtles were recaptured and sampled twice.
[b] T = plasma testosterone. When T was not available, sex was determined using VTG expression and tail length.
[c] SCL = straight carapace length from nuchal notch to posterior marginal notch.
[d] $Tail_{P-T}$ = tail length from posterior margin of plastron to tip of tail; $Tail_{C-T}$ = from cloaca to tip of tail.
[e] NT = not tested.

Table 7.2. Blood organochlorine contaminant concentrations in loggerhead sea turtles that were 'abnormally' expressing VTG compared to normal juveniles that were not expressing VTG.

	Individual 'abnormal' turtles				Precocious females[b]	Small normal females[c]	
Turtle ID	3-43[a]	3-43[a]	0024	2057			
Capture date	6/2/2000	8/11/2000	6/13/2000	7/31/2000	Summer 2000	Summers 2000 - 2002	
N[d]	1	1	1	1	2	36	
SCL[d]	52.3	53.7	61.1	95.5	< 77 cm	< 77 cm	
Sex	F	F	F	F	F	F	
VTG[d]	+	+	+	-	+	-	
pg/g ww					Mean	Mean (SD)	Median (range)
Total PCBs	11400	9870	3250	14500	6940	4540 (3610)	3580 (121 - 13700)
mirex	59.2	25.1	42.5	359	42.3	27.0 (38.5)	14.5 (<LOD - 178)
diedrin	<LOD[e]	41.0	<LOD	156	10.3	29.4 (48.3)	<LOD (<LOD - 244)
Total chlordanes	169	159	91.3	692	128	171 (113)	147 (<LOD - 432)
4,4'-DDE	867	625	278	2550	512	519 (502)	319 (<LOD - 1770)
Total DDTs	902	625	319	2550	541	534 (507)	337 (<LOD - 1770)
Total OCs	12500	10700	3700	18300	7660	5310 (4080)	4350 (168 - 14300)
% lipid	0.165	0.304	0.094	0.081	0.164	0.240 (0.0885)	0.222 (0.0898 - 0.440)
ng/g lipid					Mean	Mean (SD)	Median (range)
Total PCBs	6890	3250	3460	17900	4270	2360 (2980)	1560 (41.0 - 15000)
mirex	35.9	8.26	45.3	443	33.7	14.9 (33.4)	6.35 (<LOD - 196)
diedrin	<LOD	13.5	<LOD	193	3.38	13.3 (20.9)	<LOD (<LOD - 89.7)
Total chlordanes	103	52.3	97.3	854	87.4	79.8 (58.5)	65.1 (<LOD - 254)
4,4'-DDE	526	206	296	3150	331	231 (229)	144 (<LOD - 994)
Total DDTs	547	206	340	3150	358	239 (233)	154 (<LOD - 1020)
Total OCs	7590	3530	3950	22600	4750	2720 (3200)	1710 (57.1 - 15700)

[a] Turtle 3-43 was recaptured and sampled twice.
[b] Means were calculated using turtles 3-43 (the average of both samples) & 0024, which were below 77 cm SCL and precociously producing VTG.
[c] Combined female turtles that were below 77 cm SCL, but were not producing VTG.
[d] N = sample size; SCL = straight carapace length; VTG = presence (+) or absence (-) of plasma vitellogenin.
[e] <LOD = below the analytical limit of detection.

Table 7.3. Adipose organochlorine contaminant concentrations in a juvenile loggerhead sea turtle that was precociously expressing VTG compared to normal juveniles that were not expressing VTG.

	ID 3-43	Normal Juveniles	
Capture date	8/11/2000	Summers 2000 & 2001	
N[a]	1	43	
SCL[a]	53.7	45.7 - 74	
Sex	F	M and F	
VTG[a]	+	-	
ng/g ww	ng/g ww	Mean (SD)	Median (range)
Total PCBs	1080	237 (240)	169 (8.00 - 1360)
mirex	13.6	4.19 (3.97)	3.34 (<LOD[b] - 18.8)
diedrin	10.6	4.76 (4.01)	3.54 (<LOD - 16.7)
Total chlordanes	65.9	26.0 (20.7)	20.3 (<LOD - 87.8)
4,4'-DDE	221	60.8 (60.8)	43.2 (<LOD - 273)
Total DDTs	242	62.9 (64.0)	43.2 (<LOD - 287)
Total OCs	1420	341 (317)	263 (9.38 - 1680)
% lipid	57.2	25.5 (20.3)	25.8 (0.255 - 64.7)
ng/g lipid	ng/g lipid	Mean (SD)	Median (range)
Total PCBs	1890	2020 (2990)	938 (61.3 - 18800)
mirex	23.7	44.1 (62.8)	17.7 (<LOD - 289)
diedrin	18.6	35.7 (88.2)	19.0 (<LOD - 584)
Total chlordanes	115	249 (416)	217 (<LOD - 2620)
4,4'-DDE	387	447 (651)	246 (<LOD - 3280)
Total DDTs	423	453 (651)	254 (<LOD - 3280)
Total OCs	2480	2820 (4000)	1490 (88.2 - 25000)

[a] N = sample size; SCL = straight carapace length; VTG = presence (+) or absence (-) of plasma vitellogenin.
[b] <LOD = below the analytical limit of detection.

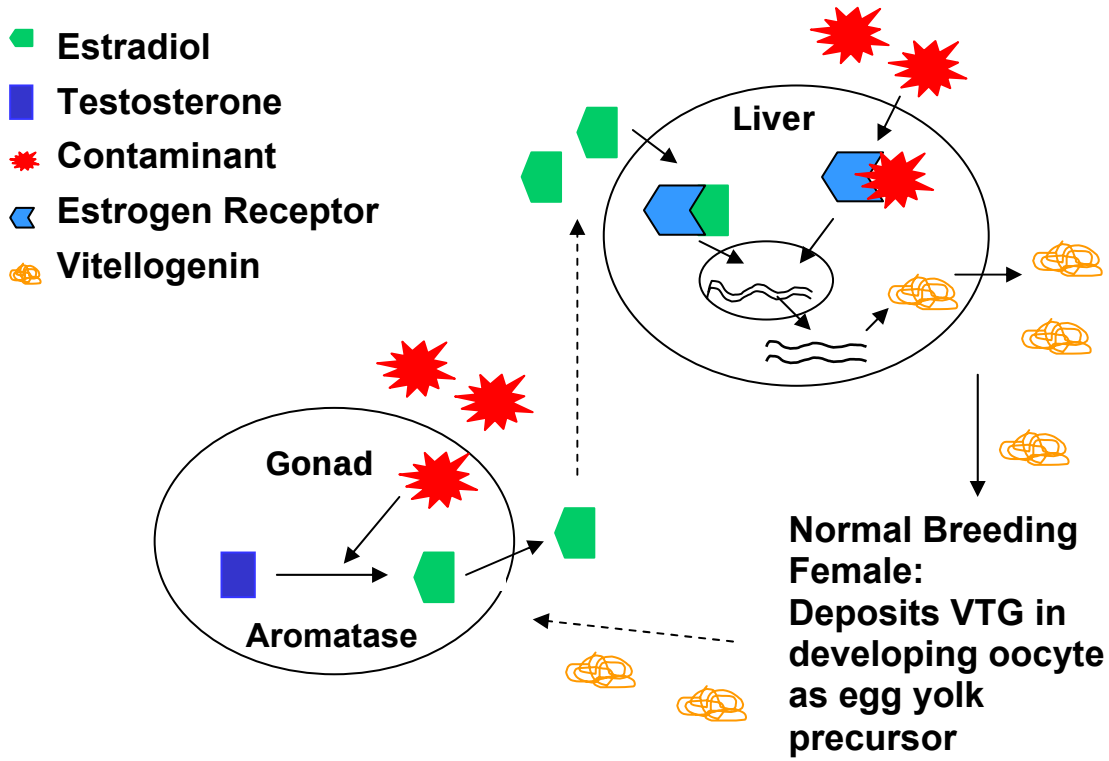


Figure 7.1. Mechanistic pathway of the normal and abnormal induction of VTG in oviparous species. In a normal, adult female, estradiol is produced in the ovaries by the enzyme cytochrome P450 aromatase. Estradiol can enter circulation and elicit estrogenic effects at target tissues, such as the liver shown here. Estradiol bound to the estrogen receptor acts as a transcription factor to turn on estrogen-responsive genes, such as VTG. Circulating VTG is then deposited into follicles as a protein precursor to egg yolk. Contaminants can induce the abnormal production of VTG in juveniles and males by many mechanisms, only two are shown. Contaminants may bind directly to the estrogen receptor, thereby mimicking the function of endogenous estradiol. Secondly, contaminants may increase the production of endogenous estrogen by increasing the expression or activity of aromatase. This increased production of estrogen may then turn on the production of VTG.

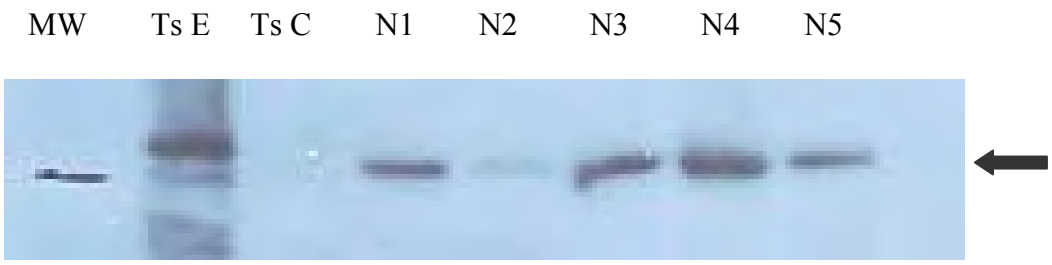


Figure 7.2. Immunoblot of plasma from nesting female loggerhead sea turtles using a polyclonal antibody raised against vitellogenin from a freshwater turtle, *T. scripta*. MW = molecular weight marker of 185 kDa; Ts E = estrogen-induced *T. scripta* (positive control); Ts C = control male *T. scripta* (negative control); N = five nesting female loggerhead sea turtles from Bald Head Island, NC.

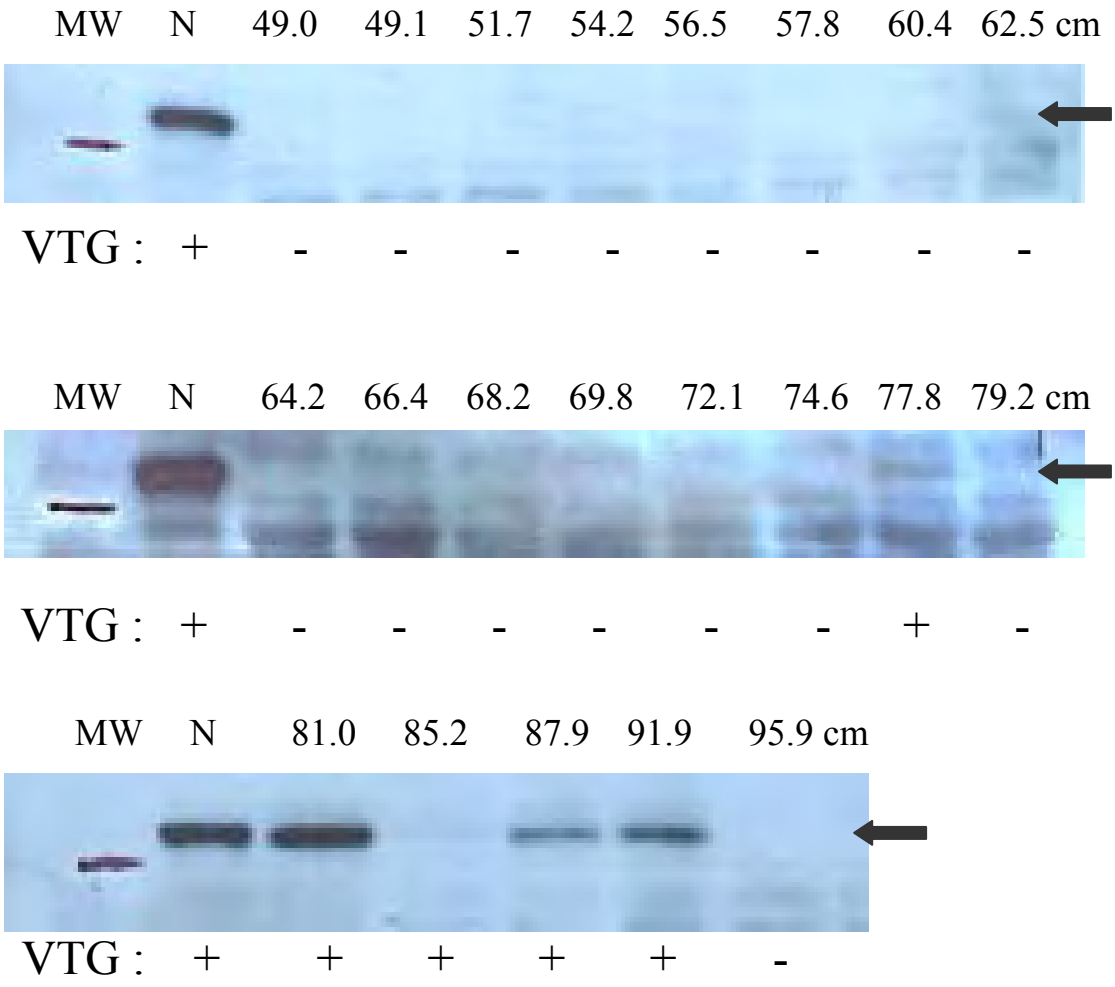


Figure 7.3. Immunoblots of plasma from female loggerhead sea turtles that ranged in size from 49.0 cm to 95.9 cm SCL. Size of each turtle is listed above each lane. MW = molecular weight marker of 185 kDa; N = nesting female loggerhead sea turtles served as a positive control on each gel. Presence (+) or absence (-) of VTG is depicted under each lane.

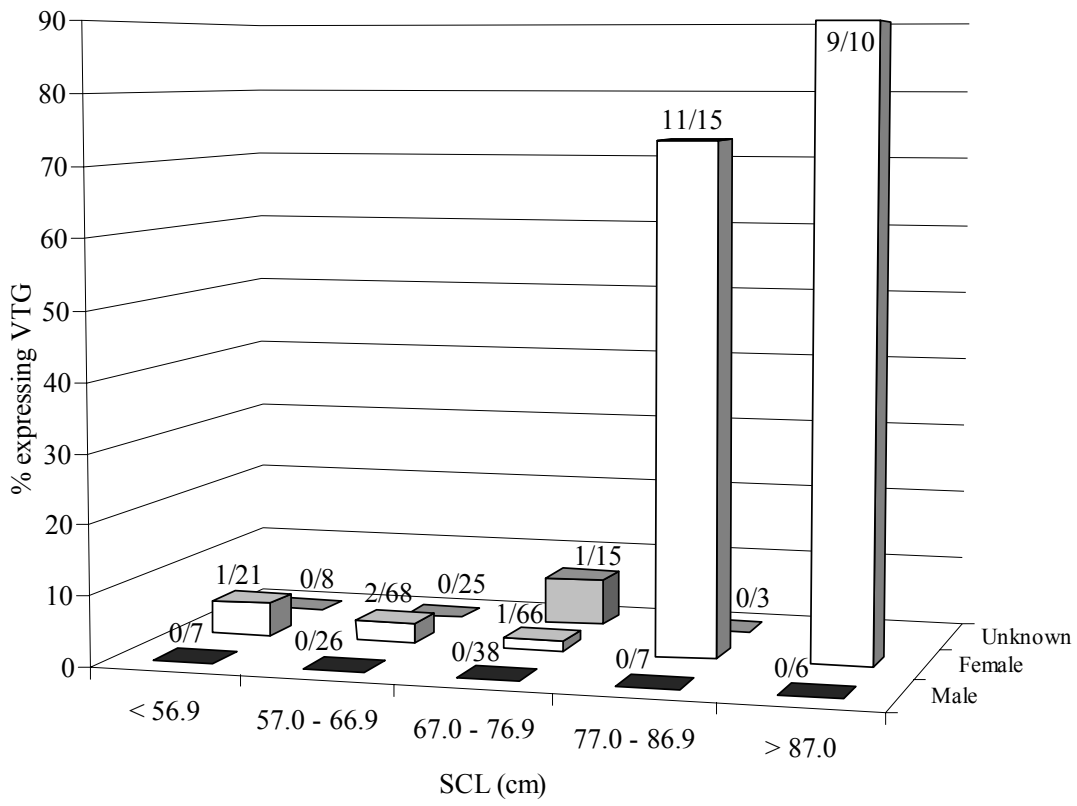


Figure 7.4. Summary of all loggerhead sea turtle plasma samples screened for VTG, excluding the 5 nesting female loggerhead turtles shown in Figure 7.2. Numbers above each bar indicate the number of turtles positive for VTG/total number of turtles screened in that category. SCL = straight carapace length from the nuchal notch to the posterior marginal notch.

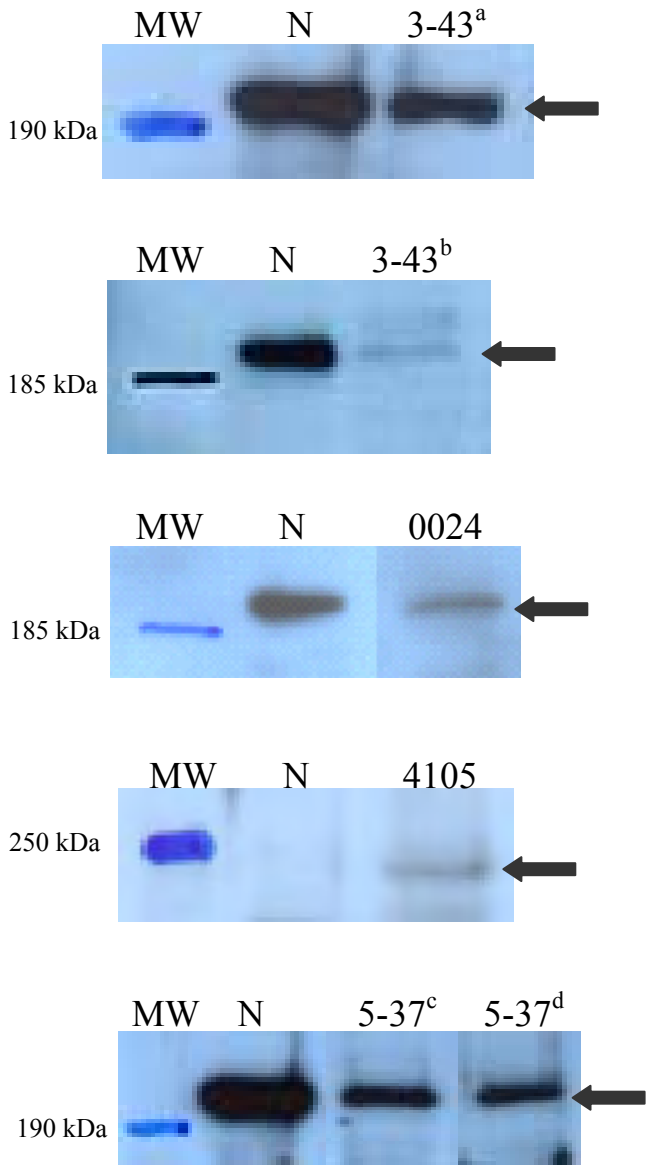


Figure 7.5. Immunoblots of juvenile loggerhead sea turtles that were smaller than 77 cm in SCL and 'abnormally' expressing VTG. Turtle ID numbers are shown above their respective lanes. Turtle ID 3-43 was captured on a) 6/2/2000 and recaptured on b) 8/11/2000. Turtle 5-37 was captured on c)10/21/2002 and recaptured on d) 11/1/2002. MW = molecular weight marker; N = nesting female loggerhead sea turtles served as a positive control on each gel. The positive control did not work on the gel containing turtle ID 4105, but this turtle had a protein at the same MW as VTG in the other turtles.

CHAPTER EIGHT

Characterization of cytochrome P450 aromatase activity in a green sea turtle testis cell line as an *in vitro* model for examining endocrine-disrupting contaminants

ABSTRACT

Understanding how contaminants disrupt the endocrine system of endangered sea turtles is important for predicting and managing the recovery of these species. However, intentional exposure of these animals to test compounds is not allowed due to their endangered status; therefore, we investigated the use of a green sea turtle testis cell line (GST-TS) as an *in vitro* model for examining the effects of contaminants on aromatase activity. Aromatase, which converts testosterone to estradiol, is critical for proper development and reproduction. Aromatase activity in the GST-TS cells, measured by the release of [^{3}H]water from [1β-^{3}H]androstenedione, followed Michaelis-Menton kinetics with a Vmax of 15 fmol/mg/hr and a Km of 201 nM [^{3}H]androstenedione. Product was formed linearly up to 12 hr of substrate incubation, and 30 ^{o}C was the optimal temperature. A potent aromatase inhibitor, 4-androstenoldione (4-OHA), decreased the activity of this enzyme. Dexamethasone, a known inducer, significantly increased aromatase activity, but only at an exposure of 1000 nM for 21 hr. Two other known inducers, human chorionic gonadotropin and 8Br-cyclic AMP, did not significantly increase aromatase activity. Two environmental contaminants were tested for disruption of aromatase activity. Atrazine significantly elevated aromatase activity at low concentrations, but only slightly and only at 24 hr of exposure. 4,4'-DDE significantly decreased aromatase activity after a 24 hr treatment, but only at cytotoxic concentrations (100 uM). These findings suggest that GST-TS cells can be used as an *in vitro* model to test compounds that are suspected inhibitors of aromatase, but induction of activity by suspected contaminants needs further investigation.

INTRODUCTION

In recent decades studies have provided convincing evidence that environmental contaminants disrupt the endocrine system, resulting in reproductive abnormalities in humans and wildlife and potentially increasing the risk of human breast and prostate cancers (Crisp *et al*. 1998). Proper levels of steroid hormones are critical for development and reproduction, especially during the neonatal and pubertal developmental stages.

A key enzyme in the production of steroid hormones is cytochrome P450 aromatase. Aromatase converts testosterone to estradiol and is expressed primarily in the gonad, brain, and mammalian placenta. Little is known about the expression and activity of this enzyme in endangered sea turtles, and nothing is known about how contaminants may alter this enzyme. Understanding the normal and contaminant-affected functions of the endocrine system is important for the conservation and recovery of sea turtles.

Many reptiles have a unique mechanism of sexual differentiation called temperature-dependent sex determination (TSD). During a critical embryonic stage the nest temperature cues steroid hormone production which then directs differentiation of males or females. In sea turtles, warmer nest temperatures produce females, while cooler temperatures produce males with a pivotal temperature of 28.26 ^{o}C for green sea turtles (*Chelonia mydas*; Ackerman 1997). The mechanism behind TSD involves, at least in part, temperature-influenced expression and activity of cytochrome P450 aromatase. Aromatase has been shown to be regulated by temperature in the embryonic brain and gonads of several freshwater turtle species

(Willingham *et al*. 2000; Place *et al*. 2001), of alligators (*Alligator mississippiensis*; Milnes et al. 2002), and of leatherback sea turtles (*Dermochelys coriacea*; Desvages et al. 1993). In all of these species, female-producing temperatures resulted in higher aromatase activity than male-producing temperatures. In this way, temperature signals the production of the correct steroid hormone milieu required for sexual differentiation.

When the delicate balance of temperature and steroids is disrupted, sex reversal can occur. Freshwater turtle eggs incubated at male-producing temperatures and exposed to estradiol result in females (Wibbels *et al*. 1991). Sex reversal by estrogen has also been shown in the olive ridley sea turtle (*Lepidochelys olivacea*; Merchant-Larios *et al*. 1997). Females also result from eggs that are incubated at male temperatures and are exposed to organochlorine (OC) contaminants that have estrogenic activity, including polychlorinated biphenyls (PCBs) and pesticides such as 4,4'-DDE and chlordanes (Willingham and Crews 2000; Bergeron *et al*. 1994). Mixtures of contaminants produce even stronger sex reversal, and this effect does not follow the typical "threshold dose" concept (Bergeron *et al*. 1994; Sheehan *et al*. 1999). Even one molecule of a contaminant can, in theory, reverse the sex of developing reptiles. In a few studies, however, 4,4'-DDE failed to reverse the sex of snapping turtles (*Chelydra serpentina*; Portelli *et al*. 1999) and green sea turtles (Podreka *et al*. 1998). This effect appears to depend on the species, concentration, and temperature chosen. The mechanism of sex reversal by contaminants is not fully known, but may include a disruption of aromatase.

Several compounds are known to alter aromatase activity either through gene expression or due to direct influence of enzyme activity. An increase in aromatase activity can lead to feminization and estrogenic effects, whereas an inhibition may lead to masculinization. Numerous pharmaceutical compounds, such as 4-androstenoldione (4-OHA), fadrozole, and tamoxifen have been developed as potent inhibitors of aromatase activity and are used in the treatment of estrogen-dependent breast cancers. Fadrozole was shown to inhibit aromatase in the brain, ovary, and pituitary of red-eared slider turtles (*Trachemys scripta*; Tsai *et al*. 1994). Similarly, environmental contaminants can alter the activity of aromatase.

Atrazine, the most heavily-used herbicide in many countries, has been shown to induce aromatase activity in human cell lines, including adrenocortical carcinoma cells (H295R) and placental choriocarcinoma cells (JEG-3; Sanderson *et al*. 2000, Sanderson *et al*. 2001). Rats exposed to atrazine displayed delayed puberty, elevated estrogen, and decreased testosterone, indicative of induced aromatase activity (Stoker *et al*. 2000). Likewise, aromatase activity was elevated, although not significantly, in male alligators exposed *in ovo* to atrazine (Crain *et al*. 1997). A 3-fold increase in aromatase activity was observed in tadpoles that were grown in water containing ppb concentrations of atrazine (Tyrone Hayes, pers. communication). This effect may explain the demasculinization observed in frogs exposed to atrazine (Hayes *et al*. 2002).

Imidazole fungicides, including clotrimazole, ketoconazole, clotrimazole, and penconazole are potent aromatase inhibitors. Inhibition was observed in rainbow trout ovaries and human H295R cells (Shilling *et al*. 1999, Sanderson *et al*. 2002).

Flavonoids and phytoestrogens, found in fruits, vegetables, and soy products, also inhibit aromatase activity. Flavones, such as aminoglutethimide, quercetin, equol, and flavone decreased aromatase activity in rainbow trout (*Oncorhynchus mykiss*) ovarian microsomes (Pelissero *et al*. 1996; Shilling *et al*. 1999). The phytoestrogens biochanin A and genistein also decreased activity, but to a lesser extent (Pelissero *et al*. 1996).

OC contaminants, such as PCBs and pesticides, have been shown to alter aromatase activity in everything from cell lines to wildlife. In the H295R cell line, 4,4'-DDT, 2,4'-DDT, and 2,4'-DDE decreased aromatase but only at cytotoxic concentrations (Sanderson *et al*. 2002). Toxaphene and chlordane decreased aromatase activity by antagonizing an orphan receptor (ERRα-1) in a hepatoma cell line (Chen *et al*. 2001). PCB congeners and metabolites decreased aromatase activity in human placenta cell lines (JEG-3 and JAR), but most were also cytotoxic (Letcher *et al*. 1999). Similarly, cytotoxic concentrations of TCDD and PCB 126 decreased aromatase activity in JEG-3 cells, but Aroclor 1016 and toxaphene had no effect on aromatase activity (Drenth *et al*. 1998). Rainbow trout ovarian microsomes incubated with 2,4'-DDE and 4,4'-DDE showed elevated aromatase activity, but not to a statistically significant extent (Shilling *et al*. 1999).

In whole animal experiments, OC compounds altered aromatase activity. When rats were fed PCB-contaminated fish from the Great Lakes, the aromatase activity was decreased in the ovaries of their offspring (Gerstenberger *et al*. 2000). Rats exposed to a relevant PCB mixture, similar to that found in human milk, and to Aroclor 1254 showed decreased brain aromatase activity and altered behavior, and

their offspring had altered hormone profiles (Hany *et al*. 1999; Kaya *et al*. 2002). *In vivo* exposure to 4,4'-DDE in male rats increased liver aromatase expression and activity, and serum estrogen levels were elevated but not to a statistically significant extent (You *et al*. 2001).

The effects of OC contaminants on aromatase have also been noted in reptilian wildlife. Alligators collected from an OC-contaminated lake, Lake Apopka in Florida, exhibited significantly reduced aromatase activity in the gonadal-adrenal mesonephros (GAM) compared to animals from a reference lake (Crain *et al* 1997). In the red-eared slider turtle, Aroclor 1242 increased embryonic aromatase in the brain and gonad, but chlordane did not change aromatase (Willingham and Crews 2000).

OC contaminants, such as PCBs and pesticides have been found in tissues of endangered sea turtles (Keller *et al*. in prep), but their sensitivity to these compounds is unknown. It is important to know which contaminants and concentrations disrupt the production of steroid hormones in these species, as hormones are critical to their normal development and reproductive capacity. Exposure of sea turtles to contaminants is not possible due to their endangered status. Therefore, our study investigated a potential *in vitro* model for examining endocrine disruption in sea turtles.

MATERIALS AND METHODS

Cell culture conditions

The availability of reptile cell lines is very limited. Currently 13 known sea turtle cell lines have been established and characterized from an immature male green

sea turtle (Lu *et al*. 1999). Green sea turtle testis cells (GST-TS) were obtained (a gift from Dr. Yuanan Lu) and maintained under appropriate conditions (Lu *et al*. 1999). They were grown until almost confluent in Costar® flasks (Corning Inc., Corning, NY) in RPMI-1640 media without phenol red or L-glutamine (Mediatech, Inc., Herndon, VA) and supplemented with final concentrations of 10 % charcoal-stripped fetal bovine serum (FBS; Hyclone, Logan, UT), 2 mM L-glutamine (Mediatech, Inc., Herndon, VA), 100 IU/ml penicillin, and 100 ug/ml streptomycin (Mediatech, Inc., Herndon, VA) in a humidified environment at 30 ^{o}C and 5 % CO_2.

GST-TS cells were trypsinized and plated in Costar® 6 well plates (Corning Inc., Corning, NY) at 1.3 x 10^5 cells/well. The media was changed on the following day to serum-free RPMI 1640 media. At this time, 3 or 4 wells were exposed to each concentration of test compound or vehicle 21 or 24 hr prior to measuring aromatase activity. Some compounds were tested for additional exposure durations as well as during the 6 hr substrate incubation period when aromatase activity was measured. The aromatase inhibitor, 4-androsten-4-ol-3,17-dione (4-OHA; Sigma A9295, St. Louis, MO), was diluted in ethanol and tested for the 21 hr prior to replacing the media with media containing the aromatase substrate (^{3}H-androstenedione) or for only the 6 hr substrate incubation (co-exposure of the cells to 4-OHA and ^{3}H-androstenedione). Three known inducers were tested. Dexamethasone (Dex; Sigma D2915, St. Louis, MO) was diluted directly into serum-free media and tested for 7, 14, 16, 21, 48, and 72 hr prior to substrate incubation as well as 10 hr prior plus 6 hr during the substrate incubation (16 hr total). Human chorionic gonadotropin (HCG;

Sigma CG-10, St. Louis, MO) was diluted in serum-free media and tested for 24 hr prior to substrate incubation. 8Br-cyclic AMP (cAMP; Sigma, St. Louis, MO) was diluted in DMSO and tested for 24 hr prior to substrate incubation and during the 6 hr substrate incubation. Two environmental contaminants were investigated for their effect on aromatase activity. Atrazine (EM Science, Gibbstown, NJ) was diluted in DMSO and tested for 24, 48, and 72 hr prior to substrate incubation and during the 6 hr substrate incubation. 4,4'-DDE was diluted in DMSO and tested for 24 hr prior to substrate incubation and during the 6 hr substrate incubation.

Aromatase Assay

Aromatase activity was measured by the [^{3}H]water release assay (Thompson and Siiteri 1974). Briefly, the 1β hydrogen from [1β-^{3}H]androstenedione is removed as aromatase converts the substrate to estradiol. The product, radioactive water, is counted following extraction of the remaining substrate.

The activity within the GST-TS cells was characterized for substrate concentration (20 nM to 1000 nM), time (30 min to 24 hr), presence of FBS, and temperature (25, 30, and 35 ^{o}C). Once optimal conditions were determined, experiments involving exposure to test compounds were performed. After exposure of the cells to the test compounds, the media was replaced with serum-free media (750 ul/well) containing 100 uM [1β-^{3}H]androstenedione (NEN, Boston, MA). For exposure of the cells to test compounds during the substrate incubation, the media was replaced with serum-free media (750 ul/well) containing the ^{3}H-androstenedione and the test compound. Cells were incubated with substrate for 6 hr. Substrate incubation

was stopped by transferring media (650 ul/well) to a tube containing 325 ul 30% trichloroacetic acid (TCA) to precipitate proteins. Tubes were mixed for 1 min by vortex and centrifuged at 14000 x g for 20 min at 4^{o}C. The supernatant (850 ul) was transferred to a new tube containing 2 ml chloroform, mixed for 1 min, and centrifuged at 1300 x g for 20 min at 4 ^{o}C. The aqueous phase (600 ul) was transferred to a new tube containing 600 ul 5 % dextran-coated charcoal, mixed for 1 min, and placed at 4 ^{o}C. The following day, tubes were centrifuged at 14000 x g for 20 min at 4 ^{o}C. The supernatant (900 ul) was thoroughly mixed with 6 ml Ecolume scintillation fluid (ICN, Costa Mesa, CA) and counted for [^{3}H]water. For each experiment, three or more wells containing no cells were treated identically to wells with cells, and these served as controls for background radioactivity.

Protein determination

Immediately after removing the reaction media, the wells were rinsed twice with 1 ml PBS. Cells were solubilized with 100 ul/well of 0.1% SDS and diluted with 3 ml 50 mM Tris (pH 7.6). Protein concentration was determined by Bradford (1976).

Temperature-dependent aromatase assay

Homogenates of the GST-TS cells were tested for the temperature optimum of aromatase activity. Cells were grown in 75 cm^{2} Falcon flasks with media containing charcoal-stripped FBS without phenol red. At 80 to 100 % confluence, the cells were rinsed twice with PBS, scraped from the flask, and centrifuged at 400 x g for 2 min. The cell pellet was resuspended in homogenization buffer (50 mM Tris pH 7.4, 100 mM KCl, 1 mM EDTA, 1 mM DTT, 1mM PMSF, 10 % glycerol) and homogenized with a hand-held all-glass homogenizer for 1 to 2 min. Protein concentrations were

measured by Bradford (1976) and then tested for aromatase activity by incubating samples with 200 nM [1β-^{3}H]androstenedione and 10 mM NADPH in PBS at 25, 30, and 35 ^{o}C. The reaction was stopped by adding 250 ul of 30 % TCA. Tubes were mixed by vortex and 1 ml chloroform was added to this mixture. Following centrifugation at 850 x g for 15 min, 600 ul aqueous layer was removed, mixed with 600 ul 5 % dextran coated charcoal, incubated for 1 to 2 hr, and centrifuged at 14000 x g for 20 min. Supernatant (600 ul) was counted in 5 ml Ecolume.

Aromatase activity of placental microsomes

Three full-term human placentas were obtained from the Duke University Hospital. Within 15 min of delivery, the placentas were placed on ice packs or kept at 4 ^{o}C until processing. Time between delivery and the start of processing did not exceed 12 hr. Microsomes were prepared using a procedure similar to Thompson and Siiteri (1974). Tissue was separated from membranes and large vessels and rinsed with cold 0.15 M KCl. The tissue was homogenized in 0.25 M sucrose and 100 mM PMSF using a Polytron homogenizer for 1 to 2 min and centrifuged at 20,000 x g for 30 min at 4 ^{o}C. The supernatent was centrifuged at 148,000 x g for 45 min at 4 ^{o}C. The pellet was resuspended in a 0.05 M potassium phosphate buffer (pH 7.4) containing 100 mM PMSF and 4 μg/ml leupeptin. Microsomes were stored at -80 ^{o}C.

Placental microsomes were tested for aromatase activity as a positive control by the method of Purba and Bhatnagar (1990). Briefly, 100 ug of microsomal protein was incubated with 400 uM [1β-^{3}H]androstenedione and 0.24 mM NADPH in PBS (final concentrations in a final volume of 500 ul) for 20 min at 37 ^{o}C. Simultaneously,

replicate samples were also incubated with 10 μM 4-OHA. Extraction and measurement of [^{3}H]water was performed as described above in the "temperature-dependent aromatase assay" section.

Statistics

All statistical tests were performed using Systat 8.0 software (SPSS, Inc, Chicago, IL). Analysis of variance (ANOVA) and Least Significant Difference tests were used to compare more than two groups of data, whereas pooled t-tests were used to compare data with two groups.

RESULTS

Aromatase activity was characterized in the GST-TS cell line for substrate concentration, incubation time, use of serum, and temperature. Aromatase activity in this cell line followed Michaelis-Menton kinetics with a Vmax of 15 fmol/mg/hr and Km of 201 nM ^{3}H-androstenedione (Fig. 8.1). Product formation was linear up to 12 hr of substrate incubation (Fig. 8.2). A substrate incubation of 6 hr was chosen for all experiments as it provided adequate product formation well within the linear range. Aromatase activity was inhibited by the presence of FBS in the media, therefore all experimental exposures were performed with serum-free media (Fig. 8.3A). Aromatase activity was lower in cell homogenates that were incubated at 35 ^{o}C (Fig. 8.3B). No statistically significant difference was detected between 25 and 30 ^{o}C, but 30 ^{o}C was used for all future experiments as it resulted in the highest overall activity.

Due to the low activity measured in these cell lines, we believed it was necessary to use a positive control to verify our experimental and extraction

procedures. Human placenta is known to have extremely high levels of aromatase activity and the kinetics are well-characterized (Purba and Bhatnagar 1990). Aromatase activity of the placental microsomes was 954 ± 215 pmol/mg/hr (mean ± standard deviation). This activity value was very similar to the reported Vmax value of 1270 pmol/mg/hr obtained using nearly identical conditions (Purba and Bhatnagar 1990). Placental aromatase activity was significantly inhibited by 10 uM 4-OHA (198 ± 65.6 pmol/mg/hr).

GST-TS cells were exposed to a known aromatase inhibitor (4-OHA) for either 6 hr during a co-exposure with the ^{3}H-androstenedione substrate or 21 hr prior to the addition of ^{3}H-androstenedione. Aromatase activity was inhibited when the cells were exposed to 4-OHA during the 6 hr substrate incubation period (Fig. 8.4). No inhibition was observed when the cells were exposed to 4-OHA for the 21 hr period prior to substrate incubation (data not shown), suggesting that 4-OHA competes directly with the ^{3}H-androstenedione substrate.

GST-TS cells were exposed to three known aromatase inducers. Dexamethasone (Dex) significantly induced aromatase activity at 1000 nM for a 21 hr exposure prior to adding ^{3}H-androstenedione (Fig. 8.5A). The other concentrations and exposure durations of Dex and all other inducers failed to increase aromatase activity in the GST-TS cells. Dex concentrations of 10, 100, and 1000 nM failed to increase aromatase activity after 7, 14, 16, 48, and 72 hr of exposure prior to the incubation with ^{3}H-androstenedione (data not shown). Exposure of the GST-TS cells to these Dex concentrations for 10 hr prior to the incubation with ^{3}H-androstenedione plus the 6 hr of substrate incubation (16 hr total) also did not elicit an increase of

aromatase activity (data not shown). A 24 hr exposure of 0.1 to 100 IU/ml of human chorionic gonadotropin (HCG) prior to the incubation with ^{3}H-androstenedione did not alter the aromatase activity (Fig. 8.5B). Similarly, 100 uM 8Br-cAMP did not significantly induce aromatase activity in these cells after a 24 hr exposure prior to the incubation with ^{3}H-androstenedione or a 6 hr co-exposure with ^{3}H-androstenedione. The 24 hr control vs. cAMP-exposed activity was 20.1 ± 4.5 and 25.8 ± 7.3, respectively. The 6 hr control vs. cAMP-exposed activity was 24.0 ± 3.3 and 19.8 ± 3.7, respectively. These data indicate that these cells are not sensitive to potent known inducers and suggest that part of the pathway necessary for induction of aromatase is missing in these cells.

The effects of two environmental contaminants were tested on aromatase activity in the GST-TS cell line. A 24 hr exposure to atrazine, known to induce aromatase in many systems, prior to the incubation with ^{3}H-androstenedione statistically increased aromatase activity (Fig. 8.6). Aromatase activity was not altered following a 48 or 72 hr exposure prior to the substrate incubation or during a 6 hr co-exposure with ^{3}H-androstenedione (data not shown). Exposure to a high concentration of 4,4'-DDE (100 uM) for 6 hr during or 24 hr prior to the substrate incubation decreased aromatase activity (Fig. 8.7A). Cellular protein was also reduced by 100 uM 4,4'-DDE after 24 hr confirming our visual observations of cytoxicity (Fig. 8.7B).

DISCUSSION

Aromatase activity was low in the GST-TS cell line (Vmax = 15 fmol/mg/hr) compared to other biological systems (Table 8.1). The GST-TS aromatase activity

was especially lower than aromatase activity in embryonic reptiles (Crain *et al*. 1997, Smith and Joss 1994), in testes of immature chickens (Callard *et al*. 1978), and in a Leydig tumor cell line from a rat (Young *et al*. 1997). The low aromatase activity in the GST-TS cells may be explained by the sex and developmental stage of the turtle from which these cells were derived. The cell line was obtained from an immature male green sea turtle with a straight carapace length (SCL) of 72.3 cm (Lu *et al*. 1999). Gonadal aromatase activity is low in immature animals, because active steroid production by gonads is dormant between the embryonic stage and the onset of puberty. The age (or size) at which male green sea turtles enter puberty is not known, but this turtle was much smaller than adults, at least adult nesting females which have a mean SCL of 99 cm (Miller 1997). Because of the relatively small size of this turtle and its immature age class, it is plausible that it had not entered puberty and would have low aromatase activity.

Certain characteristics of this cell line may also contribute to lower aromatase activity. The proportional composition of the two testicular cell types is unknown in this cell line. Leydig cells produce androgens upon stimulation by luteinizing hormone, and Sertoli cells are responsible for spermatogenesis. A controversy exists over which cell type is primarily responsible for testicular aromatization (Valladares and Payne 1979). A low percentage of the responsible cell type, whichever that may be, in this cell line may explain the low aromatase activity. In addition, aromatase activity is lower in cell lines than their representative tissue homogenates (Table 8.1). For example, activity in a placental cell line was approximately 20 pmol/mg/hr (Sanderson *et al*. 2001; Letcher *et al*. 1999) which was much lower than the activity

(near 1000 pmol/mg/hr) in placental microsomes (this study and Purba and Bhatnagar 1990).

Serum has been shown previously to inhibit the cAMP-stimulation of aromatase activity in several cell types (Lephart and Simpson 1991). In the absence of FBS, aromatase activity of human placenta cells (JEG-3) was higher and more sensitive to the inhibitory effects of OC contaminants than in cells that were incubated with FBS (Letcher *et al*. 1999, Drenth *et al*. 1998). This was also seen with the GST-TS cells. Aromatase activity measured in cells grown without FBS was 18 fmol/mg/hr, while addition of FBS or charcoal-stripped FBS resulted in activity levels of 6 to 7 fmol/mg/hr. This indicates that some factor present in the FBS inhibited or interfered with the activity of the constitutive enzyme.

The optimal temperature for this enzymatic reaction in the GST-TS cells was 30 ^{o}C. This temperature was also the optimal temperature for the growth of this cell line (Lu *et al*. 1999) and similar to water temperatures that sea turtles prefer (Spotila *et al*. 1997).

Aromatase activity in the GST-TS cells was inhibited by a potent aromatase inhibitor (4-OHA). However, the known inducers (Dex, HCG, or cAMP) failed to increase aromatase activity in these cells, except for 1000 nM Dex at a 21 hr exposure duration. Failure of positive controls to mimic *in vivo* events is common in cell culture research, as many mechanistic pathways are missing. The isolation of one cell type eliminates signaling from other organs or tissues, and many intracellular mechanisms can be lost. For example, *in vivo* exposure to 4,4'-DDE increased liver expression of aromatase, but *in vitro* exposure of primary cultures of hepatocytes to

4,4'-DDE failed to induce aromatase (You *et al*. 2001). In addition, exposure of a human prostate cancer cell line to Dex or cAMP did not induce aromatase activity (Block *et al*. 1996).

Environmental contaminants altered aromatase activity in the GST-TS cell line. A 24 hr exposure to relatively low concentrations of atrazine increased aromatase activity. The induction was statistically significant, but the biological significance is not known. Maximal induction at 10 uM atrazine resulted in only a 1.4 fold increase, whereas the same atrazine concentration increased aromatase activity in human adrenocortical cells (H295R) by approximately 2.7 fold (Sanderson *et al*. 2000). This relatively low increase in activity could be greatly significant in sea turtles, which have a low metabolic activity in general. It is also plausible that the minor induction observed in the GST-TS is due to a missing link in the induction pathway. This latter hypothesis may explain the failure of the positive controls to elicit a response.

4,4'-DDE inhibited aromatase activity at the highest concentration tested (100 uM), but this concentration was cytotoxic to the cells as determined by visual inspection and cellular protein levels. Sanderson *et al*. (2002) also found that 10 uM of DDT compounds (4,4'-DDT, 2,4'-DDT, and 2,4'-DDE) inhibited aromatase in H295R cells after 24 hr of exposure, but this concentration was cytotoxic. They did not observe an inhibitory effect of either 1 uM or 10 uM of 4,4'-DDE, which is the most abundant metabolite of DDT in the environment. Aromatase activity in the GST-TS cells was similarly not affected by 10 uM 4,4'-DDE, but was decreased at 100 uM exposure for 6 and 24 hr. Evidence of cytotoxicity by visual inspection or

decreased cellular protein was observed at 24 hr, but not at 6 hr. This may indicate a rapid inhibition of enzyme activity prior to cytotoxic effects of 4,4'-DDE. In contrast to this inhibition, *in vivo* exposure to this metabolite has been shown to induce hepatic aromatase activity in male rats (You *et al*. 2001).

OC contaminants have been detected in sea turtle tissues, but compared to fish-eating wildlife and snapping turtles, sea turtles accumulate relatively low concentrations. 4,4'-DDE is found on average at 64.4 ng/g wet mass in adipose tissue of loggerhead sea turtles (Keller *et al*. in prep). The adipose concentrations of 4,4'-DDE in green sea turtles are even lower at 9 ng/g wet mass (Mckenzie *et al*. 1999). These levels are 3 to 4 orders of magnitude lower than the concentration shown by the current study to be cytotoxic and inhibitory to their testicular aromatase. Sea turtle tissues have not been analyzed for atrazine concentrations, thus it is not known whether the concentrations tested in this study are environmentally relevant for these animals. It is likely that they are exposed to atrazine, because concentrations in the coastal waters of the NC and SC where many sea turtles feed ranged from 3.1 to 12.6 ng/L (Alegria *et al*. 2000). The sensitivity of the endocrine system of this species to environmental contaminants requires further clarification.

In conclusion, we have characterized the cytochrome P450 aromatase activity in a green sea turtle testis cell line and have shown that it can be decreased by known inhibitors. Attempts to increase activity using classical inducing agents were unsuccessful. This may be due to the lack of interaction of the compounds with molecular or biochemical pathways in the cells or the absence of critical signaling pathways that were lost during culturing. These findings suggest that this cell line

may be a useful model for examining endocrine-disrupting contaminants that are thought to inhibit aromatase, but its use to screen compounds that are suspected inducers could be limited. Atrazine, a known inducer and heavily-used herbicide, slightly increased aromatase in these sea turtle cells, but we do not know the biological significance of this increase. 4,4'-DDE inhibited aromatase, but only at concentrations that were cytotoxic and not thought to be environmentally relevant.

Table 8.1. Comparison of aromatase activity in different species, tissues, and cell lines.

Common name	Age/stage/sex	Tissue or cell type[a]	Aromatase activity[b]	Study
Rainbow trout	female	ovarian microsomes	4266[c]	Shilling *et al.* 1999
Green sea turtle	immature male	testis cell line	15[c]	this study
Green sea turtle	immature	hypothalamus/preoptic area	1380[d]	Callard *et al.* 1978
Green sea turtle	immature	amygdala	940[d]	Callard *et al.* 1978
Red-eared slider turtle	adult female	ovarian homogenate	25	Tsai *et al.* 1994
Red-eared slider turtle	adult female	brain homogenate	27	Tsai *et al.* 1994
Red-eared slider turtle	adult female	pituitary homogenate	3.5	Tsai *et al.* 1994
American alligator	hatchling/female temp.	GAM from contaminated lake	7549[e]	Crain *et al.* 1997
American alligator	hatchling/female temp.	GAM from reference lake	11424[e]	Crain *et al.* 1997
Saltwater crocodile	embryo/female temp.	GAM	492.59	Smith and Joss 1994
Lizard	adult female	ovarian follicles	1.5[c]	Assisi *et al.* 2001
Snake	female	forebrain	2825[f]	Callard *et al.* 1978
Chicken	mature female	gonadal homogenate	4640[g]	Callard *et al.* 1978
Chicken	immature male	gonadal homogenate	6711[g]	Callard *et al.* 1978
Chicken	immature female	gonadal homogenate	74433[g]	Callard *et al.* 1978
Zebra finch	female hatchling	ovarian homogenate	2352 - 23196	Schlinger and Arnold 1992
Zebra finch	male hatchling	testicular homogenate	ND - 436.2	Schlinger and Arnold 1992
Rat	adult female	ovarian homogenate	2900[c]	Purba and Bhatnagar 1990
Rat	60 day old female	ovarian microsomes	6000	Gerstenberger *et al. 2000*
Rat	male	Leydig tumor cell line (H540)	9400 - 51700	Young *et al.* 1997
Rat	immature male	Sertoli cells in culture	0.00067	Gore-Langton *et al.* 1980
Rat	adult male	testicular homogenate	not detectable	Valladares and Payne 1979
Rat	male	liver microsomes	24000	You *et al.* 2001
Human	female	placental microsomes	954000 ± 215000	this study
Human	female	placental microsomes	1270000[c]	Purba and Bhatnagar 1990
Human	female	JEG-3	16000	Sanderson *et al.* 2001
Human	female	JEG-3	23000	Letcher *et al.* 1999
Human	female	MCF-7 homogenate	80[c]	Sun *et al.* 1997
Human	female	T-47D homogenate	10[c]	Sun *et al.* 1997
Human	adult female	sc adipose stromal cells	133 - 500[c]	Ackerman *et al.* 1981
Human	adult female	omental adiopose stromal cells	26.7 - 112[c]	Ackerman *et al.* 1981
Human	male	LNCaP	760[c]	Block *et al.* 1996
Human		H295R	1100	Sanderson *et al.* 2000

[a] GAM = gondal adrenal mesonephros; JEG-3 = placental choriocarcinoma cell line; MCF-7 and T-47D = breast cancer cell lines; LNCaP = prostate cancer cell line; H295R = adrenocortical carcinoma cell line.
[b] Values are reported as fmol/mg protein/hr unless otherwise stated.
[c] Values are Vmax.
[d] fmol of E1/hr/g wet weight of tissue.
[e] fmol/g wet weight/hr.
[f] fmol of E1+E2α/hr/g wet weight of tissue.
[g] fmol of E1+E2β+E2α/hr/g of wet weight of tissue.

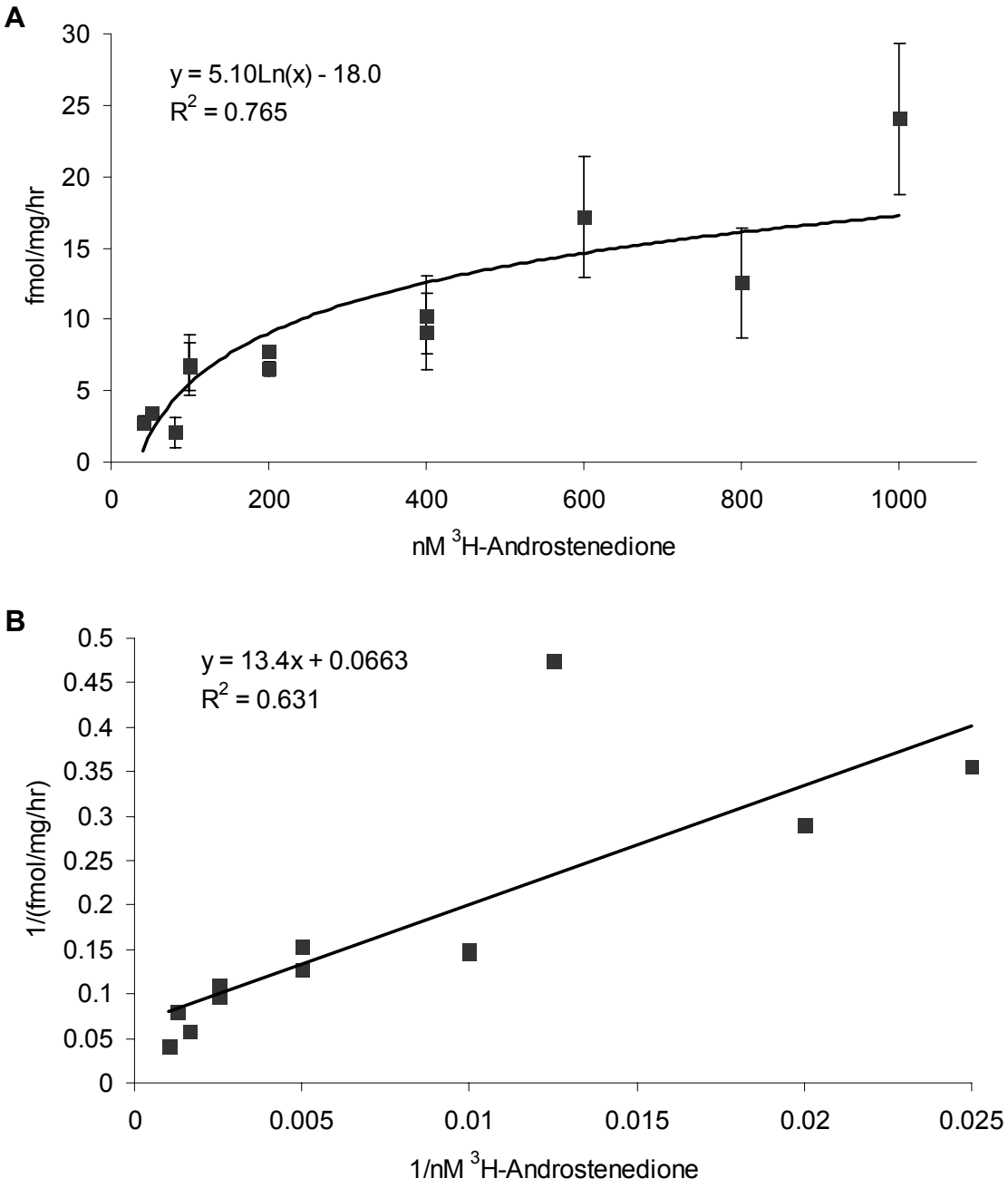


Figure 8.1. GST-TS aromatase activity measured at several substrate concentrations. Activity appears to follow Michaelis-Menton kinetics (A) and using a Lineweaver Burk plot (B) Vmax was calculated as 15 fmol/mg/hr and Km as 201 nM ^{3}H-androstenedione.

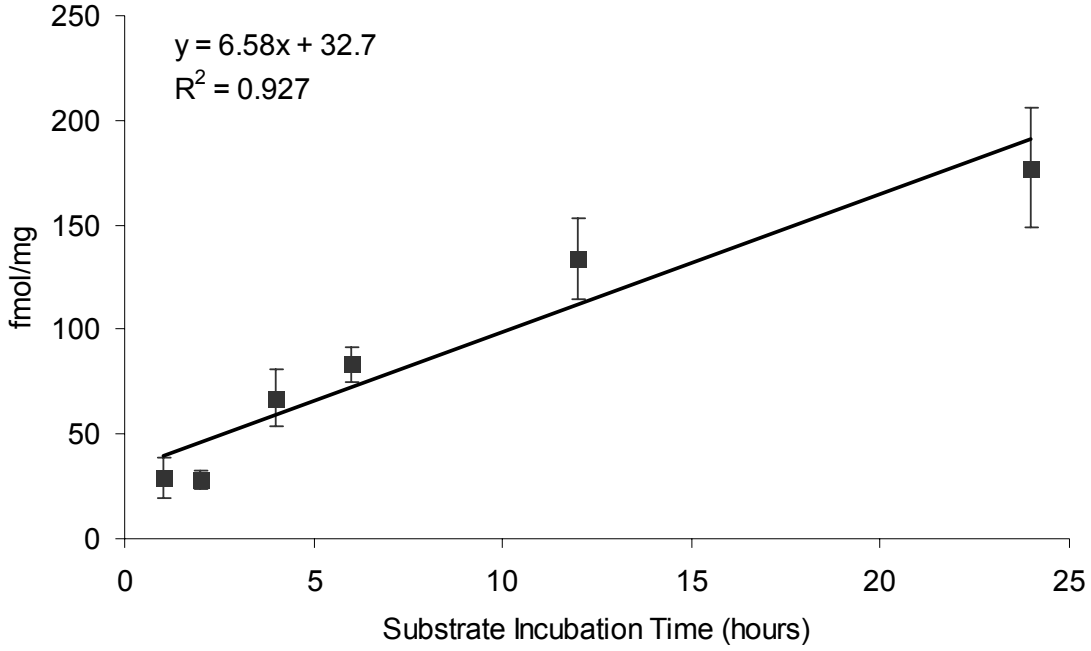


Figure 8.2. Linear formation of ^{3}H-water by aromatase in GST-TS cells with increasing substrate incubation duration.

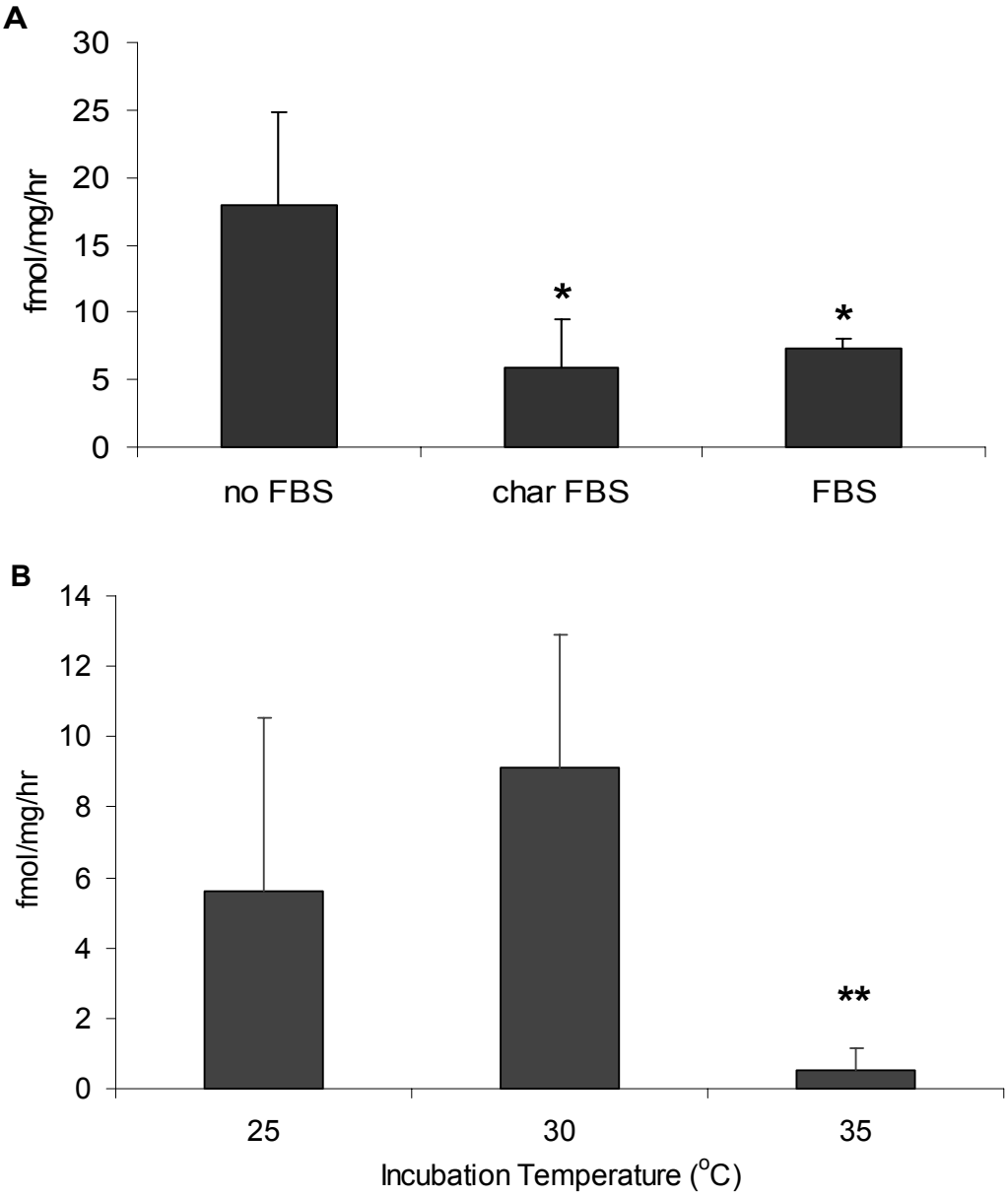


Figure 8.3. Effect of A) presence and type of fetal bovine serum (FBS) and B) temperature on GST-TS aromatase activity. A) Cells were seeded in 6 well plates with either 10 % charcoal-stripped FBS (for the 'no FBS' and 'char FBS' treatments) or 10 % FBS (FBS). The following day, the media was replaced with serum-free media (no FBS), 10 % charcoal-stripped FBS (char FBS), or 10 % FBS (FBS). Aromatase activity was tested on the third day by incubating cells with 400 nM ^{3}H-androstenedione for 6 hrs. * indicates a statistically significant difference from the no FBS treatment (ANOVA $p = 0.035$ with Least Significant Difference test $p < 0.05$). B) Homogenates of cells were incubated with 200 nM ^{3}H-androstenedione for 2 hr at 3 temperatures. ** indicates a difference from 30 ^{o}C (ANOVA $p = 0.068$ with Least Significant Difference test $p = 0.026$).

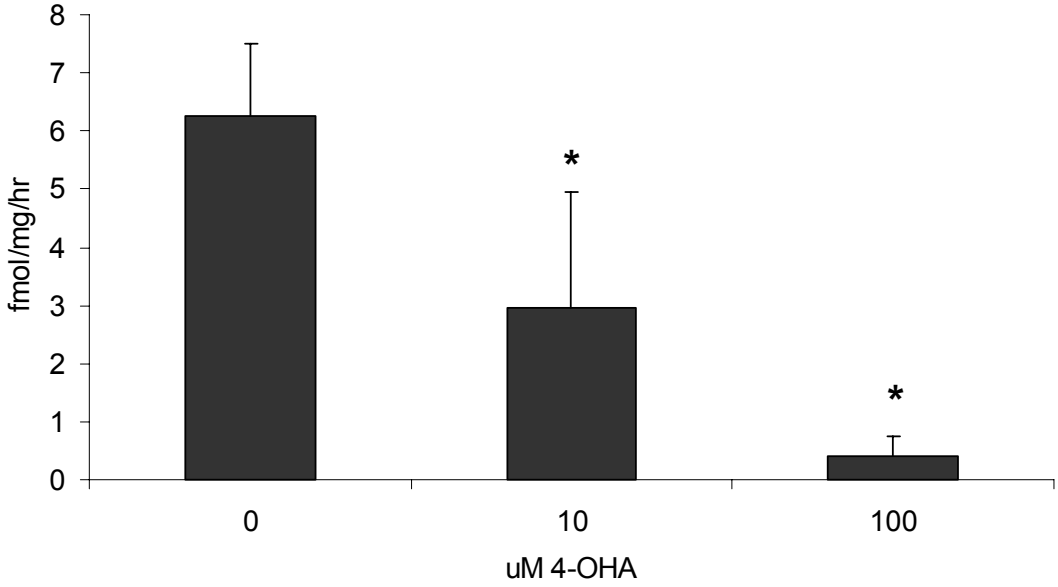


Figure 8.4. Inhibition of GST-TS aromatase activity by 4-OHA. Cells were exposed to 4-OHA during the 6 hr substrate incubation. * indicates a difference from the control (ANOVA $p < 0.001$ with Bonferroni $p < 0.005$).

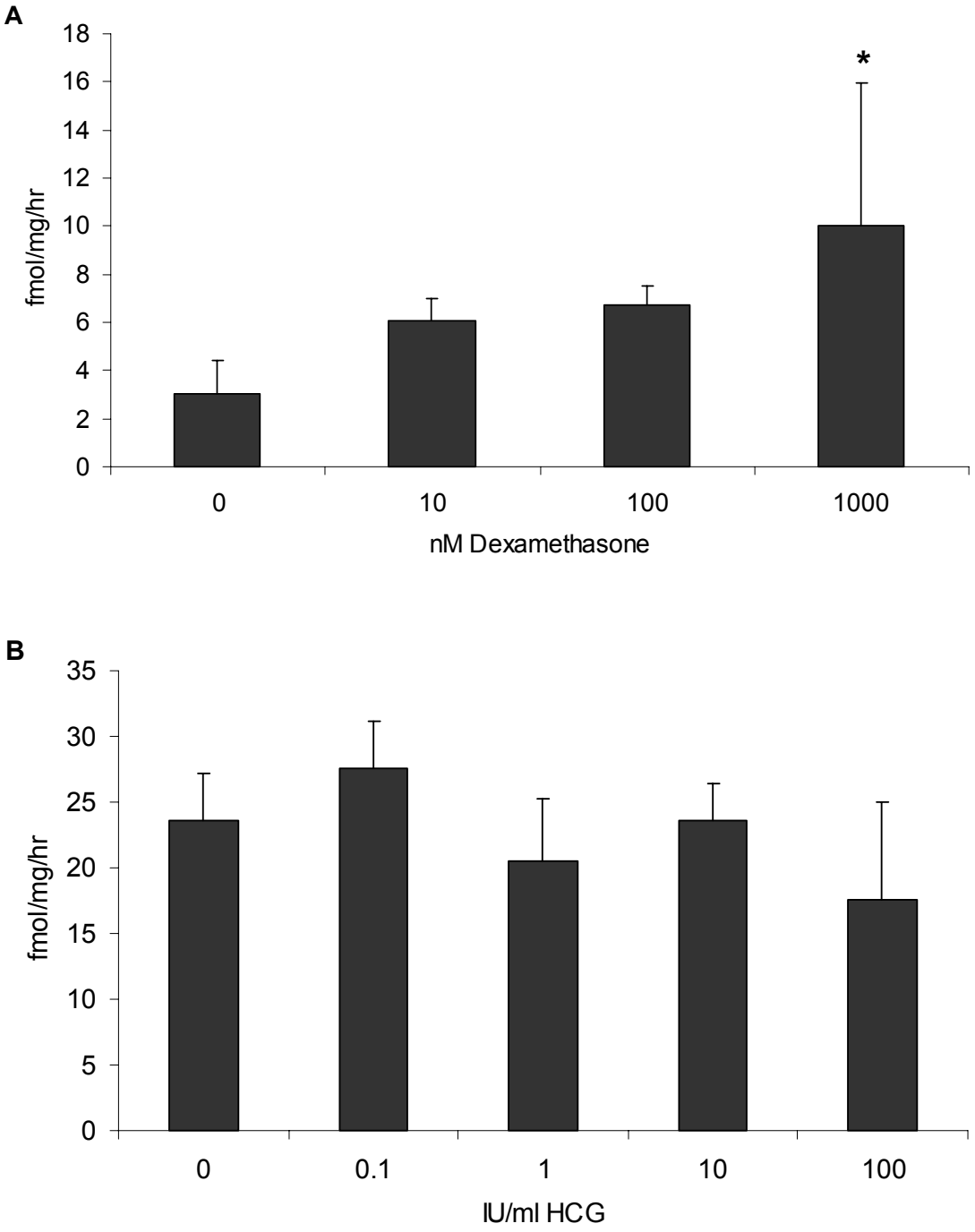


Figure 8.5. Lack of induction of GST-TS aromatase activity by A) dexamethasone (Dex), and B) human chorionic gonadotropin (HCG). Cells were exposed to Dex for 21 hr and to HCG for 24 hr before the exposure media was removed and replaced with media containing ^{3}H-androstenedione for a 6 hr substrate incubation. * indicates a difference from the control (ANOVA $p = 0.128$ with Least Significant Difference test $p = 0.025$).

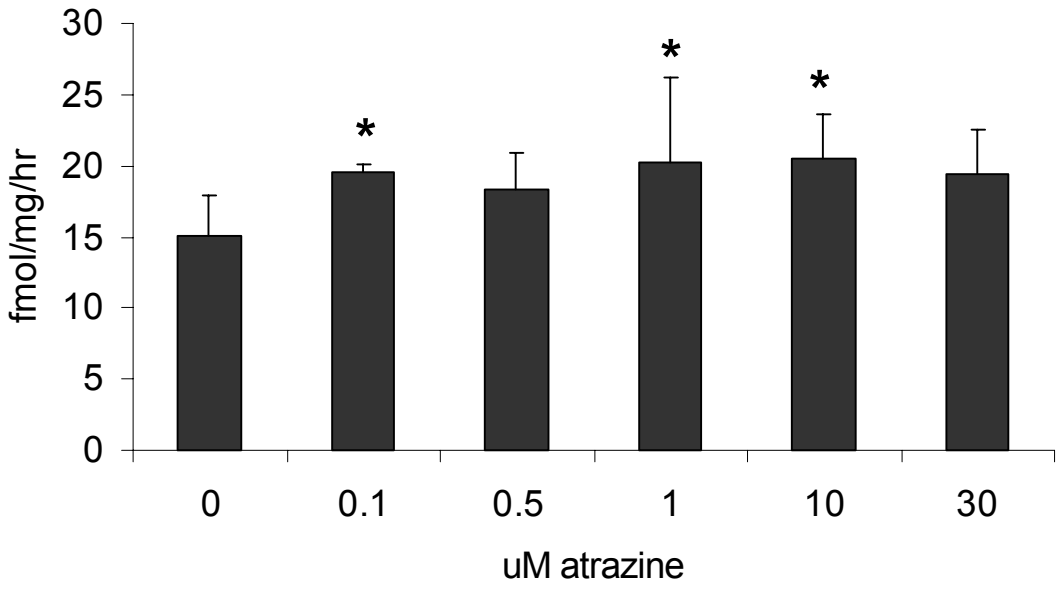


Figure 8.6. Effect of atrazine on GST-TS aromatase activity. Cells were exposed to atrazine for 24 hr prior to substrate incubation. * indicates a difference from the 24 hr control (ANOVA $p = 0.032$ with Least Significant Difference test $p < 0.05$).

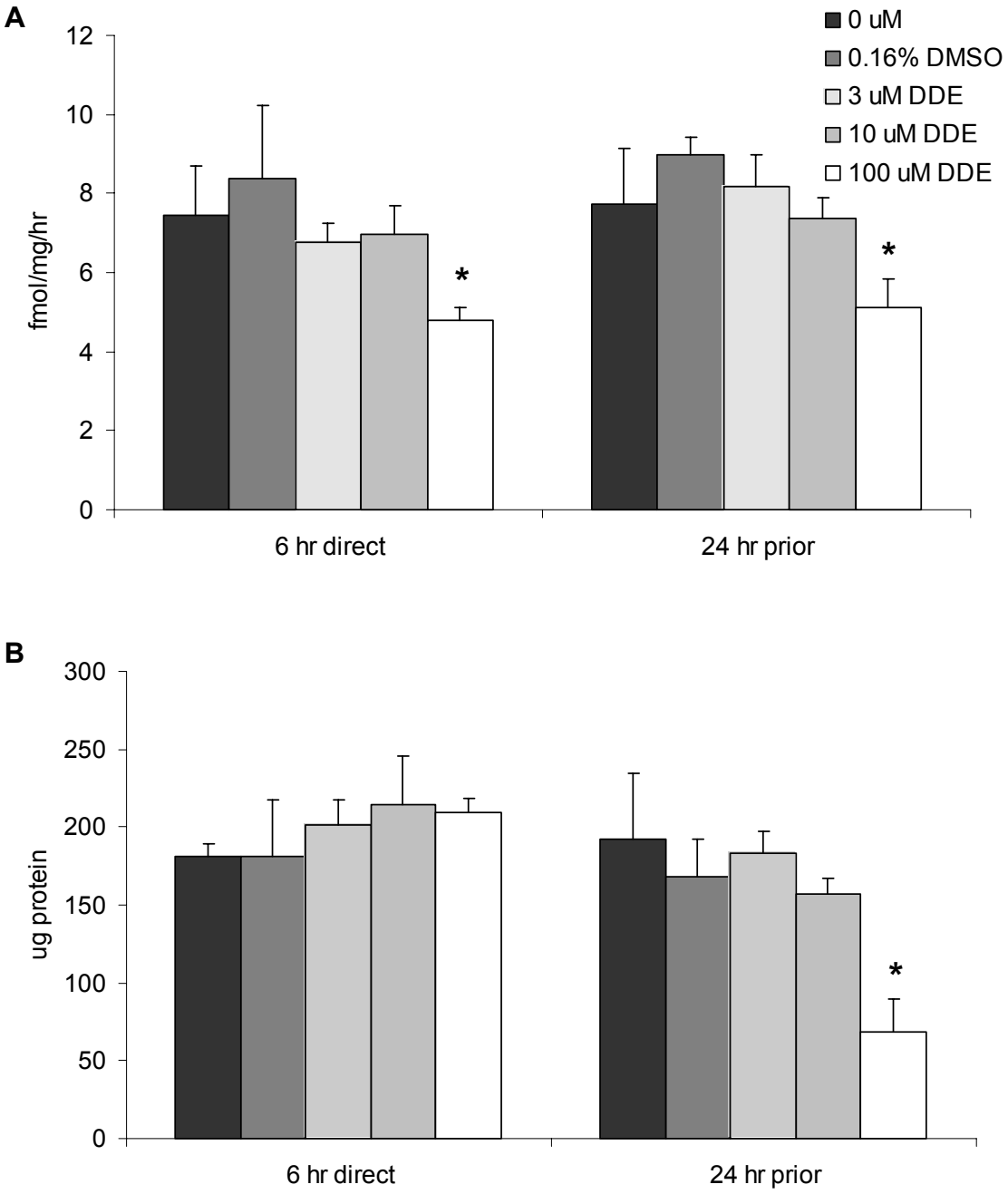


Figure 8.7. Effect of 4,4'-DDE on A) GST-TS aromatase activity and B) cellular protein. Cells were exposed to 4,4'-DDE for either 6 hr during the substrate incubation (6 hr direct) or 24 hr prior to the substrate incubation (24 hr prior). * indicates a difference from the control and DMSO control within the same exposure duration (ANOVA $p < 0.05$ with Least Significant Difference test $p < 0.05$).

CONCLUSIONS

Mounting evidence suggests that organochlorine (OC) contaminants affect the immune and endocrine systems of wildlife to the extent that they may have caused population declines. By suppressing immune functions, OC compounds may have reduced disease resistance and led to mass mortality of marine mammals during viral epizootics (Ross *et al*. 1996) and reduced an alligator population from Lake Apopka, Florida (Gross *et al*. 1996). Disrupted endocrine systems can lead to reduced reproduction, as seen in bald eagles of the Great Lakes and possibly in alligators from Lake Apopka (Grasman *et al*. 1998; Guillette *et al*. 1995). Have OC contaminants played a role in the population declines of endangered and threatened sea turtle populations?

Sea turtles accumulate these compounds, but at levels lower than fish-eating shorebirds and marine mammals. Until now, only one study had examined the effects of OCs on sea turtles, in which only one compound, 4,4'-DDE, was tested for its ability to reverse the sex of embryonic green sea turtles (Podreka *et al*. 1998). Many other compounds need to be examined for their toxicity on this species as well as the six other sea turtle species and other life stages.

This dissertation sought to address three main issues concerning OC contaminants and sea turtles: accumulation of OCs in blood and adipose, effects on the immune system, and effects on the endocrine system.

Accumulation of OCs in the blood and adipose

Loggerhead and Kemp's ridley sea turtles accumulated detectable concentrations of PCBs, 4,4'-DDE, chlordanes, mirex, and dieldrin in blood. These

concentrations correlated to levels measured in paired adipose tissue, suggesting that blood OCs are in equilibrium with those in fatty tissues. This finding indicates that simple, non-invasive blood sampling can now be used to measure OC contaminant concentrations in sea turtles, making it feasible to incorporate OC monitoring in sea turtle projects around the world.

The OC concentrations in adipose of the loggerhead, Kemp's ridley, and green sea turtles were similar to previously reported levels for these species. Interestingly, the blubber layer of a single leatherback sea turtle had much higher concentrations than the body fat from the same animal and from any other sea turtle examined in this study. Future studies should investigate the accumulation and mobilization of OCs in this species. Other sea turtle populations should be analyzed, especially those likely to be exposed to higher concentrations, such as turtles near the mouth of the Mississippi River or in waters near highly urbanized areas.

Effects on the immune system

Although the average loggerhead sea turtle accumulates lower levels of OCs than other fish-eating wildlife, this study has provided evidence that the current level of OC exposure may negatively affect sea turtle health. Significant correlations were observed between OC concentrations and indicators of poorer health, including blood chemistry values (↑ blood urea nitrogen, ↑ total protein, ↓ albumin:globulin ratio, ↓ glucose, ↑ osmolality, ↑ sodium, ↓ magnesium, ↑ AST, ↓ ALP), hematology (↑ total WBC, ↑ heterophil:lymphocyte ratio), and immune function (↑ lymphoproliferation responses to T-cell and B-cell mitogens). Moreover, three turtles exhibiting signs of wasting syndrome had two orders of magnitude higher blood OC concentrations than

the average healthy loggerhead turtle. These findings suggest that sea turtles do accumulate levels of OCs that may be detrimental to their immune system and overall health. These wasting turtles still had OC levels that were lower than concentrations in other apparently healthy wildlife (snapping turtles, birds, and marine mammals). Therefore, it is plausible that sea turtles may be more sensitive to these compounds than previously suspected. Future studies should investigate additional immune function parameters, such as natural killer cell activity, oxidative burst, and antibody production. These functions, as seen with the lymphocyte proliferation test, may be influenced by seasonal changes and contaminant exposure.

Effects on the endocrine system

Three percent of juvenile female loggerhead turtles (smaller than 77 cm straight carapace length) were expressing an egg yolk precursor protein, vitellogenin (VTG). This precocious expression may be considered abnormal. The blood PCB concentrations of these precocious females were significantly higher than normal juvenile females of the same size class that were not expressing VTG. These data support the hypotheses that VTG may be useful as a biomarker of exposure to estrogenic contaminants and that current OC contamination may affect the loggerhead sea turtle endocrine system.

This study provides the first evidence that suggests that OC contaminants may be negatively impacting sea turtles. However, the data are solely correlative and therefore circumstantial. Since experimental exposures are not feasible with endangered and threatened sea turtle species, cell culture offers a valuable alternative for experimental manipulation. For this reason, this study sought to develop an *in*

vitro cell culture model for assessing the effect of contaminants on the sea turtle endocrine system. The activity of cytochrome P450 aromatase, the enzyme responsible for the conversion of testosterone to estradiol, was characterized in a green sea turtle testis cell line. Aromatase activity was significantly increase by a 24 hr exposure to low concentrations of atrazine, a heavily used agricultural herbicide. 4,4'-DDE exposure inhibited aromatase activity in these cells, but only at a cytotoxic concentration. Additional classes of contaminants, especially PCBs should be examined for their capacity to alter sea turtle aromatase activity and other endocrine parameters. This effect may be vitally important to the sex ratio of sea turtle populations. If contaminants alter the activity or expression of this enzyme during the temperature-sensitive period of embryonic development then these compounds may alter the development of gonads and possibly reverse the sex of turtles.

Implications

Overall, the data presented here provide evidence that sea turtles are affected by current chronic levels of OC contaminants. This additional risk to their survival and reproduction should now be considered in the management of these species. Future experiments should be designed to aid in the extrapolation of these sublethal effects to values that can be used in population modeling, such as a percentage of turtles that will die or fail to reproduce due to current contamination. This type of risk assessment will help managers more accurately predict the recovery or continual decline of certain sea turtle populations.

APPENDIX

Appendix Table A1. Comparison of measured organochlorine concentrations (ng/g wet mass) to certified and reference values of NIST SRM 1945 Organics in Whale Blubber.

	Certified values[a] (ng/g wet mass)	Reference values[b] (ng/g wet mass)	Measured values[c] (ng/g wet mass)		Certified values[a] (ng/g wet mass)	Reference values[b] (ng/g wet mass)	Measured values[c] (ng/g wet mass)
PCB 18	4.48 (0.88)		3.10 (0.57)	PCB 187	105.1 (9.1)		135 (19)
PCB 28		14.1 (1.4)	11.8 (1.4)	PCB 193			9.74 (0.32)
PCB 50+31		3.12 (0.69) for 31	3.91 (0.61)	PCB 194	39.6 (2.5)		65.0 (10.3)
PCB 44	12.2 (1.4)		13.8 (1.1)	PCB 195	17.7 (4.3)		22.6 (3.8)
PCB 49	20.8 (2.8)		23.4 (6.6)	PCB 201	16.96 (0.89)		23.0 (4.3)
PCB 52	43.6 (2.5)		42.3 (4.8)	PCB 206	31.1 (2.7)		49.2 (0.7)
PCB 66	23.6 (1.6)		21.6 (1.7)	PCB 209	10.6 (1.1)		20.9 (3.1)
PCB 70+76			10.1 (1.2)	Total PCBs			1800 (139)
PCB 74			44.4 (4.1)				
PCB 87	16.7 (1.4)		23.0 (3.4)	α-HCH	16.2 (3.4)		15.0 (5.5)
PCB 92+84+89			18.0 (2.5)	β-HCH		8.0 (1.4)	1.87 (0.76)
PCB 95	33.8 (1.7)		12.7 (1.4)	γ-HCH	3.30 (0.81)		2.32 (0.59)
PCB 99	45.4 (5.4)		64.1 (6.7)	Total HCHs			19.1 (6.5)
PCB 101+90	65.2 (5.6)		73.3 (40.4)				
PCB 105	30.1 (2.3)		28.4 (5.3)	HCB	32.9 (1.7)		31.2 (3.8)
PCB 107			4.27 (1.36)	Heptachlor epoxide	10.8 (1.3)		16.9 (13.7)
PCB 110	23.3 (4.0)		33.9 (4.6)	Dieldrin		37.5 (3.9)	44.0 (11.2)
PCB 118	74.6 (5.1)		89.0 (14.3)	Mirex	28.9 (2.8)		35.9 (1.9)
PCB 128	23.7 (1.7)		31.8 (5.8)				
PCB 132			19.6 (1.8)	*trans* -chlordane			8.67 (2.16)
PCB 138+163	131.5 (7.4)		184 (22)	*cis* -chlordane	46.9 (2.8)		45.2 (8.5)
PCB 146			46.4 (1.2)	*trans* -nonachlor	231 (11)		143 (21)
PCB 149	106.6 (8.4)		78.0 (5.8)	*cis* -nonachlor	48.7 (7.6)		38.4 (4.9)
PCB 151+82	28.7 (5.2) for 151		38.5 (4.4)	oxychlordane	19.8 (1.9)		27.1 (2.2)
PCB 153	213 (19)		253 (54)	Total chlordanes			263 (31)
PCB 154			4.93 (0.60)				
PCB 156	10.3 (1.1)		12.2 (1.4)	2,4'-DDD	18.1 (2.8)		14.7 (3.1)
PCB 157			5.64 (6.15)	4,4'-DDD	133 (10)		112 (24)
PCB 158			10.8 (0.9)	2,4'-DDE	12.28 (0.87)		13.7 (1.8)
PCB 170+190	40.6 (2.6)		37.8 (1.5)	4,4'-DDE	445 (37)		521 (88)
PCB 174			24.8 (3.2)	2,4'-DDT	106 (14)		112 (13)
PCB 180	106.7 (5.3)		169 (30)	4,4'-DDT	245 (15)		239 (50)
PCB 183	36.6 (4.1)		41.8 (3.1)	Total DDTs			1090 (89)

[a] Weighted means of results from 3 analytical techniques (95 % confidence interval for the true concentration including an allowance for differences between the analytical methods (See NIST Certificate of Analysis).

[b] Weighted means of results from 1 or 2 analytical techniques (95 % confidence interval for the true concentration; some include an allowance for differences between the analytical methods (See NIST Certificate of Analysis).

[c] Mean (SD) obtained from 4 one gram samples of NIST SRM 1945.

Appendix Table A2. Comparison of mean (SD) organochlorine concentrations in fat biopsies of 14 male and 30 female juvenile loggerhead sea turtles.

	ng/g wet mass			ng/g lipid		
	Females	Males	p-values[a]	Females	Males	p-values[a]
Total PCBs	277 (306)	210 (168)	0.930	2000 (3400)	2040 (1800)	0.423
α-HCH[b]	0.230 (1.05)	<LOD	NT[b]	0.396 (1.76)	<LOD	NT
β-HCH	0.151 (0.483)	0.204 (0.534)	1.000	0.288 (0.896)	0.508 (1.29)	0.489
γ-HCH	2.57 (7.26)	<LOD	NT	4.79 (12.6)	<LOD	NT
HCB[b]	1.25 (2.85)	0.421 (0.949)	0.944	3.12 (7.37)	1.40 (3.50)	0.631
mirex	4.74 (4.58)	3.70 (3.15)	0.980	37.3 (56.6)	57.2 (73.0)	0.631
dieldrin	4.78 (3.28)	5.13 (5.53)	0.444	39.1 (104)	27.1 (27.0)	0.216
heptachlor epoxide	2.77 (2.61)	2.28 (2.84)	0.512	9.29 (9.37)	12.9 (16.9)	0.324
trans -nonachlor	15.8 (11.3)	11.5 (8.87)	0.402	110 (169)	101 (91)	0.607
cis -nonachlor	1.51 (1.61)	1.28 (1.87)	0.178	5.64 (6.58)	4.16 (6.70)	0.19
oxychlordane	11.7 (11.5)	9.50 (12.0)	0.535	129 (319)	146 (164)	0.466
Total Chlordanes	29.1 (22.2)	22.3 (19.1)	0.655	244 (478)	251 (223)	0.136
4,4'-DDE	64.6 (65.3)	64.1 (66.1)	0.444	402 (652)	539 (637)	0.11
2,4'-DDT	2.62 (5.35)	2.49 (4.25)	0.990	6.39 (12.4)	8.38 (14.8)	NT
Total DDTs	67.2 (69.6)	66.5 (69.5)	0.444	408 (652)	547 (636)	0.11
Percent lipid	28.8 (21.4)	20.8 (18.6)	NT			

[a] Mann-Whitney t-test. All p-values were > 0.05, so there were no differences between males and females.
[b] HCH = hexachlorohexane; HCB = hexachlorobenzene; NT = not tested.

Appendix Table A3. Available information on juvenile loggerhead sea turtles captured in inshore waters of North Carolina that were included in analyses in this dissertation.

Turtle ID	Capture Date	Location	PIT tag[a]	SCL (cm)[a]	Weight (kg)	T (pg/ml)[a]	Sex[a]	Lap sex[a]	VTG[a]	Comments[b]	Blood OC[c]	Adipose OC[c]
3-25	7/31/2000	Core Sound, NC	502E637A44	45.7	14.4	1.6	F	F	-		Y	Y
3-26	7/31/2000	Core Sound, NC	500E116D39	53.7	22.6	7.6	F	F	-		Y	Y
3-27	7/28/2000	Core Sound, NC	502E1F396B	49.9	18.6	5.6	F	F	-		Y	Y
3-28	7/28/2000	Core Sound, NC	500F213753	63.3	34.0	114.1	M	M	-		Y	Y
3-29	8/4/2000	Core Sound, NC	502E5C6522	65.9	32.6	1.8	F	F	-		Y	Y
3-30	8/4/2000	Core Sound, NC	407A73410A	52.6	20.6	6.2	F	F	-		Y	Y
3-31	8/4/2000	Core Sound, NC	502E5C6B4A	51.2	20.0	9.9	F	F	-		Y	Y
3-32	8/4/2000	Core Sound, NC	503157334C	62.3	34.2	12.8	F	F	-		Y	Y
3-33	8/7/2000	Core Sound, NC	500F2A2F4F	66.3	40.0	129.4	M	M	-		Y	Y
3-34	8/7/2000	Core Sound, NC	500E364720	74.0	56.6	7.3	F	F	-		Y	Y
3-35	8/9/2000	Core Sound, NC	500E0A5247	64.6	42.2	132.3	M	M	-		Y	Y
3-36	8/9/2000	Core Sound, NC	500F1A1967	70.4	49.0	4.3	F	F	-		Y	Y
3-37	8/9/2000	Core Sound, NC	502E797470	66.5	43.6	0.67	F	F	-		Y	Y
3-38	8/9/2000	Core Sound, NC	500F2B4C36	59.7	29.2	11.3	F	F	-		Y	Y
3-39	8/9/2000	Core Sound, NC	407B187E0A	64.0	45.6	232	M	M	-		Y	Y
3-40	8/9/2000	Core Sound, NC	407A065E3D	63.4	35.0	188.4	M	M	-		Y	Y
3-41	8/9/2000	Core Sound, NC	502E7A0B4C	50.8	18.4	3.5	F	F	-		Y	Y
3-42	8/11/2000	Core Sound, NC	411F370358	64.6	37.0	12.6	F	F	-		Y	Y
3-43	8/11/2000	Core Sound, NC	412C323B3D	53.7	32.4	8.3	F	F	+	3-6	Y	Y
3-44	8/11/2000	Core Sound, NC	411F210C14	71.4	49.0	7.3	F	F	-		Y	Y
3-45	8/11/2000	Core Sound, NC	411F19430A	64.5	42.0	37.2	M	M	-		Y	Y
490	7/13/2001	Core Sound, NC	407A4D6E05	61.2	41.4	7.2	F	F	-	PR	Y	Y
1180	7/16/2001	Core Sound, NC	41442A477E	71.2	52.6	8.2	F	F	-	PR	Y	Y
1301	7/13/2001	Core Sound, NC	4254481B7B	57.8	30.8	96.9	M	M	-	PR	Y	Y
1304	7/13/2001	Core Sound, NC	4237633439	60.7	36.6	83.7	M	M	-	PR	Y	Y
1332	7/20/2001	Core Sound, NC	4235006341	67.8	49.6	2.7	F	F	-	PR	Y	Y
772	7/16/2001	Core Sound, NC	4134384832	62.7	39.6	5.5	F	F	-		Y	Y
923	7/10/2001	Core Sound, NC	414435173D	63.7	37.0	95.2	M	M	-		Y	Y
956	7/10/2001	Core Sound, NC	41344E1121	58.8	31.0	5	F	F	NT[a]		Y	Y
982	7/18/2001	Core Sound, NC	4144384078	66.3	48.8	10.5	F	F	-		Y	Y
1057	7/18/2001	Core Sound, NC	502E562E3A	71.3	52.4	94.3	M	M	-	3-9	Y	Y
1110	7/16/2001	Core Sound, NC	41450B1130	58.5	30.0	5.2	F	F	-	4-16	Y	Y
1165	7/17/2001	Core Sound, NC	423571434C	64.1	42.0	3	F	F	-	4-48	Y	Y

Appendix Table A3. Con't

Turtle ID	Capture Date	Location	PIT tag[a]	SCL (cm)[a]	Weight (kg)	T (pg/ml)[a]	Sex[a]	Lap sex[a]	VTG[a]	Comments[b]	Blood OC[c]	Adipose OC[c]
1235	7/16/2001	Core Sound, NC	42347E0640	67.1	40.8	44.5	M	M	-		Y	Y
1269	7/13/2001	Core Sound, NC	42346B501D	56.2	26.8	70.8	M	M	-		Y	Y
1307	7/17/2001	Core Sound, NC	4143670D14	63.3	35.8	4.8	F	F	-		Y	Y
1308	7/17/2001	Core Sound, NC	41450B2F25	65.1	38.6	47.1	M	M	-	Died	Y	Y
1311	7/17/2001	Core Sound, NC	4134210F69	64.7	39.4	5	F	F	-		Y	Y
1325	7/16/2001	Core Sound, NC	4234661C77	50.6	20.0	96.8	M	M	-		Y	Y
1328	7/16/2001	Core Sound, NC	4234660A27	64.7	32.4	3.3	F		-	Impaired	Y	Y
1377	7/17/2001	Core Sound, NC	4144331675	61.7	34.6	4.4	F	F	-		Y	Y
1379	7/16/2001	Core Sound, NC	414B186433	57.7	30.2	5	F	F	-	4-22	Y	Y
1392	7/20/2001	Core Sound, NC	422D755D7A	61.3	32.4	7.1	F	F	-		Y	Y
1393	7/20/2001	Core Sound, NC	4236176056	49.0	16.0	7.6	F	F	-		Y	Y
1310	7/18/2001	Core Sound, NC	4232442700	59.3	30.8	3.3	F		-		Y	N
1349	7/13/2001	Core Sound, NC	422E607142	62.5		3.3	F		-		Y	N
839/1377	7/13/2001	Core Sound, NC	40797C4012	74.1		(6.7)	F		-	3-7	Y	N
1385	7/13/2001	Core Sound, NC	4232486A5D	77.3		9.1	F		+		Y	N
2-3	7/2/1999	Core Sound, NC	4144223B71	83.2		16.3	F		-		N	N
2-8	10/13/1999	Pamlico Sound, NC	41434F4F19	71.6		4.4	F		-		N	N
2-13	10/25/1999	Pamlico Sound, NC	41447C1B43	78		NT	U		-		N	N
2-16	11/2/1999	Pamlico Sound, NC	41441B1716	69.2		NT	U		-		N	N
2-24	11/29/1999	Core Sound, NC	411C63781F	61.3	32.8	14.5	F		NT	Impaired	Y	N
3-6	6/2/2000	Core Sound, NC	412C323B3D	52.3		4.8	F		+	3-43	Y	N
3-7	6/5/2000	Core Sound, NC	40797C4012	72.1		6.7	F		-	839/1377	Y	N
3-9	6/16/2000	Core Sound, NC	502E562E3A	71.2		153.7	M		-	1057	Y	N
3-12	6/16/2000	Core Sound, NC	500E0D477F	75.3		2.7	F		-		N	N
3-15	6/19/2000	Core Sound, NC	407A140443	69.6		4.6	F		-		N	N
3-16	7/14/2000	Core Sound, NC	500E1D1520	69		3.2	F		-		N	N
3-20	7/21/2000	Core Sound, NC	500F303C7A	68		17.5	F		-		N	N
3-49	8/21/2000	Core Sound, NC	407B197830	75.2		2.18	F		-		N	N
3-53	9/18/2000	Core Sound, NC	500E126E5D	78.2		225.3	M		-		N	N
3-58	9/18/2000	Core Sound, NC	500F25021B	69.3		53	M		-		N	N
4-16	6/15/2001	Core Sound, NC	41450B1130	58.3	31.0	23.9 (5.2)	F		NT	1110	Y	N
4-22	6/29/2001	Core Sound, NC	414B186433	57.6		0	F		NT	1379	Y	N
4-48	10/8/2001	Core Sound, NC	423571434C	65.2		3	F		NT	1165	Y	N

Appendix Table A3. Con't

Turtle ID	Capture Date	Location	PIT tag[a]	SCL (cm)[a]	Weight (kg)	T (pg/ml)[a]	Sex[a]	Lap sex[a]	VTG[a]	Comments[b]	Blood OC[c]	Adipose OC[c]
5-2	5/16/2002	Core Sound, NC	42557F761E	65.7		NT	U		-		N	N
5-3	5/16/2002	Core Sound, NC	425D034963	71.7		NT	U		-		N	N
5-4	5/20/2002	Core Sound, NC	42521A1D14	70.6		NT	U		-		N	N
5-5	5/20/2002	Core Sound, NC	4061563F56	57.6	31	NT	U		-		N	N
5-6	5/20/2002	Core Sound, NC	4255314529	62.3	37.4	NT	U		-		N	N
5-7	5/20/2002	Core Sound, NC	410C2B150A	65.2		NT	U		-		N	N
5-8	5/20/2002	Core Sound, NC	4255620B46	71.1		NT	U		-		N	N
5-9	5/20/2002	Core Sound, NC	425518657E	82.6	65.0	NT	U		-	Impaired	Y	N
5-10	5/20/2002	Core Sound, NC	425078754E	67		NT	U		-		N	N
5-11	5/20/2002	Core Sound, NC	413C736F73	57.2	29.4	NT	U		-		N	N
5-12	6/28/2002	Core Sound, NC	42523D3F4B	73.7		NT	U		-		N	N
5-13	6/28/2002	Core Sound, NC	42515B2915	58.9		NT	U		-		N	N
5-14	6/28/2002	Core Sound, NC	42535A040A	56.3		NT	U		-		N	N
5-15	6/28/2002	Core Sound, NC	425D1C6C1D	62		NT	U		-		N	N
5-16	6/28/2002	Core Sound, NC	4232476C5B	70.3		NT	U		-		N	N
5-21	8/26/2002	Core Sound, NC	425336374D	56.1	25.8	NT	U		-		N	N
5-22	8/26/2002	Core Sound, NC	4254344476	61.4		NT	U		-		N	N
5-23	8/26/2002	Core Sound, NC	4256091A5A	64.7		NT	U		-		N	N
5-24	8/26/2002	Core Sound, NC	4235026112	66.5		NT	U		-		N	N
5-25	9/23/2002	Core Sound, NC	425C62470D	62.7		NT	U		-		N	N
5-26	9/23/2002	Core Sound, NC	43117C3445	66.4		NT	U		-		N	N
5-27	9/27/2002	Core Sound, NC	4254651D0D	63.9		NT	U		-		N	N
5-28	9/30/2002	Pamlico Sound, NC	4351583B4C	56.5		NT	U		-	Satellite tag	N	N
5-29	9/30/2002	Pamlico Sound, NC	43510F3829	64.7		NT	U		-	Satellite tag	N	N
5-30	9/30/2002	Pamlico Sound, NC	4312311C3D	61.8		NT	U		-	Satellite tag	N	N
5-31	9/30/2002	Pamlico Sound, NC	431407043E	51.8		NT	U		-	Satellite tag	N	N
5-32	10/7/2002	Core Sound, NC	500F1B014B	70.6		NT	U		-	Tar covered	N	N
5-33	10/7/2002	Core Sound, NC	42557A367F	61.2		NT	U		-	Tar covered	N	N
5-34	10/7/2002	Core Sound, NC	414360271A	55.4		NT	U		-		N	N
5-36	10/21/2002	Core Sound, NC	412C225543	65.5		NT	U		-		N	N
5-37	10/21/2002	Core Sound, NC	407A065E3D	68.1		NT	U		+	5-39	N	N
5-38	10/21/2002	Core Sound, NC	42551D1675	41		NT	U		-		N	N
5-39	11/1/2002	Core Sound, NC	407A065E3D	68		NT	U		+	5-37	N	N

Appendix Table A3. Con't

[a] PIT tag injected into left front flipper; SCL = straight carapace length from nuchal notch to posterior marginal notch; T = testosterone, T measured from a sample collected on a different capture date are shown in parentheses; Sex = determined by T (F = female; M = male; U = unknown); Lap sex = determined by laparoscopy; VTG = presence (+) or absence (-) of plasma vitellogenin; NT = not tested.
[b] When turtles were recaptured, the corresponding turtle ID was listed. PR = OC contaminants were also measured in plasma and RBCs (see Chapter 2). Died = died after the laparoscopic procedure, trematode egg masses found in various organs. Impaired = 3 turtles that showed signs of extreme wasting (see Chapter 4). Satellite tagging performed by Catherine McClellan. A few turtles were covered in tar apparently from the tar-dipped pound nets.
[c] Whether organochlorine (OC) contaminants were measured in the blood or adipose tissue, Y = yes, N = no.

Appendix Table A4. Available information on loggerhead sea turtles captured in offshore waters of the southeast coast of the U.S. that were included in analyses in this dissertation.

Turtle ID	Capture Date	Location[a]	SCL (cm)[b]	Weight (kg)	T (pg/ml)[b]	Sex[b]	VTG[b]	Blood OC[c]	Adipose OC[c]
CC0008	6/6/2000	SC	70.7	52.2	NT[b]	M	-	N	N
CC0009	6/6/2000	SC	61.4	36.3	6	F	-	N	N
CC0010	6/6/2000	SC	63.6	38.6	7	F	-	N	N
CC0011	6/7/2000	SC	58.7	29.5	13	F	-	N	N
CC0012	6/7/2000	SC	74.1	63.5	NT	M	-	N	N
CC0013	6/7/2000	SC	63.1	49.9	NT	F	-	N	N
CC0014	6/7/2000	SC	55.3	31.8	NT	F	-	N	N
CC0015	6/7/2000	SC	67.3		NT	F	-	N	N
CC0016	6/7/2000	SC	88.6	124.7	NT	F	+	Y	N
CC0017	6/9/2000	SC	67.1	49.9	NT	F	-	N	N
CC0018	6/9/2000	SC	54.3	27.2	NT	M	-	N	N
CC0019	6/12/2000	SC	68.8	59.0	105	M	-	N	N
CC0020	6/12/2000	SC	72.4	59.0	21	U	-	N	N
CC0021	6/12/2000	SC	55.5	27.2	3	F	-	N	N
CC0022	6/12/2000	SC	63.8	47.6	3	F	-	N	N
CC0023	6/12/2000	SC	64.3	45.4	93	M	-	N	N
CC0024	6/13/2000	SC	61.1	40.8	8	F	+	Y	N
CC0025	6/13/2000	SC	59.8	38.6	NT	U	-	N	N
CC0026	6/13/2000	SC	85.2	95.3	9	F	+	N	N
CC0027	6/13/2000	SC	66.4	52.2	NT	U	-	N	N
CC0028	6/14/2000	SC	91.1	113.4	253	M	-	Y	N
CC0029	6/15/2000	SC	77.8	77.1	15	F	+	Y	N
CC0030	6/20/2000	SC	91.9	129.3	18	F	+	Y	N
CC0031	6/21/2000	SC	58.3	43.1	17	F	-	N	N
CC0032	6/23/2000	SC	63.2	24.9	16	F	-	N	N
CC0033	6/23/2000	SC	57.1	34.0	30	M	-	N	N
CC0034	6/27/2000	SC	60.7	40.8	6	F	-	N	N
CC0035	6/27/2000	SC	55.4	36.3	3	F	-	N	N
CC0036	6/27/2000	SC	54.8	38.6	56	M	-	N	N
CC0037	6/28/2000	SC	70.3		178	M	-	N	N
CC0038	6/28/2000	SC	94.7	131.5	310	M	-	Y	N
CC0039	6/30/2000	SC	70.8	56.7	164	M	-	N	N
CC0040	6/30/2000	SC	79.2	83.9	13	F	-	Y	N
CC0041	6/30/2000	SC	64.7	50.8	96	M	-	Y	N
CC0042	7/6/2000	SC	87.9	106.6	6	F	+	Y	N
CC0043	7/7/2000	SC	69.4	61.2	15	F	-	N	N
CC0044	7/7/2000	SC	62.7	54.4	23	U	-	N	N
CC0045	7/10/2000	SC	51.3	34.0	11	F	-	N	N
CC0046	7/10/2000	SC	56.5	38.6	6	F	-	N	N
CC0047	7/10/2000	SC	56.7	36.3	17	F	-	N	N
CC0048	7/10/2000	SC	88.4	117.9	1330	M	-	Y	N
CC0049	7/11/2000	SC	60.4	52.2	13	F	-	N	N
CC0071	6/20/2001	SC	89.0	117.0	94.4	M	NT	Y	N
CC0072	6/28/2001	SC	89.5	136.4	19.9	F	NT	Y	N

Appendix Table A4. Con't

Turtle ID	Capture Date	Location[a]	SCL (cm)[b]	Weight (kg)	T (pg/ml)[b]	Sex[b]	VTG[b]	Blood OC[c]	Adipose OC[c]
CC0077	6/28/2001	SC	86.2	106.0	57.8	M	NT	Y	N
CC0078	6/29/2001	SC	61.8	34.0	8.9	F	NT	Y	N
CC0079	6/29/2001	SC	69.7	46.0	3.5	F	NT	Y	N
CC0080	7/2/2001	SC	90.7	111.1	10.1	F	NT	Y	N
CC0081	7/2/2001	SC	75.5	61.0	5.8	F	NT	Y	N
CC0082	7/2/2001	SC	66.5	45.0	5.2	F	NT	Y	N
CC0083	7/2/2001	SC	> 95.5	> 140.9	325.0	M	NT	Y	N
CC0084	7/2/2001	SC	70.7	59.0	89.3	M	NT	Y	N
CC0089	7/12/2001	SC	88.1	141.0	10.2	F	NT	Y	N
CC0090	7/12/2001	SC	91.4	140.9	7.7	F	NT	Y	N
CC0096	7/18/2001	SC	91.2	131.5	8.0	F	NT	Y	N
CC0097	7/19/2001	SC	64.1	42.0	9.4	F	NT	Y	N
CC0101	5/31/2002	SC	71.4	52.0	NT	U	-	N	N
CC0102	5/31/2002	SC	71.0	56.0	87.3	M	-	N	N
CC0106	6/4/2002	SC	73.5	63.0	83.4	M	-	N	N
CC0112	6/7/2002	SC	72.4	62.0	3.2	F	-	N	N
CC0114	6/7/2002	SC	76.7	81.7	17.6	F	-	N	N
CC0116	6/11/2002	SC	70.0	53.0	7.39	F	-	N	N
CC0117	6/11/2002	SC	69.7	55.0	11.6	F	-	N	N
CC0119	6/12/2002	SC	73.2	60.0	53.5	M	-	N	N
CC0122	6/13/2002	SC	69.0	55.0	10.9	F	-	N	N
CC0125	6/18/2002	SC	75.0	58.0	115.2	M	-	N	N
CC0126	6/18/2002	SC	86.7	109.0	11.9	F	+	N	N
CC0127	6/18/2002	SC	84.6	95.3	56.3	M	-	N	N
CC0129	6/19/2002	SC	74.5	65.0	3.62	F	-	N	N
CC0130	6/19/2002	SC	92.3	136.2	86	F	+	N	N
CC0131	6/20/2002	SC	72.1	53.0	7.8	F	-	N	N
CC0134	6/24/2002	SC	73.6	60.0	86.8	M	-	N	N
CC0135	6/25/2002	SC	72.3	61.0	4.05	F	-	N	N
CC0137	6/26/2002	SC	72.3	55.0	10.9	F	-	N	N
CC0138	6/26/2002	SC	93.4	122.6	71.9	M	-	N	N
CC0139	6/27/2002	SC	103.5	> 136.2	101.3	F	+	N	N
CC0140	6/27/2002	SC	74.2	45.0	2.25	F	-	N	N
CC0141	6/28/2002	SC	70.9	50.0	40.4	M	-	N	N
CC0143	7/1/2002	SC	69.3	48.0	9.66	F	-	N	N
CC0144	7/2/2002	SC	77.9	90.8	16.4	F	-	N	N
CC0146	7/8/2002	SC	82.8	113.5	21.1	U	-	N	N
CC2008	7/11/2000	GA	53.0	25.0	13	F	-	N	N
CC2009	7/11/2000	GA	59.7	39.0	17	F	-	N	N
CC2010	7/11/2000	GA	64.8	51.0	47	M	-	N	N
CC2011	7/11/2000	GA	61.1	38.0	1	F	-	N	N
CC2012	7/12/2000	GA	63.2	40.0	19	F	-	N	N
CC2013	7/12/2000	GA	61.6	44.0	23	U	-	N	N
CC2014	7/12/2000	GA	70.8	55.0	36	M	-	N	N
CC2015	7/12/2000	GA	52.4	25.0	110	M	-	N	N
CC2016	7/12/2000	GA	51.7	25.0	5	F	-	N	N

Appendix Table A4. Con't

Turtle ID	Capture Date	Location[a]	SCL (cm)[b]	Weight (kg)	T (pg/ml)[b]	Sex[b]	VTG[b]	Blood OC[c]	Adipose OC[c]
CC2017	7/12/2000	GA	68.9	54.0	44	M	-	N	N
CC2018	7/12/2000	GA	89.2	109.1	2	F	+	N	N
CC2019	7/12/2000	GA	71.7	60.0	239	M	-	N	N
CC2020	7/12/2000	GA	49.1		13	F	-	N	N
CC2021	7/13/2000	GA	62.7	42.0	14	F	-	N	N
CC2022	7/13/2000	GA	61.3	39.0	34	M	-	N	N
CC2023	7/13/2000	GA	60.1	32.0	90	M	-	N	N
CC2024	7/13/2000	GA	59.4	38.0	11	F	-	N	N
CC2025	7/13/2000	GA	68.7	49.0	22	U	-	N	N
CC2026	7/13/2000	GA	63.6	44.0	11	F	-	N	N
CC2027	7/14/2000	GA	59.9	33.0	5	F	-	N	N
CC2033	7/17/2000	GA	68.5	39.0	4	F	-	N	N
CC2034	7/17/2000	GA	86.8	100.0	472	M	-	N	N
CC2035	7/18/2000	GA	74.2		16	F	-	N	N
CC2036	7/18/2000	GA	67.6	50.0	13	F	-	N	N
CC2037	7/19/2000	GA	76.9	72.7	36	F	+	Y	N
CC2038	7/19/2000	GA	54.2	28.0	14	F	-	N	N
CC2039	7/19/2000	GA	67.3	50.0	468	M	-	N	N
CC2040	7/19/2000	GA	68.2	47.0	18	F	-	N	N
CC2041	7/20/2000	GA	63.2	37.0	79	M	-	N	N
CC2043	7/20/2000	GA	59.7	33.0	17	F	-	N	N
CC2044	7/24/2000	GA	59.3	35.0	5	F	-	N	N
CC2045	7/24/2000	GA	66.9	46.0	8	F	-	N	N
CC2046	7/24/2000	GA	62.6	38.0	7	F	-	N	N
CC2047	7/24/2000	GA	71.9	65.0	15	F	-	N	N
CC2048	7/24/2000	GA	66.4	50.0	7	F	-	N	N
CC2049	7/25/2000	GA	67.9	44.0	11	F	-	N	N
CC2050	7/25/2000	GA	74.6	63.0	9	F	-	Y	N
CC2051	7/26/2000	GA	58.5	31.0	15	F	-	N	N
CC2052	7/26/2000	GA	55.0	29.0	112	M	-	N	N
CC2053	7/26/2000	GA	73.2	60.0	264	M	-	N	N
CC2054	7/27/2000	GA	66.3	50.0	23	U	-	N	N
CC2055	7/31/2000	NE FL	63.8	37.0	124	M	-	N	N
CC2056	7/31/2000	NE FL	59.1	30.0	74	M	-	N	N
CC2057	7/31/2000	NE FL	95.5	113.6	15	F	-	Y	N
CC2058	7/31/2000	NE FL	58.9	27.5	1	F	-	N	N
CC2059	8/1/2000	NE FL	70.2	56.0	22	U	-	N	N
CC2060	8/1/2000	NE FL	65.6	41.0	100	M	-	N	N
CC2061	6/1/2000	NE FL	62.6	45.0	167	M	-	N	N
CC2062	8/1/2000	NE FL	87.5	95.5	0	F	+	N	N
CC2064	8/1/2000	NE FL	64.2	37.0	12	F	-	N	N
CC2065	8/1/2000	NE FL	59.6	32.0	NT	U	-	N	N
CC2066	8/1/2000	NE FL	62.5	39.0	17	F	-	N	N
CC2067	8/2/2000	NE FL	55.6	28.0	NT	U	-	N	N
CC2068	8/2/2000	NE FL	58.7	29.0	6	F	-	N	N
CC2069	8/2/2000	NE FL	58.5	29.0	62	M	-	N	N

Appendix Table A4. Con't

Turtle ID	Capture Date	Location[a]	SCL (cm)[b]	Weight (kg)	T (pg/ml)[b]	Sex[b]	VTG[b]	Blood OC[c]	Adipose OC[c]
CC2070	8/2/2000	NE FL	44.8	16.0	26	U	-	N	N
CC2071	8/3/2000	NE FL	65.7	45.0	21	U	-	N	N
CC2072	8/3/2000	NE FL	59.7	38.0	131	M	-	N	N
CC2073	8/3/2000	NE FL	61.3	37.0	18	F	-	N	N
CC2074	8/3/2000	GA	62.7		4	F	-	N	N
CC2075	8/3/2000	GA	86.4	95.5	160	M	-	N	N
CC2076	8/3/2000	GA	69.9	47.0	127	M	-	N	N
CC2077	8/3/2000	GA	57.8	31.0	5	F	-	N	N
CC2078	8/3/2000	GA	63.5	40.0	3	F	-	N	N
CC2079	8/3/2000	GA	65.9	44.0	6	F	-	N	N
CC2080	8/4/2000	GA	81.0	97.7	16	F	+	N	N
CC2081	8/9/2000	GA	62.5	37.0	2	F	-	N	N
CC2082	8/9/2000	GA	66.7	48.0	212	M	-	N	N
CC2083	8/9/2000	GA	61.3	37.0	4	F	-	N	N
CC2084	8/9/2000	GA	67.8	37.0	4	F	-	N	N
CC2085	8/11/2000	GA	57.0	30.0	12 (24)	F	-	N	N
CC2086	8/11/2000	GA	61.2	38.0	258	M	-	N	N
CC2087	8/11/2000	GA	67.5	45.0	8	F	-	N	N
CC2088	8/11/2000	GA	69.6	53.0	13	F	-	N	N
CC2089	8/11/2000	GA	58.6	30.0	13	F	-	N	N
CC2090	8/14/2000	GA	66.5	47.0	4	F	-	N	N
CC2091	8/14/2000	GA	59.4	38.0	14	F	-	N	N
CC2092	8/14/2000	GA	51.6	24.0	12	F	-	N	N
CC2093	8/16/2000	GA	58.1	34.0	14	F	-	N	N
CC2094	8/16/2000	GA	58.5	38.0	4	F	-	N	N
CC2122	6/22/2001	GA	87.0	105.0	152.4	M	NT	Y	N
CC2140	6/28/2001	NE FL	90.3	101.0	355.5	M	NT	Y	N
CC2143	6/28/2001	NE FL	58.8	28.0	4.1	F	NT	Y	N
CC2160	7/13/2001	GA	64.6	40.0	107.3	M	NT	Y	N
CC2161	7/13/2001	GA	61.3	38.0	9.1	F	NT	Y	N
CC2174	7/20/2001	GA	58.6	34.0	4.9	F	NT	Y	N
CC2183	7/25/2001	GA	59.3	34.0	5.9	F	NT	Y	N
CC2192	7/27/2001	GA	71.4	50.0	1.6	F	NT	Y	N
CC2207	6/13/2002	GA	72.6	61.0	95.2	M	-	N	N
CC2210	6/17/2002	GA	69.4	50.0	10	F	-	N	N
CC2211	6/17/2002	GA	70.5	52.0	17.8	F	-	N	N
CC2212	6/17/2002	GA	69.3	52.0	3.8	F	-	N	N
CC2216	6/17/2002	GA	97.9	> 100	7.4	F	+	N	N
CC2223	6/19/2002	GA	69.2	54.0	9.7	F	-	N	N
CC2225	6/19/2002	GA	79.5	> 100	18.2	F	+	N	N
CC2229	6/20/2002	GA	68.4	49.0	50.7	M	-	N	N
CC2231	6/24/2002	GA	68.9	56.0	10.4	F	-	N	N
CC2233	6/26/2002	NE FL	68.9	52.0	9.7	F	-	N	N
CC2240	6/28/2002	GA	69.7	53.0	17.2	F	-	N	N
CC2241	6/28/2002	GA	70.6	55.0	93.1	M	-	N	N
CC2247	7/9/2002	NE FL	67.6	58.0	18	F	-	N	N

Appendix Table A4. Con't

Turtle ID	Capture Date	Location[a]	SCL (cm)[b]	Weight (kg)	T (pg/ml)[b]	Sex[b]	VTG[b]	Blood OC[c]	Adipose OC[c]
CC2266	7/15/2002	GA	81.6	80.0	31.4	M	-	N	N
CC2267	7/15/2002	GA	76.0	69.0	352.3	M	-	N	N
CC2268	7/15/2002	GA	77.4	75.0	12.4	F	-	N	N
CC2269	7/15/2002	GA	71.6	57.0	11.2	F	-	N	N
CC2270	7/15/2002	GA	72.4	67.0	377.3	M	-	N	N
CC2273	7/15/2002	GA	69.1	55.0	13.2	F	-	N	N
CC2274	7/16/2002	GA	68.0	49.0	20.2	U	-	N	N
CC2275	7/16/2002	GA	70.7	54.0	8.3	F	-	N	N
CC2281	7/17/2002	GA	69.3	53.0	11	F	-	N	N
CC2285	7/17/2002	GA	71.3	54.0	11.1	F	-	N	N
CC2289	7/18/2002	GA	79.4	76.0	24.2	F	+	N	N
CC2291	7/18/2002	GA	71.0	52.0	283.9	M	-	N	N
CC2292	7/18/2002	GA	70.2	53.0	7.6	F	-	N	N
CC2295	7/18/2002	GA	76.1	66.0	9.7	F	-	N	N
CC2300	7/22/2002	GA	71.8	59.0	97	M	-	N	N
CC2302	7/23/2002	GA	80.9	78.0	15.7	F	+	N	N
CC2305	7/24/2002	GA	68.7	62.0	10.2	F	-	N	N
CC2306	7/24/2002	GA	69.1	62.0	74.4	M	-	N	N
CC2308	7/24/2002	GA	69.1	58.0	89.8	M	-	N	N
CC2309	7/24/2002	GA	87.6	108.0	324.5	M	-	N	N
CC4009	7/11/2000	SC	69.8	54.4	11	F	-	N	N
CC4010	7/11/2000	SC	77.2	74.8	495	M	-	N	N
CC4011	7/12/2000	GA	69.5	59.0	481	M	-	N	N
CC4012	7/12/2000	GA	60.5	43.1	13	F	-	N	N
CC4013	7/12/2000	GA	56.5	36.3	3	F	-	N	N
CC4014	7/13/2000	GA	67.2	45.4	126	M	-	N	N
CC4015	7/14/2000	SC	68.2	52.2	4	F	-	N	N
CC4016	7/17/2000	SC	72.1	57.8	10	F	-	N	N
CC4017	7/18/2000	GA	52.5	20.4	5	F	-	N	N
CC4018	7/19/2000	GA	65.1	49.9	11	F	-	N	N
CC4019	7/19/2000	GA	63.3	43.1	12	F	-	N	N
CC4021	7/20/2000	GA	64.8	47.6	10	F	-	N	N
CC4022	7/20/2000	GA	71.6	57.6	182	M	-	N	N
CC4023	7/20/2000	GA	64.5	45.4	9	F	-	N	N
CC4024	7/20/2000	GA	66.8	52.6	7	F	-	N	N
CC4025	7/21/2000	GA	66.9	54.4	16	F	-	N	N
CC4026	7/24/2000	SC	81.5	90.7	28	F	+	N	N
CC4027	7/24/2000	SC	66.6	54.4	23	U	-	N	N
CC4028	7/24/2000	SC	71.9	68.0	15	F	-	N	N
CC4029	7/25/2000	SC	60.2	34.0	7	F	-	N	N
CC4030	7/27/2000	SC	52.6	22.7	135	M	-	N	N
CC4031	7/27/2000	SC	64.3	43.1	206	M	-	N	N
CC4032	7/27/2000	SC	73.7	61.2	551	M	-	N	N
CC4033	7/27/2000	SC	80.4	81.6	1322	M	-	N	N
CC4034	7/28/2000	GA	62.3	38.6	11	F	-	N	N
CC4035	7/28/2000	GA	67.9	61.2	27	U	-	N	N

Appendix Table A4. Con't

Turtle ID	Capture Date	Location[a]	SCL (cm)[b]	Weight (kg)	T (pg/ml)[b]	Sex[b]	VTG[b]	Blood OC[c]	Adipose OC[c]
CC4060	6/21/2001	GA	94.5	130.2	8.2	F	NT	Y	N
CC4062	6/21/2001	GA	94.9	131.5	140.9	M	NT	Y	N
CC4066	6/27/2001	GA	92.5	115.7	165.4	M	NT	Y	N
CC4071	6/3/2002	SC	70.0	48.0	11	F	-	N	N
CC4075	6/5/2002	GA	68.7	54.5	6.1	F	-	N	N
CC4079	6/6/2002	SC	72.4		6.7	F	-	N	N
CC4080	6/6/2002	SC	80.0	90.8	4.7	F	+	N	N
CC4081	6/7/2002	SC	69.7	56.8	7	F	-	N	N
CC4082	6/10/2002	SC	67.0	59.0	57.9	M	-	N	N
CC4084	6/11/2002	GA	70.0	59.0	5.2	F	-	N	N
CC4085	6/11/2002	GA	71.0	54.5	5.4	F	-	N	N
CC4086	6/12/2002	GA	70.5	70.4	61.4	M	-	N	N
CC4088	6/12/2002	SC	89.4	118.0	93.9	M	-	N	N
CC4089	6/12/2002	SC	73.9	72.6	10	F	-	N	N
CC4090	6/13/2002	SC	93.0	> 136.2	8.1	F	+	N	N
CC4091	6/13/2002	SC	83.9	99.9	3.9	F	+	N	N
CC4092	6/13/2002	SC	73.4	63.6	44.2	M	-	N	N
CC4095	6/18/2002	SC	73.3	70.4	104.6	M	-	N	N
CC4102	6/20/2002	GA	68.4	68.1	11.4	F	-	N	N
CC4103	6/20/2002	GA	70.1	72.6	13	F	-	N	N
CC4105	6/24/2002	GA	66.0	47.7	7.5	F	+	N	N
CC6030	7/10/2001	SC	72.4	52.0	6.0	F	NT	Y	N
CC6031	7/10/2001	SC	63.0	38.0	11.1	F	NT	Y	N
CC6032	7/10/2001	SC	62.6	37.5	10.0	F	NT	Y	N
CC6033	7/10/2001	SC	56.7	30.0	13.0	F	NT	Y	N
CC6034	7/10/2001	SC	64.2	45.0	190.1	M	NT	Y	N
CC6035	7/11/2001	SC	57.9	25.0	6.4	F	NT	Y	N
CC6036	7/11/2001	SC	57.9	32.5	9.9	F	NT	Y	N
CC6037	7/12/2001	SC	70.1	42.0	84.8	M	NT	Y	N
CC6038	7/12/2001	SC	64.1	39.5	11.4	F	NT	Y	N
CC6039	7/13/2001	SC	63.3	41.0	109.3	M	NT	Y	N
CC6040	7/13/2001	SC	59.7	34.5	67.8	M	NT	Y	N
CC6041	7/14/2001	SC	55.5	38.5	7.4	F	NT	Y	N
CC6042	7/14/2001	SC	64.9		89.9	M	NT	Y	N
CC6043	7/15/2001	SC	64.8	36.5	5.4	F	NT	Y	N
CC6044	7/15/2001	SC	59.4	34.0	67.8	M	NT	Y	N
CC6045	7/15/2001	SC	63.5	44.5	9.0	F	NT	Y	N
CC6046	7/15/2001	SC	59.5	33.0	3.1	F	NT	Y	N

[a] Offshore waters of South Carolina (SC), Georgia (GA), and northeast FL (NE FL).

[b] SCL = straight carapace length from nuchal notch to posterior marginal notch; T = testosterone, T measured from a sample collected on a different capture date are shown in parentheses; Sex = determined by T, tail length, and presence of VTG (F = female; M = male; U = unknown); VTG = presence (+) or absence (-) of plasma vitellogenin; NT = not tested.

[c] Whether organochlorine (OC) contaminants were measured in the blood or adipose tissue, Y = yes, N = no.

Appendix Table A5. Available information on additional sea turtles that were included in analyses in this dissertation.

Turtle ID	Species[a]	Status	Capture Date	Location[b]	SCL (cm)[c]	Weight (kg)	T (pg/ml)[c]	Sex[c]	Blood OC[d]	Adipose OC[d]
Lk-99-794	Lk	Dead	11/1999	Cape Cod, MA	31.31		NT[c]	U	Y	Y
Lk-99-754	Lk	Dead	11/1999	Cape Cod, MA	23.1		NT	U	Y	Y
Lk-99-759	Lk	Dead	11/1999	Cape Cod, MA	27.6		NT	U	Y	Y
Lk-99-770	Lk	Dead	11/1999	Cape Cod, MA	26.8		NT	U	Y	Y
Lk-99-771	Lk	Dead	11/1999	Cape Cod, MA	21.9		NT	U	Y	Y
Lk-99-780	Lk	Dead	11/1999	Cape Cod, MA	28.2		NT	U	Y	Y
Lk-99-789	Lk	Dead	11/1999	Cape Cod, MA	22.1		NT	U	Y	Y
Lk-99-798	Lk	Dead	11/1999	Cape Cod, MA	30.4		NT	U	Y	Y
Lk1	Lk	Dead	4/26/1998	offshore NC	45.6	NT	NT	F	N	Y
Lk2	Lk	Dead	7/11/2000	Neuse River, NC	29.7	NT	NT	M	N	Y
Lk2015	Lk	Live	6/28/2001	offshore NE FL	58.2	35	1.6	F	Y	N
Lk2016	Lk	Live	6/28/2001	offshore NE FL	44.7	14	1.4	F	Y	N
Lk2017	Lk	Live	6/28/2001	offshore NE FL	47.2	17	59.6	M	Y	N
Lk2019	Lk	Live	7/19/2001	offshore GA	42.6	12	3.0	F	Y	N
Lk4002	Lk	Live	6/14/2001	offshore SC	45.4	8	4.3	F	Y	N
Lk4006	Lk	Live	6/29/2001	offshore SC	53.4	27.2	226.3	M	Y	N
Cc1	Cc	Dead	8/16/1999	Bald Head Island, NC	53.7	20.0	NT	M	N	Y
Cm3	Cm	Dead	8/7/2000	Core Sound, NC	24.2	1.8	NT	M	N	Y
Dc1	Dc	Euthanized	6/9/1999	Long Beach, NC	176.0[e]	NT	NT	F	N	Y[e]

[a] Lk = Kemp's ridley; Cc = loggerhead; Cm = green; Dc = leatherback.

[b] MA = Massachusetts; NC = North Carolina; NE FL = northeast Florida; GA = Georgia; SC = South Carolina.

[c] SCL = straight carapace length from nuchal notch to posterior marginal notch; T = testosterone; NT = not tested; Sex = determined by T for live turtles and by visual examination of gonads for dead turtles during necropsy.

[d] Whether organochlorine (OC) contaminants were measured in the blood or adipose tissue, Y = yes, N = no.

[e] Length of the leatherback was curved carapace length; both subcutaneous fat and the carapace blubber layer were analyzed for OC contaminants.

Appendix Table A6. Concentrations of organochlorine contaminants (pg/g wet mass) in plasma, red blood cells, and whole blood of individual loggerhead sea turtles.[a]

	Plasma					Red blood cells					Whole blood				
Turtle ID	490	1180	1301	1304	1332	490	1180	1301	1304	1332	490	1180	1301	1304	1332
PCB 8	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
PCB 18	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
PCB 49	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
PCB 52	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
PCB 56	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
PCB 63	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
PCB 66	19.4	42.0	47.4	17.6	45.0	14.7	31.8	20.5	0	15.0	19.1	56.9	52.3	0	0
PCB 70+76	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
PCB 74	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
PCB 87+81	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
PCB 92+84+89	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
PCB 95	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
PCB 99	283	1020	1120	84.5	269	59.7	190	180	15.1	51.6	150	560	709	71.2	192
PCB 101+90	0	60.7	0	0	0	42.8	0	0	0	0	0	21.3	0	0	0
PCB 105	71.9	168	239	32.1	98.5	0	48.2	80.4	0	39.0	41.6	127	205	25.9	82.4
PCB 107	0	57.6	25.4	0	0	0	26.1	13.2	0	0	0	43.0	28.8	0	20.3
PCB 110	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
PCB 118	240	695	872	87.6	326	55.7	192	281	29.9	120	131	447	717	62.3	264
PCB 128	49.6	116	138	0	68.5	0	42.3	67.0	0	31.3	39.8	103	136	13.4	55.2
PCB 132	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
PCB 138	1020	2330	3130	407	870	150	471	634	63.8	199	602	1430	2390	271	567
PCB 146	153	486	197	35.8	125	0	95.8	31.4	0	18.3	69.7	291	154	28.8	102
PCB 149	16.3	51.5	0	0	0	27.4	0	13.5	0	0	0	31.6	15.4	0	0
PCB 151+82	0	101	0	0	0	0	0	32.8	0	0	65.9	68.5	0	0	13.8
PCB 153	1080	2590	4580	405	1840	183	588	1070	64.8	488	609	1630	3560	303	1420
PCB 154	13.4	49.9	26.3	0	0	0	0	0	0	0	0	38.3	14.7	0	0
PCB 156	19.4	46.3	38.2	0	30.2	0	0	24.6	0	0	0	31.1	44.7	0	26.1
PCB 157	0	14.5	24.4	0	0	0	0	0	0	0	0	16.9	32.1	0	0
PCB 158	20.9	33.9	57.7	0	30.5	0	0	20.4	0	0	0	40.0	52.5	0	27.6
PCB 163	116	353	285	26.3	152	21.2	122	114	0	79.3	70.1	251	253	21.3	119
PCB 170	55.9	118	173	31.8	177	0	40.9	74.5	0	72.8	33.7	90.2	174	19.7	140
PCB 174	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
PCB 180	346	695	1330	178	840	57.2	179	372	43.4	235	197	474	1190	52.2	630
PCB 183	84.9	186	339	25.5	184	18.5	57.2	102	0	53.9	66.9	157	296	25.6	142
PCB 187	315	822	473	105	345	20.9	246	159	0	107	249	570	406	97.5	276
PCB 193	118	232	280	33.7	68.8	15.6	68.2	89.3	0	18.8	57.5	167	260	20.7	65.1
PCB 194	36.5	87.6	140	38.7	97.7	0	39.6	68.7	0	48.7	26.8	67.1	131	0	66.4
PCB 195	0	14.3	0	0	0	0	0	0	0	0	0	21.6	22.7	0	0
PCB 201	16.1	35.4	41.5	17.3	23.3	16.1	21.5	14.8	14.4	17.7	13.2	24.3	29.2	0	44.6
PCB 206	52.3	157	146	13.5	79.2	0	61.9	75.4	0	39.1	29.0	121	142	0	63.5
PCB 209	0	27.4	20.8	0	0	0	0	16.4	0	0	0	42.2	24.8	0	0
ΣPCBs	4130	10600	13700	1540	5670	682	2520	3560	231	1630	2470	6920	11000	1010	4320
mirex	0	0	116	0	66.3	0	0	108	0	39.9	0	26.4	178	0	74.5
dieldrin	21.9	28.0	44.3	0	0	0	17.8	26.8	0	0	39.0	78.3	57.0	0	55.3
heptachlor epoxide	0	0	0	0	0	0	0	0	0	0	0	36.7	62.4	0	56.9
t-chlordane	0	0	15.2	0	0	0	29.8	20.5	0	43.8	23.6	33.1	44.4	0	32.0
c-chlordane	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
t-nonachlor	59.5	113	148	45.4	148	0	48.7	90.0	0	116	56.6	108	163	44.2	176
c-nonachlor	14.8	24.8	0	13.1	0	0	0	0	0	0	15.4	30.2	16.3	0	0
oxychlordane	55.7	57.0	322	39.6	133	20.1	21.6	182	0	100	52.2	53.0	307	17.7	126
Σchlordanes	130	195	485	98.2	281	20.1	100	292	0	260	148	224	531	61.9	334
4,4'-DDE	395	997	532	236	713	189	526	477	107	941	347	901	599	194	837
2,4'-DDD	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
4,4'-DDD	0	0	16.8	0	0	47.4	0	0	0	0	17.5	16.9	0	0	0
2,4'-DDT	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
ΣDDTs	395	997	549	236	713	236	526	477	107	941	365	918	599	194	837
% lipid	0.426	0.259	0.259	0.238	0.166	0.271	0.291	0.262	0.238	0.343	0.361	0.348	0.362	0.427	0.209

[a] Values shown as zero were below the limit of detection.

Appendix Table A7. Blood concentrations of organochlorine contaminants (pg/g wet mass) in individual loggerhead sea turtles.[a]

	3-25	3-26	3-27	3-28	3-29	3-30	3-31	3-32	3-33	3-34	3-35	3-36	3-37
PCB 8	0	0	0	0	13.6	0	0	0	14.8	0	0	0	0
PCB 18	0	0	0	0	0	0	0	0	47.2	0	0	0	0
PCB 49	0	0	0	0	0	0	0	0	258	0	0	0	0
PCB 52	0	0	0	0	0	0	0	0	288	0	0	0	0
PCB 56	0	0	0	0	0	0	0	0	26.3	0	0	0	0
PCB 63	0	0	0	0	0	0	0	0	0	0	0	0	0
PCB 66	0	35.4	0	13.8	14.2	0	0	0	268	0	14.9	0	17.2
PCB 70 + 76	0	0	0	0	0	0	0	0	96.4	0	0	0	0
PCB 74	0	0	0	0	0	0	0	0	300	0	0	0	0
PCB 87+81	0	0	0	0	0	0	0	0	341	0	0	0	0
PCB 92+84+89	0	33.1	0	0	0	0	0	0	105	0	0	0	15.2
PCB 95	0	0	0	0	0	0	0	0	217	0	0	0	0
PCB 99	40.3	492	121	361	268	245	143	109	1290	0	176	62.4	361
PCB 101+90	0	0	0	0	0	0	0	0	0	0	0	0	0
PCB 105	18.2	144	47.5	126	71.6	63.8	48.9	44	273	0	45.8	37.2	129
PCB 107	0	15.4	0	0	0	0	0	0	81.0	0	0	0	0
PCB 110	0	0	0	0	0	0	0	0	541	0	0	0	0
PCB 118	38.5	389	116	353	208	152	110	106	1050	0	122	93.6	378
PCB 128	0	114	34.3	86.7	71.2	51.1	32.0	19.0	200	0	28.6	41.0	133
PCB 132	0	0	0	0	0	0	0	0	102	0	0	0	0
PCB 138	290	2230	822	1860	1430	1500	897	746	3350	173	842	914	1970
PCB 146	22.5	328	90.3	155	157	61.1	38.1	46.9	426	0	122	119	198
PCB 149	0	0	0	22.3	63.7	17.2	0	0	421	0	0	0	0
PCB 151+82	0	0	0	0	0	0	0	0	170	0	0	0	0
PCB 153	254	2020	733	1930	1186	1132	773	608	3990	206	659	873	2240
PCB 154	0	47.4	0	13.2	13.6	0	0	0	75.6	0	0	0	15.5
PCB 156	0	40.5	18.1	36.5	20.1	0	0	10.5	83.0	0	0	25.6	51.3
PCB 157	0	31.3	0	30.8	21.3	22.6	0	12.9	64.4	0	10.8	16.6	33.8
PCB 158	0	50.0	15.6	40.6	29.9	22.0	11.7	0	121	0	17.4	18.0	52.9
PCB 163	28.8	297	95.2	189	154	95.3	56.7	48.1	462	15.4	107	131	230
PCB 170	18.3	154	69.6	140	91.1	71.5	45.7	24.4	313	14.6	50.5	140	266
PCB 174	0	0	0	0	0	0	0	0	46.3	0	0	0	0
PCB 180	104	787	351	770	452	444	299	202	1700	76.6	269	568	1140
PCB 183	13.1	191	66.0	176	105	89.7	48.6	36.4	413	0	53.5	97.7	245
PCB 187	57.0	624	206	366	334	188	129	99.9	785	45.9	251	265	439
PCB 193	16.4	174	77.9	131	124	126	67.5	62.6	202	20.9	100	129	144
PCB 194	0	61.2	18.4	46.2	31.8	12.9	0	0	262	0	20.1	28.3	98.3
PCB 195	0	22.5	0	13.4	0	0	0	0	76.6	0	0	0	29.4
PCB 201	0	0	0	0	0	0	0	0	0	0	0	0	0
PCB 206	0	93.5	25.5	47.6	39.1	0	0	0	623	0	51.4	0	91.2
PCB 209	0	12.9	0	0	0	0	0	0	72.0	0	0	0	18.1
ΣPCBs	901	8390	2910	6910	4900	4290	2700	2180	19200	552	2940	3560	8300
mirex	0	54.7	10.1	116	12.5	0	17.7	19.9	157	0	0	0	61.4
dieldrin	0	244	0	161	94.2	88.3	74.2	74.7	60.6	51.3	73.9	52.1	54.0
heptachlor epoxide	0	0	0	98.5	17	31.9	33.1	0	27.7	0	0	0	0
t-chlordane	0	34.6	10.9	36.1	33.8	26.1	26.3	13.2	30.1	21.0	30.8	0	33.1
c-chlordane	0	0	0	0	13.9	13.3	0	0	0	13.8	0	0	0
t-nonachlor	72.4	251	81.8	218	175	122	88.9	44.5	80.2	150	57.3	57.4	185
c-nonachlor	0	31.6	19.9	0	49.3	39.8	42.0	15.2	29.2	38.8	28.3	20.9	31.7
oxychlordane	22.4	110	38.7	158	41.3	73.7	54.7	50.2	96.8	0	26.6	0	82.5
Σchlordanes	94.8	427	151	412	313	275	212	123	236	224	143	78.3	332
4,4'-DDE	125	1770	321	1160	721	316	212	41.9	754	0	423	994	1690
2,4'-DDD	0	0	0	0	0	0	10.6	0	0	0	0	0	0
4,4'-DDD	0	0	0	0	48.1	37.2	35.8	0	14.5	37.2	13.9	0	16.4
2,4'-DDT	0	0	0	0	0	0	0	0	32.0	0	0	0	0
ΣDDTs	125	1770	321	1160	769	353	258	41.9	801	37.2	437	994	1710
% lipid	0.222	0.272	0.291	0.218	0.214	0.185	0.123	0.193	0.253	0.329	0.286	0.300	0.370

[a] Values shown as zero were below the limit of detection.

Appendix Table A7. Con't

	3-38	3-39	3-40	3-41	3-42	3-43	3-44	3-45	772	923	956	982	1057
PCB 8	0	0	0	0	0	15.0	0	0	0	0	0	0	0
PCB 18	0	0	0	0	0	17.1	0	0	0	0	0	0	0
PCB 49	0	0	0	0	0	0	0	0	0	0	0	0	0
PCB 52	0	0	0	0	0	0	0	0	0	0	0	0	0
PCB 56	0	0	0	0	0	0	0	0	0	0	0	0	0
PCB 63	0	0	0	0	0	0	0	0	0	0	0	0	0
PCB 66	0	37.3	0	0	0	28.4	0	0	0	23.0	0	31.7	50.1
PCB 70 + 76	0	0	0	0	0	0	0	0	0	0	0	0	0
PCB 74	0	0	0	0	0	0	0	0	0	0	0	0	0
PCB 87+81	0	18.5	0	0	0	0	0	0	0	0	0	0	0
PCB 92+84+89	0	16.2	0	0	0	16.4	0	0	0	0	0	0	0
PCB 95	0	0	0	0	0	0	0	0	0	0	0	0	0
PCB 99	88.7	306	220	108	107	418	0	29.2	347	268	269	273	1000
PCB 101+90	0	0	0	0	0	0	0	0	0	30.9	0	25.2	17.8
PCB 105	30.9	75.8	58.9	44.5	34.8	98.8	0	23.8	108	121	121	91.5	351
PCB 107	0	0	0	0	0	13.4	0	0	26.3	19.9	0	28.4	83.3
PCB 110	0	0	0	0	0	0	0	0	0	0	0	0	0
PCB 118	58.8	235	164	119	96.1	304	0	55.6	363	371	366	288	1180
PCB 128	21.8	61.6	39.0	27.3	19.0	73.2	0	23.9	91.2	98.7	85.9	80.1	341
PCB 132	0	0	0	0	0	0	0	0	0	0	0	0	0
PCB 138	642	1660	1380	853	734	2930	72.3	704	1710	1090	1560	921	4250
PCB 146	55.9	283	146	78.6	51.3	381	0	87.5	275	137	88.4	211	639
PCB 149	0	58.8	0	0	0	32.0	0	0	0	0	0	66.4	50.7
PCB 151+82	0	55.7	0	0	0	14.2	0	0	0	0	0	71.8	33.0
PCB 153	493	1380	1050	726	651	2100	59.9	651	1860	1690	2040	1120	5790
PCB 154	0	37.6	0	0	0	47.0	0	0	24.1	0	0	18.7	82.9
PCB 156	0	24.9	16.5	15.2	0	25.8	0	16.7	33.3	34.7	33.9	30.0	123
PCB 157	0	25.3	20.7	15.9	15.8	27.5	0	0	25.5	13.5	21.5	0	76.1
PCB 158	10.3	34.6	18.5	15.2	0	49.5	0	10.5	37.3	30.7	33.6	36.1	139
PCB 163	60.5	207	125	88.5	63.9	278	0	95.0	252	170	168	196	639
PCB 170	43.1	106	60.5	58.5	28.0	143	0	96.3	139	124	158	126	466
PCB 174	0	0	0	0	0	0	0	0	0	0	0	19.7	0
PCB 180	268	526	406	356	226	1020	39.3	439	820	700	957	529	2390
PCB 183	43.1	131	83.6	63.7	40.2	230	0	65.3	189	150	199	130	610
PCB 187	139	536	315	181	133	898	0	206	663	298	272	478	1600
PCB 193	52.7	176	149	82.0	60.2	374	0	79.9	310	111	178	152	499
PCB 194	0	52.4	23.2	10.9	0	89.9	0	16.0	150	94.0	81.7	104	379
PCB 195	0	21.6	0	0	0	27.7	0	0	19.9	0	0	24.9	158
PCB 201	0	0	0	0	0	0	0	0	24.6	20.7	21.0	30.4	63.9
PCB 206	0	149	63.8	0	0	192	0	0	294	105	63.8	193	692
PCB 209	0	15.2	0	0	0	25.5	0	0	45.0	26.4	17.2	57.6	179
ΣPCBs	2010	6230	4340	2840	2260	9870	172	2600	7810	5730	6740	5330	21900
mirex	0	11.2	15.5	16.5	0	25.1	0	0	46.2	167	111	30.2	296
dieldrin	49.8	134	55.1	46.1	43.1	41.0	0	36.7	0	0	0	0	127
heptachlor epoxide	0	27.8	0	21.2	0	0	0	0	16.9	37.0	0	0	84.3
t-chlordane	19.1	33.8	20.4	20.8	17.5	26.9	0	0	19.0	34.0	14.0	26.5	103
c-chlordane	0	0	0	0	0	0	0	0	0	0	0	0	0
t-nonachlor	69.3	110	55.1	71.9	27.4	71.8	10.3	26.2	107	157	108	188	426
c-nonachlor	28.0	39.7	25.1	17.2	21.7	26.3	10.2	13.3	18.6	17.5	14.3	48.3	35.7
oxychlordane	27.1	38.7	34.9	36.2	25.2	33.5	0	0	40.8	101	102	45.9	211
Σchlordanes	145	222	136	146	91.8	159	20.5	39.5	185	310	238	309	776
4,4'-DDE	265	715	244	221	0	625	0	449	603	678	414	1170	2420
2,4'-DDD	0	0	0	0	0	0	0	0	0	0	0	0	0
4,4'-DDD	0	12.2	0	0	0	0	0	0	0	0	0	32.8	23.8
2,4'-DDT	0	0	0	0	0	0	0	0	0	0	0	0	0
ΣDDTs	265	727	244	221	0	625	0	449	603	678	414	1200	2440
% lipid	0.222	0.276	0.184	0.218	0.136	0.304	0.260	0.206	0.285	0.191	0.278	0.440	0.385

[a] Values shown as zero were below the limit of detection.

Appendix Table A7. Con't

	1110	1165	1235	1269	1307	1308	1311	1325	1328	1377	1379	1392	1393
PCB 8	0	0	0	0	0	0	0	0	0	0	0	0	0
PCB 18	0	0	0	0	0	0	0	0	0	0	0	0	0
PCB 49	0	0	0	0	0	0	0	0	0	0	0	0	0
PCB 52	0	0	0	0	0	0	0	0	0	0	0	0	0
PCB 56	0	0	0	0	0	0	0	0	0	0	0	0	0
PCB 63	0	0	0	0	0	0	0	0	0	0	0	0	0
PCB 66	19.0	15.2	19.0	18.4	0	0	39.0	0	30.7	0	17.8	0	18.9
PCB 70 + 76	0	0	0	0	0	0	0	0	0	0	0	0	0
PCB 74	0	0	0	0	0	0	0	0	0	0	0	0	0
PCB 87+81	0	0	0	0	0	0	0	0	0	0	0	0	0
PCB 92+84+89	0	0	0	0	0	0	0	0	0	0	0	0	0
PCB 95	0	0	0	0	0	0	0	0	0	0	0	0	0
PCB 99	360	126	408	253	0	190	322	15.7	1090	22.2	181	16.5	159
PCB 101+90	0	0	0	0	0	0	0	0	22.4	0	0	0	0
PCB 105	113	42.9	175	69.5	0	68.2	104	15.6	460	17.8	47.0	0	86.0
PCB 107	27.3	0	36.7	0	0	18.1	35.2	0	97.4	0	0	0	18.9
PCB 110	0	0	0	0	0	0	0	0	0	0	0	0	0
PCB 118	283	130	539	194	0	215	361	36.2	1290	40.6	176	31.0	226
PCB 128	92.9	26.9	155	40.3	0	46.6	92.3	0	368	0	53.4	13.4	57.3
PCB 132	0	0	0	0	0	0	0	0	0	0	0	0	0
PCB 138	2140	715	1740	882	48.2	810	1170	183	4860	211	897	187	872
PCB 146	289	127	257	138	0	137	299	26.6	701	25.0	238	19.0	145
PCB 149	22.1	0	0	0	0	0	33.6	0	19.9	0	17.1	0	0
PCB 151+82	44.4	22.9	13.2	14.1	0	19.3	85.4	0	26.4	14.8	26.4	0	0
PCB 153	1830	662	2830	923	40.5	1140	1580	202	6360	208	1210	207	842
PCB 154	16.7	0	20.3	14.2	0	0	31.2	0	41.9	0	36.3	0	0
PCB 156	35.5	0	65.1	16.6	0	20.9	37.8	0	154	0	26.8	0	23.6
PCB 157	47.5	0	37.6	0	0	0	16.1	0	107	0	0	0	0
PCB 158	67.8	0	63.3	22.5	0	18.7	36.4	0	182	0	28.6	0	21.6
PCB 163	283	112	322	108	0	140	256	26.4	806	27.1	153	20.2	155
PCB 170	166	43.2	314	49.1	0	109	131	19.3	685	22.2	111	20.8	59.7
PCB 174	23.0	0	0	0	0	0	0	0	0	0	0	0	0
PCB 180	1120	310	1480	440	32.3	602	658	123	3290	138	627	115	352
PCB 183	209	58.7	322	86.7	0	106	171	19.2	660	17.6	185	14.0	73.9
PCB 187	642	268	696	257	0	310	661	81.1	1540	57.1	812	49.6	309
PCB 193	329	115	205	108	0	119	161	21.5	475	23.3	204	28.1	103
PCB 194	91.7	35.4	212	34.4	0	73.3	130	14.1	296	20.2	247	14.2	43.2
PCB 195	0	0	70.0	0	0	0	39.5	0	83.6	0	89.1	0	0
PCB 201	28.7	0	27.3	14.1	0	20.0	36.7	0	53.1	0	42.3	0	16.2
PCB 206	108	41.3	280	56.7	0	85.4	278	0	145	0	1180	0	17.5
PCB 209	16.0	0	53.7	30.6	0	25.3	67.7	0	30.4	0	185	0	0
ΣPCBs	8400	2850	10300	3770	121	4270	6830	784	23900	845	6790	736	3600
mirex	35.4	0	243	0	0	38.9	47.7	0	211	0	69.2	0	19.9
dieldrin	0	0	19.5	28.1	0	0	0	0	111	0	0	0	0
heptachlor epoxide	0	0	38.5	31.3	0	0	0	0	85.3	0	0	0	0
t-chlordane	0	0	42.2	0	0	15.7	46.7	0	99.0	0	0	0	0
c-chlordane	0	0	0	0	0	0	0	0	0	0	0	0	0
t-nonachlor	136	50.8	287	72.8	0	113	139	32.7	659	59.3	83	35.5	89.1
c-nonachlor	20.1	14.9	21.0	18.1	0	20.2	39.4	0	21.2	13.3	28.4	0	15.3
oxychlordane	36.3	0	127	47.5	0	49.0	32.0	0	209	0	15.5	0	27.9
Σchlordanes	192	65.7	477	138	0	198	257	32.7	988	72.6	127	35.5	132
4,4'-DDE	675	231	1130	634	47.3	507	1110	127	3800	198	475	116	326
2,4'-DDD	0	0	0	0	0	0	0	0	0	0	0	0	0
4,4'-DDD	13.0	0	13.8	0	0	23.4	14.2	0	0	13.7	24.8	18.1	27.2
2,4'-DDT	0	0	0	0	0	0	0	0	0	0	0	0	0
ΣDDTs	688	231	1140	634	47.3	530	1120	127	3800	212	500	134	353
% lipid	0.226	0.208	0.235	0.164	0.295	0.275	0.220	0.166	0.0979	0.313	0.315	0.413	0.247

[a] Values shown as zero were below the limit of detection.

Appendix Table A7. Con't

	1310	1349	839/1377	1385	2-24	3-6	3-7	3-9	4-16	4-22	4-48	5-9	0016
PCB 8	0	0	0	0	0	0	0	0	0	0	0	0	0
PCB 18	0	0	0	0	0	0	0	0	0	0	0	0	0
PCB 49	0	0	0	0	0	0	0	0	0	0	0	0	0
PCB 52	0	0	0	0	0	0	0	0	0	0	0	0	0
PCB 56	0	0	0	0	0	0	0	0	0	0	0	152	0
PCB 63	0	0	0	0	0	0	0	0	0	0	0	212	0
PCB 66	0	0	31.3	50.9	84.4	28.5	38.0	33.4	26.8	0	13.3	158	0
PCB 70 + 76	0	0	0	0	0	0	0	0	0	0	0	0	0
PCB 74	0	0	21.2	0	0	0	0	0	0	0	0	0	0
PCB 87+81	0	0	0	0	0	0	0	0	0	0	0	0	0
PCB 92+84+89	0	0	0	0	0	0	0	0	0	0	0	35.8	0
PCB 95	0	0	0	0	0	0	0	0	0	0	0	25.8	0
PCB 99	0	0	567	416	355	362	426	415	416	76.7	182	24500	88.1
PCB 101+90	0	0	0	0	0	23.7	28.2	13.8	15.1	0	0	952	0
PCB 105	0	15.3	138	113	134	127	161	131	183	19.6	53.1	6120	44.9
PCB 107	0	0	39.1	0	15.9	31.4	44.6	34.0	33.1	0	0	1020	26.1
PCB 110	0	0	0	0	0	0	0	0	0	0	0	0	0
PCB 118	0	16.3	477	464	484	393	497	434	460	67.5	152	21000	135
PCB 128	0	0	94.3	105	91.9	114	130	120	153	16.9	31.1	7980	31.9
PCB 132	0	0	0	0	0	0	0	0	0	0	0	0	0
PCB 138	123	140	2390	2070	1190	2270	2200	1720	2320	443	1060	86000	400
PCB 146	0	0	482	427	97.2	409	437	311	330	80.7	194	10300	61.2
PCB 149	0	0	55.3	0	0	84.9	107	19.7	35.5	0	26.2	19.9	0
PCB 151+82	0	0	114	0	0	66.2	164	25.0	51.6	15.0	54.0	66.8	0
PCB 153	115	61.7	2590	2640	3220	2360	2310	2030	2360	527	926	90300	520
PCB 154	0	0	42.5	0	15.3	30.9	32.3	36.1	18.2	0	0	1070	0
PCB 156	0	0	38.3	56.1	41.3	43.6	53.8	40.8	69.4	0	0	1830	0
PCB 157	0	0	38.7	0	46.8	32.2	39.0	25.7	45.8	0	0	1090	22.9
PCB 158	0	0	31.2	0	62.3	63.0	64.8	51.4	78.0	0	18.2	3030	31.5
PCB 163	0	0	405	315	182	356	399	273	363	59.6	164	16900	88.7
PCB 170	0	0	138	170	307	262	238	152	317	41.3	59.4	6070	29.4
PCB 174	0	0	0	0	0	24.9	27.4	0	0	0	0	0	22.5
PCB 180	67.4	68.7	989	1050	1510	1550	1180	797	1560	245	438	25000	135
PCB 183	0	0	231	278	313	306	253	212	286	48.3	87.2	8140	37.8
PCB 187	24.1	25.6	1040	1120	278	1200	1080	710	824	207	434	24200	126
PCB 193	13.4	0	410	343	92.0	503	461	279	363	89.6	161	3770	68.8
PCB 194	61.2	60.8	116	239	113	212	211	124	202	37.2	36.4	3750	23.4
PCB 195	0	0	19.5	77.8	32.8	65.8	60.6	43.7	42.8	0	0	660	0
PCB 201	0	0	0	56.6	0	44.2	48.1	28.6	54.3	0	14.8	775	0
PCB 206	0	0	221	719	67.6	323	380	214	227	88.4	58.2	1990	29.2
PCB 209	0	0	31.9	158	0	67.9	113	48.6	33.0	0	0	622	0
ΣPCBs	404	389	10800	10900	8730	11400	11200	8320	10900	2060	4160	348000	1920
mirex	0	0	22.4	0	85.8	59.2	40.2	102	76.6	0	0	1320	27.2
dieldrin	0	0	15.4	952	0	0	0	35.5	0	0	0	904	0
heptachlor epoxide	0	0	0	0	14.2	0	0	41.3	0	42.9	52.4	490	20.5
t-chlordane	0	0	29.2	307	0	0	0	39.7	0	0	0	2050	0
c-chlordane	0	0	0	137	0	0	0	0	0	0	0	97.3	0
t-nonachlor	22.6	30.5	111	209	95.1	123	184	165	321	33.1	51.7	10500	52.7
c-nonachlor	14.3	15.0	28.6	60.6	16.7	10.1	43.6	35.4	32.4	14.3	18.7	392	0
oxychlordane	0	0	26.1	0	110	35.9	30.4	75.3	78.3	0	0	3220	59.2
Σchlordanes	36.9	45.5	195	713	221	169	258	315	432	47.4	70.4	16300	112
4,4'-DDE	115	110	753	941	305	867	1170	1030	1690	205	266	40500	318
2,4'-DDD	0	0	0	0	31.4	0	0	0	0	0	0	27.7	0
4,4'-DDD	30.4	22.3	22.4	87.2	23.9	34.9	63.1	0	38.5	0	0	21.1	0
2,4'-DDT	0	0	0	0	0	0	0	0	0	0	0	0	0
ΣDDTs	145	132	775	1030	360	902	1230	1030	1730	205	266	40600	318
% lipid	0.0898	0.104	0.101	0.251	0.0299	0.165	0.199	0.266	0.170	0.195	0.191	0.0389	0.347

[a] Values shown as zero were below the limit of detection.

Appendix Table A7. Con't

	0024	0028	0029	0030	0038	0040	0041	0042	0048	0071	0072	0077	0078
PCB 8	0	0	0	0	0	0	0	0	0	0	0	0	0
PCB 18	0	0	0	0	0	0	0	0	0	0	0	0	0
PCB 49	0	0	0	0	0	0	0	0	0	0	0	0	0
PCB 52	0	0	0	0	0	0	0	0	0	0	0	0	0
PCB 56	0	0	0	0	0	0	0	0	0	0	0	0	0
PCB 63	0	0	0	0	0	0	0	0	0	0	0	0	0
PCB 66	57.8	15.1	0	18.7	0	0	75.0	0	0	0	63.7	0	0
PCB 70 + 76	0	0	0	0	0	0	0	0	0	0	0	0	0
PCB 74	0	0	0	0	0	0	0	0	0	0	0	0	0
PCB 87+81	0	0	0	0	0	0	0	0	0	0	0	0	0
PCB 92+84+89	0	0	0	0	0	0	0	0	0	0	0	0	0
PCB 95	0	0	0	0	0	0	0	0	0	0	0	0	0
PCB 99	132	171	13.2	346	46.4	136	79.6	42.7	34.4	33.9	286	0	109
PCB 101+90	0	0	0	0	0	0	0	0	0	0	0	0	0
PCB 105	41.5	80.8	23.5	154	31.4	72.3	25.6	18.9	18.1	51.6	121	31.0	49.7
PCB 107	0	0	0	15.0	0	0	0	0	0	0	26.4	0	0
PCB 110	0	0	0	0	0	0	0	0	0	0	0	0	0
PCB 118	168	228	56.9	415	104	184	88.5	49.2	51.3	86.8	657	85.7	122
PCB 128	36.0	48.4	0	107	21.0	52.5	18.3	0	0	26.8	125	27.0	28.4
PCB 132	0	0	0	0	0	0	0	0	0	0	0	0	0
PCB 138	645	600	173	1500	226	933	226	167	190	402	910	257	583
PCB 146	82.3	63.1	27.5	211	15.9	112	48.1	16.2	20.5	55.8	160	23.2	79.0
PCB 149	0	0	0	0	0	0	0	0	0	107	0	0	0
PCB 151+82	0	0	0	35.5	0	0	0	0	0	915	0	0	0
PCB 153	945	823	262	1820	451	1370	416	192	215	452	1090	496	624
PCB 154	15.6	0	0	0	0	0	0	0	0	0	0	0	0
PCB 156	14.5	19.1	0	41.5	15.6	30.6	13.1	0	0	0	42.7	23.8	0
PCB 157	24.7	0	0	24.2	0	14.8	21.0	0	0	27.0	0	0	0
PCB 158	26.7	15.2	0	63.1	0	25.2	23.9	0	0	55.2	25.6	0	13.2
PCB 163	89.4	86.9	24.8	249	35.5	129	56.2	17.9	25.7	79.9	180	48.7	95.9
PCB 170	58.8	50.8	23.6	112	37.6	119	38.0	0	0	43.2	96.8	116	48.4
PCB 174	0	0	0	0	0	0	0	0	0	0	0	0	0
PCB 180	399	270	195	603	186	860	142	49.6	67.1	272	449	377	330
PCB 183	103	67.8	47.3	221	47.7	232	36.7	0	0	114	83.2	54.3	58.8
PCB 187	217	181	188	602	63.7	583	114	58.0	61.0	148	402	90.9	205
PCB 193	112	44.2	47.1	159	21.1	219	29.5	13.3	19.5	240	140	34.1	73.8
PCB 194	36.0	35.7	114	70.4	40.4	333	18.8	0	0	147	96.4	81.8	35.4
PCB 195	14.2	0	13.0	0	0	80.8	0	0	0	0	0	0	0
PCB 201	0	0	0	24.1	0	30.1	0	0	0	91.0	26.0	18.4	0
PCB 206	30.8	36.5	484	49.1	32.6	997	26.6	0	0	68.4	73.9	40.0	22.5
PCB 209	0	0	38.2	18.0	0	62.0	0	0	0	0	35.3	0	0
ΣPCBs	3250	2840	1730	6860	1380	6580	1500	625	703	3420	5090	1800	2480
mirex	42.5	97	47.0	63.4	54.6	44.2	24.0	0	0	43.2	112	0	0
dieldrin	0	86.1	0	698	0	0	0	0	21.4	0	23.8	16.1	29.6
heptachlor epoxide	0	90.3	0	240	0	0	0	0	33.6	0	0	0	0
t-chlordane	0	33.8	0	84.1	13.4	0	0	0	0	0	57.0	0	0
c-chlordane	0	0	0	0	0	0	0	0	0	0	0	0	0
t-nonachlor	35.9	117	26.8	207	33.1	81.7	26.3	29.5	29.2	37.0	319	38.5	84.1
c-nonachlor	0	0	0	23.8	14.3	12.9	15.7	0	0	0	0	0	16.8
oxychlordane	55.4	244	0	507	103	56.7	38.4	26.3	52.6	51.1	106	23.9	26.3
Σchlordanes	91.3	395	26.8	822	164	151	80.5	55.8	81.8	88.1	482	62.3	127
4,4'-DDE	278	385	134	752	239	291	271	129	120	245	2380	381	380
2,4'-DDD	23.4	0	0	0	0	0	29.7	0	0	0	0	0	0
4,4'-DDD	17.8	0	0	0	15.5	0	22.6	0	0	17.1	16.5	18.1	0
2,4'-DDT	0	0	0	0	0	0	0	0	0	0	0	0	0
ΣDDTs	319	385	134	752	255	291	323	129	120	262	2400	399	380
% lipid	0.0938	0.187	0.101	0.224	0.328	0.0949	0.0733	0.116	0.215	0.0920	0.175	0.222	0.163

[a] Values shown as zero were below the limit of detection.

Appendix Table A7. Con't

	0079	0080	0081	0082	0083	0084	0089	0090	0096	0097	2037	2050	2057
PCB 8	0	0	0	0	0	0	0	0	0	0	0	0	0
PCB 18	0	0	0	0	0	0	0	0	0	0	0	0	0
PCB 49	0	0	0	0	0	0	0	0	0	0	0	0	0
PCB 52	0	0	0	0	0	0	0	0	0	0	0	0	0
PCB 56	0	0	0	0	0	0	0	0	0	0	0	0	0
PCB 63	0	0	0	0	0	0	0	0	0	0	0	0	0
PCB 66	0	0	0	0	0	0	0	0	0	0	85.2	0	21.9
PCB 70 + 76	0	0	0	0	0	0	0	0	0	0	0	0	0
PCB 74	0	0	0	0	0	0	0	0	0	0	0	0	0
PCB 87+81	0	0	0	0	0	0	0	0	0	0	0	0	0
PCB 92+84+89	0	0	0	0	0	0	0	0	0	0	0	0	0
PCB 95	0	0	0	0	0	0	0	0	0	0	0	0	0
PCB 99	46.8	72.7	40.8	67.4	40.4	16.0	31.3	69.1	90.6	70.7	91.9	138	749
PCB 101+90	0	0	0	0	0	0	0	0	0	0	0	0	21.8
PCB 105	26.5	44.6	18.8	35.0	29.7	13.1	32.4	74.1	86.6	36.6	44.0	85.5	422
PCB 107	0	0	0	0	0	0	0	0	0	0	14.0	0	70.7
PCB 110	0	0	0	0	0	0	0	0	0	0	0	0	0
PCB 118	68.8	141	50.3	96.1	86.9	29.4	77.1	217	231	89.7	125	250	931
PCB 128	0	23.7	0	16.9	14.1	0	15.8	49.2	54.0	21.1	27.3	70.4	303
PCB 132	0	0	0	0	0	0	0	0	0	0	0	0	0
PCB 138	282	455	190	397	270	168	328	660	691	347	345	828	1980
PCB 146	35.4	44.6	36.1	62.7	40.3	21.9	46.4	68.7	72.3	62.0	55.1	92.9	225
PCB 149	0	0	0	0	0	0	0	0	0	0	0	0	0
PCB 151+82	0	0	0	0	0	0	0	0	0	0	15.4	0	24.0
PCB 153	404	664	303	461	369	236	420	987	1050	472	641	1940	4660
PCB 154	0	0	0	0	0	0	0	0	0	0	24.0	25.8	65.4
PCB 156	0	0	0	0	0	0	0	25.7	34.3	0	16.3	32.4	72.0
PCB 157	0	0	0	0	0	0	0	0	15.4	0	25.5	27.4	61.2
PCB 158	0	0	0	0	0	0	0	19.9	20.2	0	31.8	25.1	79.6
PCB 163	39.7	71.9	38.9	64.2	41.7	19.6	55.2	108	117	60.5	68.1	109	535
PCB 170	22.2	39.4	14.6	38.4	19.7	0	42.5	99.3	112	34.2	77.2	223	342
PCB 174	0	0	0	0	0	0	0	0	0	0	0	17.6	0
PCB 180	175	280	97.5	260	155	102	237	540	592	204	323	1450	787
PCB 183	35.0	59.2	15.3	45.7	27.0	13.6	37.1	103	111	38.7	94.3	660	683
PCB 187	114	149	102	164	120	70.1	147	228	255	167	260	832	728
PCB 193	46.4	60.7	32.1	67.2	43.6	37.6	77.8	122	144	62.1	56.4	127	131
PCB 194	23.2	42.8	18.7	26.9	24.7	22.5	54.5	131	141	28.5	45.9	1330	447
PCB 195	0	0	0	0	0	0	0	0	0	0	34.4	344	0
PCB 201	0	0	0	0	0	0	16.0	22.8	28.5	0	0	43.4	37.0
PCB 206	21.5	48.3	14.0	20.3	25.4	17.6	45.9	132	147	25.6	110	4670	999
PCB 209	0	0	0	0	0	0	0	0	13.9	0	0	367	167
ΣPCBs	1340	2200	972	1820	1310	767	1660	3660	4010	1720	2610	13700	14500
mirex	0	47.7	0	22.7	20.4	0	19.8	129	155	0	35.8	178	359
dieldrin	0	54.9	16.9	46.3	65.5	35.8	0	0	76.6	0	0	0	156
heptachlor epoxide	0	0	0	0	0	0	0	0	117	0	0	0	89.4
t-chlordane	0	0	18.2	16.7	25.7	0	0	0	0	0	0	0	73.7
c-chlordane	0	0	0	0	0	0	0	0	0	0	0	0	0
t-nonachlor	25.0	38.8	30.7	59.9	43.4	20.6	18.4	120	84.0	40.9	34.4	62.1	313
c-nonachlor	0	0	0	14.8	0	14.8	0	0	0	15.5	17.7	0	0
oxychlordane	16.9	103	18.3	28.3	51.1	15.9	26.1	82.4	143	14.8	45.7	56.9	305
Σchlordanes	41.9	142	67.3	120	120	51.3	44.5	203	227	71.2	97.7	119	692
4,4'-DDE	185	206	193	384	219	152	177	543	400	354	355	265	2550
2,4'-DDD	0	0	0	0	0	0	0	0	0	0	33.3	0	0
4,4'-DDD	0	0	0	0	0	0	31.3	17.0	13.0	0	25.4	0	0
2,4'-DDT	0	0	0	0	0	0	0	0	0	0	0	0	0
ΣDDTs	185	206	193	384	219	152	208	560	413	354	413	265	2550
% lipid	0.183	0.178	0.177	0.145	0.165	0.107	0.152	0.0839	0.125	0.196	0.112	0.0909	0.0810

[a] Values shown as zero were below the limit of detection.

Appendix Table A7. Con't

	2122	2140	2143	2160	2161	2174	2183	2192	4060	4062	4066	6030	6031
PCB 8	0	0	0	0	0	0	0	0	0	0	0	0	0
PCB 18	0	0	0	0	0	0	0	0	0	0	0	0	0
PCB 49	0	0	0	0	0	0	0	0	0	0	0	0	0
PCB 52	0	0	0	0	0	0	0	0	0	0	0	0	0
PCB 56	0	0	0	0	0	0	0	0	0	0	0	0	0
PCB 63	0	0	0	0	0	0	0	0	0	0	0	0	0
PCB 66	0	39.0	0	0	0	0	0	0	0	0	0	0	0
PCB 70 + 76	0	0	0	0	0	0	0	0	0	0	0	0	0
PCB 74	0	0	0	0	0	0	0	0	0	0	0	0	0
PCB 87+81	0	0	0	0	0	0	0	0	0	0	0	0	0
PCB 92+84+89	0	0	0	0	0	0	0	0	0	0	0	0	0
PCB 95	0	0	0	0	0	0	0	0	0	0	0	0	0
PCB 99	426	903	51.8	43.1	67.4	54.5	24.6	0	196	235	17.1	0	21.7
PCB 101+90	0	36.4	0	0	0	0	0	0	0	0	0	0	0
PCB 105	204	406	35.1	22.1	24.8	29.7	14.4	0	172	203	25.1	0	13.5
PCB 107	0	48.2	0	0	0	0	0	0	18.0	13.6	0	0	0
PCB 110	0	0	0	0	0	0	0	0	0	0	0	0	0
PCB 118	613	1010	81.0	46.7	79.3	70.8	34.0	0	474	656	65.5	0	26.7
PCB 128	204	341	17.0	15.7	19.3	19.2	0	0	128	216	0	0	0
PCB 132	0	0	0	0	0	0	0	0	0	0	0	0	0
PCB 138	2560	3500	387	228	343	390	241	35.7	1460	2270	198	84.8	173
PCB 146	333	578	41.4	51.3	81.8	85.4	34.3	0	143	139	24.5	0	24.8
PCB 149	0	24.3	0	0	0	0	0	0	0	0	0	0	0
PCB 151+82	0	16.0	0	0	0	0	0	0	0	0	0	0	0
PCB 153	4550	4770	437	272	506	547	248	22.8	2640	4570	286	79.2	231
PCB 154	51.1	85.2	0	0	28.8	14.6	0	0	0	0	0	0	0
PCB 156	69.7	112	0	0	0	0	0	0	64.1	72.0	0	0	0
PCB 157	72.9	70.7	0	0	0	0	0	0	53.7	76.5	0	0	0
PCB 158	78.7	154	0	0	0	0	0	0	48.7	70.0	0	0	0
PCB 163	381	681	45.8	41.5	56.8	71.4	33.2	0	230	280	30.0	0	27.2
PCB 170	374	493	39.2	25.4	38.1	40.8	21.3	0	253	526	26.0	0	0
PCB 174	0	17.5	0	0	0	0	0	0	0	0	0	0	0
PCB 180	2980	2310	275	168	304	324	161	33.0	1630	2920	171	52.1	94.0
PCB 183	1120	739	39.5	53.8	139	109	26.6	0	421	744	31.4	0	0
PCB 187	2810	2480	133	304	662	574	146	0	829	849	123	14.7	72.7
PCB 193	529	365	62.8	54.7	88.0	83.2	43.5	0	215	472	53.0	0	29.4
PCB 194	1980	1070	38.4	72.3	156	119	35.2	0	733	1670	58.7	0	19.3
PCB 195	451	322	0	23.2	47.0	17.9	0	0	189	343	0	0	0
PCB 201	156	134	0	26.6	49.0	26.9	0	0	42.0	60.8	0	0	0
PCB 206	5810	3480	52.1	333	721	447	83.8	0	1790	3580	109	0	0
PCB 209	474	304	0	42.8	74.7	31.5	0	0	152	321	0	0	0
ΣPCBs	26200	24500	1740	1820	3490	3060	1150	91.5	11900	20300	1220	231	733
mirex	466	143	0	20.8	30.4	35.7	0	0	710	1620	75.5	0	0
dieldrin	118	860	16.3	19.1	21.5	20.9	0	0	0	104	0	0	0
heptachlor epoxide	82.1	485	0	0	0	0	0	0	0	0	0	0	0
t-chlordane	32.6	194	18.3	13.2	0	16.9	18.4	13.4	0	0	0	0	0
c-chlordane	0	0	0	0	0	0	0	0	0	0	0	0	0
t-nonachlor	109	733	78.5	27.8	27.5	39.7	34.0	0	120	91.4	23.5	0	16.6
c-nonachlor	16.7	55.1	0	0	0	0	0	0	0	0	0	0	0
oxychlordane	442	253	22.3	0	0	18.8	0	0	281	372	22.9	0	0
Σchlordanes	600	1240	119	41.0	27.5	75.3	52.4	13.4	401	463	46.4	0	16.6
4,4'-DDE	561	2210	452	192	192	238	192	77.9	443	627	150	109	143
2,4'-DDD	0	0	0	0	0	0	0	0	0	0	0	0	0
4,4'-DDD	0	12.1	0	0	0	0	0	0	12.7	13.3	13.9	0	0
2,4'-DDT	0	0	0	0	0	0	0	0	0	0	0	0	0
ΣDDTs	561	2220	452	192	192	238	192	77.9	456	640	164	109	143
% lipid	0.180	0.129	0.0995	0.151	0.172	0.134	0.111	0.0948	0.121	0.155	0.114	0.0982	0.180

[a] Values shown as zero were below the limit of detection.

Appendix Table A7. Con't

	6032	6033	6034	6035	6036	6037	6038	6039	6040	6041	6042	6043	6044
PCB 8	0	0	0	0	0	0	0	0	0	0	0	0	0
PCB 18	0	0	0	0	0	0	0	0	0	0	0	0	0
PCB 49	0	0	0	0	0	0	0	0	0	0	0	0	0
PCB 52	0	0	0	0	0	0	0	0	0	0	0	0	0
PCB 56	0	0	0	0	0	0	0	0	0	0	0	0	0
PCB 63	0	0	0	0	0	0	0	0	0	0	0	0	0
PCB 66	0	0	0	0	0	0	0	0	0	0	0	0	0
PCB 70 + 76	0	0	0	0	0	0	0	0	0	0	0	0	0
PCB 74	0	0	0	0	0	0	0	0	0	0	0	0	0
PCB 87+81	0	0	0	0	0	0	0	0	0	0	0	0	0
PCB 92+84+89	0	0	0	0	0	0	0	0	0	0	0	0	0
PCB 95	0	0	0	0	0	0	0	0	0	0	0	0	0
PCB 99	30.4	0	0	0	34.4	0	41.8	19.1	18.5	0	46.0	0	21.0
PCB 101+90	0	0	0	0	0	0	0	0	0	0	0	0	0
PCB 105	17.5	0	16.1	0	30.8	0	19.5	0	0	0	18.1	0	0
PCB 107	0	0	0	0	0	0	0	0	0	0	0	0	0
PCB 110	0	0	0	0	0	0	0	0	0	0	0	0	0
PCB 118	35.5	0	26.6	25.2	91.4	0	64.2	30.4	30.0	0	63.4	0	41.5
PCB 128	0	0	0	0	20.0	0	0	0	0	0	0	0	0
PCB 132	0	0	0	0	0	0	0	0	0	0	0	0	0
PCB 138	230	139	153	170	324	45.4	260	148	165	33.3	252	85.5	143
PCB 146	33.6	0	19.5	13.1	42.4	0	49.8	24.0	25.6	0	37.9	0	28.4
PCB 149	0	0	0	0	0	0	0	0	0	0	0	0	0
PCB 151+82	0	0	0	0	0	0	15.4	0	0	0	0	0	0
PCB 153	285	149	144	234	548	19.1	349	234	214	22.1	406	135	212
PCB 154	0	0	0	0	0	0	0	0	0	0	0	0	0
PCB 156	0	0	0	0	14.0	0	0	0	0	0	0	0	0
PCB 157	0	0	0	0	0	0	0	0	0	0	0	0	0
PCB 158	0	0	0	0	0	0	0	0	0	0	0	0	0
PCB 163	38.9	0	16.0	17.1	61.6	0	48.6	27.4	29.0	0	48.9	0	23.6
PCB 170	15.3	14.3	0	13.3	51.6	0	30.0	0	0	0	24.6	0	0
PCB 174	0	0	0	0	0	0	0	0	0	0	0	0	0
PCB 180	113	96.8	63.0	109	289	19.5	185	67.2	58.5	23.5	153	74.7	45.4
PCB 183	13.5	0	0	13.8	48.7	0	31.2	13.9	0	0	29.1	0	0
PCB 187	104	27.7	54.0	41.0	133	0	128	75.6	78.2	0	119	55.7	87.2
PCB 193	44.4	22.8	23.2	23.7	60.6	0	49.6	24.0	25.1	0	45.5	17.8	74.4
PCB 194	13.6	0	0	0	43.4	0	0	0	0	25.8	51.7	0	27.7
PCB 195	0	0	0	0	0	0	0	0	0	0	0	0	0
PCB 201	0	0	0	0	0	0	0	0	0	0	0	16.2	14.1
PCB 206	0	0	0	0	44.0	0	20.5	0	0	0	18.2	25.5	0
PCB 209	0	0	0	0	0	0	0	0	0	0	0	0	0
ΣPCBs	976	449	515	660	1840	84.1	1290	663	643	105	1310	410	718
mirex	0	0	0	0	29.9	0	0	0	0	0	19.3	0	0
dieldrin	0	30.4	0	35.0	0	0	0	0	0	0	0	0	0
heptachlor epoxide	0	0	0	0	0	0	0	0	0	0	0	0	0
t-chlordane	14.2	0	13.4	0	19.0	0	0	0	0	0	0	0	13.4
c-chlordane	0	0	0	0	0	0	0	0	0	0	0	0	0
t-nonachlor	22.7	23.1	25.4	35.3	38.1	0	58.7	16.9	19.7	0	33.3	19.3	26.1
c-nonachlor	0	15.5	13.9	0	14.8	0	0	0	0	0	0	0	0
oxychlordane	0	0	0	0	21.8	0	0	0	0	0	19.5	0	0
Σchlordanes	36.8	38.5	52.7	35.3	93.7	0	58.7	16.9	19.7	0	52.8	19.3	39.4
4,4'-DDE	181	149	142	173	204	55.5	261	130	148	40.8	225	79.2	183
2,4'-DDD	0	0	0	0	0	0	0	0	0	0	0	0	0
4,4'-DDD	0	0	0	0	0	17.5	16.6	16.0	16.7	16.9	16.7	26.9	26.7
2,4'-DDT	0	0	0	0	0	0	0	0	0	0	0	0	0
ΣDDTs	181	149	142	173	204	73.0	278	146	165	57.7	242	106	209
% lipid	0.127	0.142	0.117	0.169	0.117	0.0743	0.162	0.160	0.154	0.125	0.176	0.0914	0.169

[a] Values shown as zero were below the limit of detection.

Appendix Table A7. Con't

	6045	6046
PCB 8	0	0
PCB 18	0	0
PCB 49	0	0
PCB 52	0	0
PCB 56	0	0
PCB 63	0	0
PCB 66	0	0
PCB 70 + 76	0	0
PCB 74	0	0
PCB 87+81	0	0
PCB 92+84+89	0	0
PCB 95	0	0
PCB 99	47.1	31.6
PCB 101+90	0	0
PCB 105	18.9	14.3
PCB 107	0	0
PCB 110	0	0
PCB 118	55.5	35.0
PCB 128	0	0
PCB 132	0	0
PCB 138	235	212
PCB 146	40.1	36.0
PCB 149	0	0
PCB 151+82	0	0
PCB 153	325	302
PCB 154	0	0
PCB 156	0	0
PCB 157	0	0
PCB 158	0	0
PCB 163	41.4	39.2
PCB 170	17.9	0
PCB 174	0	0
PCB 180	127	86.9
PCB 183	30.4	0
PCB 187	201	119
PCB 193	74.0	34.3
PCB 194	28.6	61.8
PCB 195	0	0
PCB 201	0	0
PCB 206	53.0	18.3
PCB 209	0	0
ΣPCBs	1300	991
mirex	0	0
dieldrin	0	0
heptachlor epoxide	0	0
t-chlordane	0	0
c-chlordane	0	0
t-nonachlor	37.8	24.0
c-nonachlor	0	0
oxychlordane	14.8	0
Σchlordanes	52.7	24.0
4,4'-DDE	244	177
2,4'-DDD	0	0
4,4'-DDD	17.8	25.4
2,4'-DDT	0	0
ΣDDTs	262	202
% lipid	0.161	0.138

[a] Values shown as zero were below the limit of detection.

Appendix Table A8. Blood concentrations of organochlorine contaminants (pg/g wet mass) in individual Kemp's ridley sea turtles.[a]

	Lk-99-794	Lk-99-754	Lk-99-759	Lk-99-770	Lk-99-771	Lk-99-780	Lk-99-789	Lk-99-798	Lk2015	Lk2016	Lk2017	Lk2019	Lk4002	Lk4006
PCB 8	0	0	0	0	0	0	0	0	0	0	0	0	0	0
PCB 18	0	0	0	0	0	0	0	0	0	0	0	0	0	0
PCB 49	0	0	0	0	0	0	0	0	0	0	0	0	0	0
PCB 52	0	0	0	0	0	0	0	0	0	0	0	0	0	0
PCB 56	0	0	0	0	0	0	0	0	0	0	0	0	0	0
PCB 63	0	0	0	0	0	0	0	0	0	0	0	0	0	0
PCB 66	13.8	0	45.8	14.0	16.6	22.5	0	129	0	0	14.1	0	0	0
PCB 70+76	0	0	0	0	0	0	0	0	0	0	0	0	0	0
PCB 74	0	0	0	0	35.3	0	0	174	0	0	0	0	0	0
PCB 87+81	0	0	0	0	0	0	0	0	0	0	0	0	0	0
PCB 92+84+89	0	0	0	0	0	0	0	0	0	0	0	0	0	0
PCB 95	0	0	0	0	0	0	0	0	0	0	0	0	0	0
PCB 99	168	0	460	71.9	128	71.1	0	489	210	155	277	78.4	99.1	120
PCB 101+90	0	0	0	0	0	0	0	72.8	0	23.6	0	0	0	0
PCB 105	67.7	0	209	26.9	50.1	34.3	0	229	46.4	17.0	54.1	32.0	31.3	43.9
PCB 107	13.2	0	46.1	0	0	0	0	38.6	0	0	0	0	0	0
PCB 110	0	0	0	0	0	0	0	242	0	0	0	0	0	0
PCB 118	176	0	677	73.8	143	99.9	14.4	546	169	61.1	212	97.9	103	138
PCB 128	51.7	0	215	25.7	42.2	25.1	0	133	52.6	35.3	76.2	30.1	25.2	32.7
PCB 132	0	0	0	0	0	0	0	85.2	0	0	0	0	0	0
PCB 138	835	61.0	1230	255	329	311	135	2120	717	552	1180	466	444	432
PCB 146	138	0	343	41.2	82.6	58.1	0	333	184	199	316	154	126	94.0
PCB 149	17.0	0	55.1	34.2	38.0	0	0	272	0	34.8	20.2	0	0	0
PCB 151+82	63.9	0	93.9	47.6	56.7	19.1	38.4	396	52.4	58.6	48.7	25.0	23.5	0
PCB 153	845	70.4	2400	359	553	355	99.0	1400	1100	872	1900	804	617	672
PCB 154	0	0	16.8	14.4	0	0	0	0	38.2	55.0	71.8	45.4	0	0
PCB 156	0	0	72.1	26.3	40.0	0	0	44.6	32.5	26.2	49.9	20.9	17.1	18.3
PCB 157	0	0	17.9	0	14.3	0	0	0	34.9	19.5	64.2	61.7	15.4	14.5
PCB 158	14.6	0	35.1	14.8	21.1	0	0	58.9	31.6	16.2	47.5	14.6	0	15.5
PCB 163	142	0	378	50.6	80.3	61.5	0	340	127	121	182	90.8	97.6	80.2
PCB 170	72.2	0	220	34.8	59.8	17.4	0	73.4	99.5	59.9	156	56.3	45.4	53.1
PCB 174	0	0	0	0	16.5	0	0	45.9	0	0	0	0	0	0
PCB 180	412	59.0	897	168	244	99.8	48.5	519	602	466	1150	652	333	315
PCB 183	75.9	0	245	23.5	50.4	14.3	0	143	188	214	387	336	92.1	74.9
PCB 187	286	0	756	120	172	96.3	30.2	529	787	1470	1790	1810	641	362
PCB 193	114	14.0	92.2	20.3	33.4	20.5	0	166	205	132	295	174	106	118
PCB 194	36.9	30.1	125	24.8	32.7	0	0	30.6	247	282	499	555	130	98.0
PCB 195	0	0	0	0	0	0	0	0	69.5	100	156	175	24.5	0
PCB 201	20.7	0	37.9	19.2	22.0	23.6	18.8	30.4	38.9	73.7	93.1	79.3	28.7	16.7
PCB 206	0	0	69.6	0	0	0	0	16.2	655	1080	1680	2030	359	219
PCB 209	0	0	0	0	0	0	0	0	61.7	96.3	170	209	25.6	18.6
ΣPCBs	3560	234	8740	1470	2260	1330	384	8660	5750	6220	10900	8000	3390	2940
HCB	0	0	0	41.0	48.0	0	0	0	0	0	0	0	0	0
mirex	36.1	0	148	21.6	60.3	0	0	0	37.7	37.1	39.4	71.5	120	172
dieldrin	131	0	181	96.9	155	26.2	0	57.0	204	0	344	151	287	505
heptachlor epoxide	33.6	17.8	119	77.9	73.6	49.9	0	51.8	90.5	0	158	132	125	155
t-chlordane	0	0	54.9	28.4	31.4	0	0	0	0	0	0	0	0	0
c-chlordane	0	0	0	22.2	34.9	0	0	0	0	0	0	0	0	0
t-nonachlor	161	21.3	605	162	260	66.8	25.5	89.7	79.0	41.5	105	72.4	53.4	72.8
c-nonachlor	49.4	22.8	80.0	55.1	82.1	27.1	18.2	51.5	59.7	19.0	39.2	33.7	30.1	0
oxychlordane	49.6	0	256	45.6	103	55.1	0	111	65.9	18.7	79.6	46.4	46.4	114
Σchlordanes	260	44.1	996	313	511	149	43.8	252	205	79.2	224	152	130	187
4,4'-DDE	559	194	1830	940	964	232	143	646	233	112	181	113	197	161
2,4'-DDD	0	0	0	0	0	0	0	0	0	0	0	0	0	0
4,4'-DDD	19.2	15.7	13.5	39.4	85.9	13.5	17.0	23.6	0	20.2	0	0	0	0
2,4'-DDT	0	0	0	0	0	0	0	0	0	0	0	0	0	0
ΣDDTs	578	209	1840	979	1050	246	160	670	233	132	181	113	197	161
% lipid	0.409	0.321	0.373	0.624	1.17	0.232	0.301	0.250	0.212	0.154	0.140	0.169	0.125	0.210

[a] Values shown as zero were below the limit of detection.

Appendix Table A9. Concentrations of organochlorine contaminants (ng/g wet mass) in fat biopsies of individual juvenile loggerhead sea turtles from Core Sound, NC.[a]

	3-25	3-26	3-27	3-28	3-29	3-30	3-31	3-32	3-33	3-34	3-35	3-36	3-37
PCB 28	0	0	0	0	0	0	0	0	0	0	1.77	0	0
PCB 52	0	0	0	0	0	0	0	0	0	0	0	0	0
PCB 56	0	0	0	0	1.36	0	0	1.06	0	0	2.35	1.61	0
PCB 63	0	0	0	0	0	0	0	0	0	0	0	0	0
PCB 66	1.92	1.85	1.43	0	5.49	1.86	0	3.83	0	0	7.81	2.49	0
PCB 74	0	0	0	0	0	0	0	0	0	0	0	0	0
PCB 87	0	0	0	0	0	0	0	0	0	0	0	0	0
PCB 92	0	0	0	0	0	0	0	0	0	0	2.44	2.03	0
PCB 99	8.04	10.5	5.26	3.44	26.5	13.6	2.49	15.7	1.80	1.08	21.6	11.4	3.02
PCB 101+90	0	0	0	0	1.60	0	0	0	0	0	2.49	1.19	0
PCB 104	0	0	0	0	0	0	0	0	0	0	0	0	0
PCB 105	3.67	3.70	2.32	1.71	10.9	6.10	1.08	8.56	0	0	7.23	7.54	1.22
PCB 107	1.48	1.75	1.08	0	4.24	1.26	0	2.16	0	0	3.40	3.68	0
PCB 110	0	0	0	0	0	0	0	0	0	0	0	0	0
PCB 118	11.7	13.5	8.34	6.41	39.8	19.6	3.97	27.5	2.63	1.32	23.7	25.5	4.96
PCB 128	2.97	4.07	2.33	1.57	11.0	5.90	0	6.51	0	0	6.92	9.9	1.56
PCB 138	18.0	20.0	12.7	7.55	60.4	31.5	4.86	34.9	5.39	1.11	37.6	69.6	7.55
PCB 146	4.63	6.48	3.58	1.48	14.9	4.09	0	6.87	1.40	0	12.8	19.3	1.73
PCB 149	0	0	0	0	0	0	0	0	0	0	2.10	0	0
PCB 151+82	0	0	0	0	0	0	0	0	0	0	2.24	2.00	0
PCB 153	33.9	47.0	28.9	20.3	124	71.3	11.9	84.5	10.1	3.22	79.4	152	21.9
PCB 154	1.04	1.69	0	0	3.88	1.55	0	2.60	0	0	4.47	2.61	0
PCB 156	1.29	1.65	1.09	0	4.44	2.13	0	2.86	0	0	2.74	5.99	0
PCB 157	0	0	0	0	1.92	1.11	0	1.20	0	0	0	2.37	0
PCB 158	1.11	1.41	0	0	3.97	2.11	0	2.36	0	0	2.68	4.14	0
PCB 163	4.72	6.34	3.71	1.96	15.7	5.80	0	7.07	1.52	0	11.7	20.2	1.99
PCB 170	3.63	5.59	4.10	2.59	13.4	7.84	1.16	6.92	1.38	0	9.10	31.7	3.47
PCB 174	0	0	0	0	0	0	0	0	0	0	0	0	0
PCB 180	11.3	15.4	11.6	7.11	37.3	24.0	3.51	22.8	4.00	0	26.1	88.4	9.61
PCB 183	3.62	5.39	3.38	2.37	12.3	7.88	1.21	7.89	1.10	0	8.95	18.7	2.65
PCB 187	8.50	13.1	7.09	3.50	28.1	8.98	1.69	12.7	2.57	0	25.5	41.2	3.83
PCB 193	0	0	0	0	2.48	1.03	0	1.24	0	0	1.63	4.54	0
PCB 194	1.49	3.08	1.70	1.29	6.74	4.21	0	4.40	0	0	5.82	10.4	1.33
PCB 195	0	1.45	0	0	3.17	1.46	0	1.84	0	0	2.86	3.40	0
PCB 201	0	0	0	0	1.73	0	0	0	0	0	2.13	0	0
PCB 206	0	4.30	1.36	1.21	8.38	2.85	0	4.14	0	1.26	10.7	1.76	0
PCB 209	0	1.21	0	0	2.62	1.01	0	1.58	0	0	4.68	0	0
ΣPCBs	123	169	100	62.5	446	227	31.9	271	31.9	7.99	333	544	64.8
α-HCH	0	0	0	0	0	0	0	0	0	0	0	0	0
β-HCH	0	0	0	0	0	0	0	0	0	0	0	1.27	0
γ-HCH	0	0	0	0	0	0	0	0	0	0	0	0	0
HCB	1.04	0	0	0	0	0	0	0	0	0	0	1.35	0
mirex	3.34	3.50	2.25	3.44	5.50	5.28	2.08	13.8	0	0	6.18	2.11	1.25
dieldrin	7.63	3.36	3.53	2.08	8.24	7.28	1.65	8.97	2.61	1.49	13.0	8.13	1.14
heptachlor epoxide	2.77	1.65	1.45	0	3.98	3.66	0	6.91	0	0	3.57	2.32	0
oxychlordane	7.10	6.06	4.16	3.62	11.0	14.9	3.32	26.7	0	0	0	2.30	1.08
t-nonachlor	17.3	16.3	8.94	6.50	28.3	14.3	3.85	26.4	3.51	0	20.9	15.0	3.15
c-nonachlor	2.05	1.16	1.09	0	3.38	1.78	0	3.23	0	0	3.95	1.62	0
Σchlordanes	26.4	23.5	14.2	10.1	42.7	30.9	7.17	56.4	3.51	0	24.9	18.9	4.22
2,4'-DDT	0	0	0	0	11.5	5.90	0	6.84	0	0	0	14.3	0
4,4'-DDE	43.5	72.8	36.4	20.2	98.3	46.7	12.1	57.1	10.8	0	122	273	20.2
ΣDDTs	43.5	72.8	36.4	20.2	110	52.6	12.1	64.0	10.8	0	122	287	20.2
% lipid	29.7	3.73	11.2	2.20	40.9	26.6	4.92	37.9	37.6	0.255	55.6	44.5	2.84

[a] Values shown as zero were below the limit of detection.

Appendix Table A9. Con't

	3-38	3-39	3-40	3-41	3-42	3-43	3-44	3-45	490	772	923	956
PCB 28	0	0	0	0	0	1.28	0	0	0	2.21	0	0
PCB 52	0	0	0	0	0	0	0	0	0	1.03	0	0
PCB 56	0	2.77	1.29	0	0	3.21	0	1.23	0	0	0	0
PCB 63	0	0	0	0	0	1.06	0	0	0	2.23	0	0
PCB 66	0	5.53	4.40	1.04	3.11	12.5	0	2.01	2.94	9.13	0	0
PCB 74	0	1.25	0	0	0	0	0	0	0	1.42	0	0
PCB 87	0	0	0	0	0	0	0	0	0	4.34	0	0
PCB 92	0	3.33	1.00	0	0	3.26	0	1.46	0	0	0	0
PCB 99	6.51	18.2	18.5	4.42	15.4	50.7	1.54	9.11	18.1	111	0	5.33
PCB 101+90	0	2.94	1.35	0	1.24	4.18	0	1.16	1.25	8.39	0	0
PCB 104	0	0	0	0	0	0	0	0	0	0	0	0
PCB 105	3.09	7.84	8.21	2.36	8.50	18.7	0	6.90	6.45	27.8	0	2.00
PCB 107	0	4.26	3.15	0	2.77	8.12	0	3.44	2.15	12.3	0	0
PCB 110	0	1.15	0	0	0	0	0	0	0	0	0	0
PCB 118	8.43	26.1	28.2	8.39	29.2	69.6	2.29	21.3	23.5	129	1.49	7.67
PCB 128	2.67	7.39	7.92	2.07	8.18	18.0	0	8.98	6.05	26.8	0	1.72
PCB 138	16.5	42.5	41.9	11.8	30.8	122	5.95	54.8	18.6	91.2	0	5.69
PCB 146	4.16	15.2	11.6	3.08	7.51	40.7	1.47	15.8	7.86	47.6	0	0
PCB 149	0	3.70	0	0	0	2.67	0	0	1.29	4.08	0	0
PCB 151+82	0	4.17	1.02	0	0	2.92	0	1.02	0	3.27	0	0
PCB 153	31.7	90.6	94.5	27.8	93.6	282	12.3	126	89.4	493	5.43	33.2
PCB 154	0	4.44	3.53	0	2.01	10.7	0	2.07	1.32	14.3	0	0
PCB 156	1.12	3.18	3.14	1.08	2.95	7.45	0	5.47	2.22	9.22	0	0
PCB 157	0	1.14	0	0	1.13	2.05	0	1.86	0	3.30	0	0
PCB 158	1.15	3.22	3.00	0	2.58	8.97	0	3.47	2.23	10.1	0	0
PCB 163	4.06	13.2	10.8	3.42	8.97	33.5	1.80	17.8	7.71	43.5	0	1.86
PCB 170	4.42	10.3	10.0	3.77	8.70	31.6	2.41	27.0	6.70	24.0	0	1.95
PCB 174	0	0	0	0	0	1.26	0	0	0	0	0	0
PCB 180	13.9	30.8	30.2	11.1	25.4	111	6.72	73.1	20.2	79.0	1.12	6.55
PCB 183	3.69	9.14	10.0	3.08	9.00	35.3	1.73	15.4	7.13	38.8	0	2.65
PCB 187	8.60	27.2	23.1	6.71	15.8	99.1	3.40	34.8	17.3	97.0	0	2.65
PCB 193	0	1.83	1.74	0	0	6.27	0	3.05	1.17	4.40	0	0
PCB 194	1.77	5.26	6.10	1.50	5.73	24.4	0	8.49	4.93	15.2	0	0
PCB 195	0	2.28	2.85	0	2.56	10.5	0	2.86	2.55	8.04	0	0
PCB 201	0	1.91	0	0	0	7.84	0	0	1.42	6.59	0	0
PCB 206	0	7.50	9.35	0	5.89	41.2	0	1.96	6.89	26.9	0	0
PCB 209	0	1.19	2.95	0	2.32	10.8	0	0	2.41	8.02	0	0
ΣPCBs	112	360	340	91.6	293	1080	39.6	451	262	1360	8.04	71.3
α-HCH	0	0	0	0	0	0	0	0	0	0	0	0
β-HCH	0	0	0	0	0	0	0	1.75	0	0	0	0
γ-HCH	0	0	0	0	0	0	0	0	2.71	0	0	0
HCB	0	0	0	0	0	0	0	1.26	0	1.56	0	0
mirex	2.68	3.36	9.13	2.90	13.8	13.6	0	1.68	5.46	18.8	0	2.82
dieldrin	3.67	12.9	6.84	3.54	7.41	10.6	2.93	8.20	8.24	8.31	0	0
heptachlor epoxide	1.69	4.87	3.50	1.88	4.63	4.78	0	2.46	6.65	5.80	0	0
oxychlordane	4.52	8.01	12.1	4.42	18.6	15.8	0	2.45	14.2	49.4	1.34	8.19
t-nonachlor	12.5	25.2	21.1	11.9	22.1	43.3	3.11	13.0	19.9	35.3	0	2.93
c-nonachlor	1.54	5.56	2.90	0	2.22	6.92	0	1.05	2.39	3.04	0	0
Σchlordanes	18.6	38.7	36.1	16.3	42.9	65.9	3.11	16.5	36.5	87.8	1.3	11.1
2,4'-DDT	0	10.0	8.82	0	8.28	20.7	0	10.7	0	11.0	0	0
4,4'-DDE	43.2	123	97.6	29.1	79.0	221	11.4	201	66.4	187	0	7.58
ΣDDTs	43.2	133	106	29.1	87.3	242	11.4	212	66.4	198	0	7.58
% lipid	15.1	25.2	22.8	9.77	48.5	57.2	64.7	48.2	43.5	28.8	0.540	2.99

[a] Values shown as zero were below the limit of detection.

Appendix Table A9. Con't

	982	1057	1110	1165	1180	1235	1269	1301	1304	1307	1308	1311
PCB 28	1.54	0	0	1.29	0	0	3.33	0	2.33	0	2.96	0
PCB 52	0	0	0	0	0	0	0	0	0	0	0	0
PCB 56	3.62	0	1.33	0	1.00	0	2.50	0	0	0	1.84	0
PCB 63	0	0	0	0	0	0	0	0	0	0	0	0
PCB 66	8.82	1.41	1.86	3.53	3.63	0	7.66	1.59	1.16	1.02	1.20	4.39
PCB 74	3.43	0	0	0	0	0	0	0	0	0	2.09	0
PCB 87	0	0	0	1.04	1.35	0	1.10	0	0	0	0	0
PCB 92	0	0	0	0	0	0	0	0	0	0	1.16	0
PCB 99	45.9	18.4	35.2	18.2	18.2	2.94	52.9	15.3	10.1	2.53	6.42	18.3
PCB 101+90	5.99	0	2.52	1.31	1.67	1.18	1.25	0	0	0	1.38	0
PCB 104	0	0	0	0	0	0	0	0	0	0	1.26	1.93
PCB 105	13.0	5.66	9.85	6.10	4.20	1.02	11.3	5.77	3.41	0	1.40	3.86
PCB 107	5.07	1.90	3.57	3.01	2.52	0	3.61	1.41	1.07	0	1.00	1.90
PCB 110	1.80	0	0	0	0	0	0	0	0	0	0	0
PCB 118	44.5	24.7	35.5	23.8	19.3	1.92	46.6	25.1	12.1	3.11	6.08	17.5
PCB 128	11.4	6.34	9.03	5.33	3.76	0	9.03	5.13	2.58	0	1.06	3.65
PCB 138	42.2	18.4	30.6	17.6	11.8	2.79	34.0	15.5	10.2	3.91	3.60	11.7
PCB 146	22.2	7.91	14.1	9.77	6.96	0	16.5	2.76	4.05	1.61	2.10	8.09
PCB 149	7.41	0	2.44	1.57	1.18	0	1.37	0	0	0	0	1.81
PCB 151+82	7.05	0	3.69	1.44	0	0	2.46	0	1.19	0	0	1.62
PCB 153	171	107	145	82.6	57.1	13.6	167	100	41.4	15.8	21.7	62.4
PCB 154	6.11	1.60	1.35	1.40	0	0	10.2	0	0	0	0	2.29
PCB 156	4.04	2.28	3.09	2.20	1.38	0	4.02	1.82	0	0	0	1.19
PCB 157	1.54	0	1.27	0	0	0	1.54	0	0	0	0	0
PCB 158	4.71	2.22	3.73	2.13	1.61	0	4.63	1.68	1.04	0	0	1.36
PCB 163	20.4	8.15	14.2	9.65	6.43	1.29	12.7	5.14	3.58	1.74	1.74	6.99
PCB 170	12.1	7.28	10.1	5.92	2.60	0	8.55	5.35	2.59	1.26	1.18	3.34
PCB 174	1.37	0	0	0	0	0	0	0	0	0	0	0
PCB 180	40.9	21.1	32.4	17.6	8.51	3.06	35.0	18.8	9.21	4.24	3.61	10.4
PCB 183	15.3	9.18	12.9	6.57	4.06	1.17	14.0	7.66	3.45	1.31	1.66	4.75
PCB 187	43.7	18.0	27.6	19.1	11.6	2.05	29.5	6.88	7.48	2.95	3.21	15.0
PCB 193	2.36	0	1.23	0	0	0	2.03	0	0	0	0	0
PCB 194	6.86	5.46	4.32	2.89	1.66	0	4.22	3.81	0	0	0	2.12
PCB 195	3.10	2.76	1.70	1.40	0	0	2.55	1.68	0	0	0	1.09
PCB 201	3.55	1.12	1.71	1.31	0	0	2.02	0	0	0	0	1.00
PCB 206	8.93	9.20	3.60	3.59	2.78	0	6.31	4.08	0	0	0	3.59
PCB 209	2.10	3.19	0	1.05	0	0	5.15	1.39	0	0	0	0
ΣPCBs	572	283	414	251	173	31.0	503	231	117	39.5	66.7	190
α-HCH	0	0	0	0	0	0	0	0	0	5.64	0	0
β-HCH	0	0	0	0	0	0	1.11	0	0	2.14	0	0
γ-HCH	1.58	0	0	1.68	0	0	0	0	0	35.7	0	0
HCB	1.02	0	0	0	0	0	1.35	0	0	2.36	0	0
mirex	5.99	7.40	6.86	4.03	1.64	1.15	4.21	9.32	2.31	0	1.28	2.09
dieldrin	10.4	2.01	3.87	6.43	1.39	0	16.7	1.79	1.60	4.10	0	1.86
heptachlor epoxide	4.35	1.63	1.94	4.31	0	0	10.2	2.47	0	10.6	0	0
oxychlordane	17.5	10.5	23.0	10.8	4.52	2.25	43.7	26.4	6.04	2.66	7.49	7.05
t-nonachlor	34.4	9.79	26.0	19.6	5.10	1.79	26.5	8.63	8.29	6.81	2.19	5.94
c-nonachlor	4.40	0	1.72	2.15	1.17	0	3.31	0	1.09	0	0	0
Σchlordanes	57.7	20.3	50.6	32.6	10.8	4.04	73.5	35.1	15.4	9.47	9.67	13.0
2,4'-DDT	0	0	0	0	0	0	5.28	0	0	0	0	0
4,4'-DDE	155	48.7	95.9	64.6	25.5	20.6	170	21.3	23.2	24.6	9.65	38.5
ΣDDTs	155	48.7	95.9	64.6	25.5	20.6	175	21.3	23.2	24.6	9.65	38.5
% lipid	63.7	4.73	19.4	40.3	7.16	0.828	31.9	4.51	28.7	60.1	2.84	26.5

[a] Values shown as zero were below the limit of detection.

Appendix Table A9. Con't

	1325	1328	1332	1377	1379	1392	1393
PCB 28	1.74	0	0	0	2.96	0	11.5
PCB 52	0	0	0	0	1.13	0	1.83
PCB 56	0	0	0	1.11	1.39	2.02	0
PCB 63	0	0	0	0	0	0	2.00
PCB 66	3.03	0	0	1.49	5.18	1.33	13.6
PCB 74	0	0	0	1.04	1.04	2.09	0
PCB 87	0	0	0	0	1.06	0	1.92
PCB 92	0	0	0	0	0	1.06	1.25
PCB 99	10.0	9.94	1.73	11.4	34.5	13.8	51.8
PCB 101+90	1.53	0	0	0	2.38	0	3.23
PCB 104	0	1.18	0	1.27	0	1.49	1.07
PCB 105	3.04	2.75	0	3.77	6.58	3.79	14.5
PCB 107	1.48	1.27	0	1.80	3.12	1.20	4.38
PCB 110	0	0	0	0	0	0	0
PCB 118	11.3	10.0	2.48	12.6	29.8	12.9	54.4
PCB 128	2.44	1.99	0	3.08	5.88	3.03	9.87
PCB 138	9.50	7.30	1.46	13.1	30.4	14.2	37.2
PCB 146	5.22	3.12	0	5.38	18.1	6.05	19.7
PCB 149	0	0	0	0	2.79	0	2.18
PCB 151+82	1.66	0	0	0	4.56	0	3.04
PCB 153	45.0	35.6	11.1	53.6	146	60.6	157
PCB 154	0	0	0	0	6.76	0	2.17
PCB 156	1.18	0	0	1.16	2.42	1.50	3.73
PCB 157	0	0	0	0	1.02	0	1.30
PCB 158	1.01	0	0	1.29	3.11	1.30	4.04
PCB 163	4.88	3.43	0	5.69	13.1	6.15	19.3
PCB 170	2.97	1.79	0	3.83	6.87	4.39	8.78
PCB 174	0	0	0	0	0	0	0
PCB 180	9.66	5.61	2.59	13.5	26.4	14.4	27.3
PCB 183	3.76	2.46	1.01	4.63	11.3	4.92	11.1
PCB 187	10.3	5.08	1.59	10.8	39.9	10.8	35.3
PCB 193	0	0	0	0	1.92	0	2.12
PCB 194	0	0	0	1.10	4.43	1.33	4.67
PCB 195	0	0	0	0	1.94	0	1.44
PCB 201	0	0	0	0	1.75	0	1.51
PCB 206	0	0	0	0	10.5	0	2.81
PCB 209	0	0	0	0	1.60	0	0
ΣPCBs	130	91.5	22.0	152	430	168	516
α-HCH	0	0	0	0	1.25	0	0
β-HCH	0	0	0	0	1.12	0	0
γ-HCH	0	0	0	9.84	10.3	15.3	0
HCB	3.29	0	0	4.38	5.20	12.6	8.05
mirex	2.35	1.40	1.01	3.76	5.69	4.15	6.31
dieldrin	4.17	0	0	4.74	8.72	2.57	3.07
heptachlor epoxide	3.29	0	0	4.92	2.25	2.80	3.70
oxychlordane	9.07	8.40	3.73	11.7	17.6	11.4	41.5
t-nonachlor	14.1	4.33	1.84	24.4	23.4	14.5	22.8
c-nonachlor	0	0	0	1.54	2.56	0	1.27
Σchlordanes	23.1	12.7	5.57	37.6	43.6	25.9	65.6
2,4'-DDT	0	0	0	0	0	0	0
4,4'-DDE	28.0	16.0	0	49.1	69.9	30.7	62.6
ΣDDTs	28.0	16.0	0	49.1	69.9	30.7	62.6
% lipid	25.8	0.487	0.739	42.3	50.4	55.4	24.7

[a] Values shown as zero were below the limit of detection.

Appendix Table A10. Concentrations of organochlorine contaminants (ng/g wet mass) in yellow adipose tissue of individual Kemp's ridley sea turtles.[a]

	Lk-99-794	Lk-99-754	Lk-99-770	Lk-99-771	Lk-99-780	Lk-99-789	Lk-99-798	Lk1	Lk2
PCB 18	0	0	0	15.9	2.55	1.63	0	0	0
PCB 28	0	0	0	15.5	1.24	0	10.3	0	0
PCB 29	1.02	0	0	13.4	1.89	1.35	1.60	0	0
PCB 45	3.17	0	0	15.1	2.67	2.55	3.01	16.6	0
PCB 49	0	0	0	10.1	0	0	17.6	0	0
PCB 52	0	0	0	11.9	1.72	1.70	21.9	0	0
PCB 56	8.05	0	1.08	6.42	5.10	1.21	61.1	5.16	2.22
PCB 63	1.81	0	0	7.12	0	1.76	8.30	0	0
PCB 66	4.87	0	2.57	8.17	17.9	2.98	191	25.4	6.53
PCB 70	0	0	0	0	0	21.5	0	0	0
PCB 74	1.37	1.66	2.30	12.6	5.80	5.95	51.5	0	1.08
PCB 92	7.96	0	1.76	7.41	7.89	2.23	69.1	4.45	2.50
PCB 95	1.21	0	0	6.13	7.80	0	32.7	6.80	0
PCB 99	91.7	4.72	8.60	20.9	60.6	8.21	330	84.0	26.5
PCB 104	0	0	0	12.9	1.40	0	0	0	0
PCB 105	17.9	0	2.25	5.40	13.5	0	102	25.0	7.93
PCB 107	6.12	0	0	1.19	0	0	10.6	6.83	2.53
PCB 110	0	0	0	3.28	0	0	95.5	1.15	2.25
PCB 118	69.9	6.33	5.95	19.9	75.9	11.4	339	106	31.6
PCB 128	18.3	0	1.96	2.65	10.3	0	48.8	22.2	9.05
PCB 132	0	0	0	0	0	0	16.9	0	0
PCB 138	105	7.58	18.1	21.4	45.7	10.7	241	88.0	33.0
PCB 146	39.4	2.18	5.72	10.7	24.4	4.30	96.5	40.6	14.1
PCB 149	6.90	0	3.81	7.38	4.06	3.01	116	2.14	5.46
PCB 151+82	4.69	0	1.14	5.30	2.40	0	79.0	6.33	3.82
PCB 153	253	18.4	36.0	59.9	154	29.0	552	257	93.4
PCB 154	3.97	0	0	3.04	2.70	0	6.99	7.15	4.49
PCB 156	4.27	0	0	0	2.97	0	18.2	7.17	3.29
PCB 157	1.43	0	0	0	0	0	4.56	1.23	1.29
PCB 158	8.42	0	0	1.17	2.86	0	31.3	7.05	3.29
PCB 163	33.5	1.01	4.90	7.00	20.7	2.61	95.4	31.8	12.3
PCB 170	19.2	0	1.78	1.30	4.22	0	19.4	15.2	9.86
PCB 174	1.24	0	0	0	0	0	9.90	0	1.73
PCB 180	63.6	2.77	9.20	11.6	17.0	4.21	74.2	58.2	30.0
PCB 183	30.2	1.02	4.10	5.88	10.2	2.24	49.4	25.6	10.1
PCB 187	63.1	3.35	11.2	14.0	29.5	6.61	114	77.4	29.0
PCB 193	1.69	0	0	0	0	0	2.64	2.05	1.26
PCB 194	3.40	0	0	0	0	0	3.55	8.80	5.75
PCB 201	1.42	0	0	0	0	0	0	5.29	1.08
PCB 206	0	0	0	0	0	0	0	12.2	4.62
PCB 209	0	0	0	0	0	0	0	8.50	1.20
ΣPCBs	877	49.0	122	345	537	125	2920	965	368
α-HCH	2.68	3.75	0	6.13	7.58	6.95	6.04	2.69	0
β-HCH	0	0	15.7	3.35	2.25	176	101	0	0
γ-HCH	0	0	80.2	1.42	16.5	22.6	14.9	0	0
HCB	17.6	7.58	9.57	30.7	18.7	12.2	22.5	1.67	0
mirex	11.1	0	1.97	2.20	0	0	5.19	3.78	4.22
dieldrin	22.5	5.32	11.6	8.19	25.2	8.25	32.3	43.8	34.5
heptachlor epoxide	5.64	1.00	75.3	4.33	15.3	14.1	7.52	23.0	4.22
t-chlordane	0	0	5.68	0	0	0	0	0	0
c-chlordane	2.30	0	0	2.96	0	0	1.21	0	1.22
t-nonachlor	80.6	6.06	17.7	19.2	33.9	10.8	64.5	60.1	18.2
c-nonachlor	11.1	0	3.22	2.95	3.83	1.36	13.1	15.7	8.63
oxychlordane	63.6	6.64	10.4	33.4	96.8	10.1	226	84.4	12.7
Σchlordanes	158	12.7	37.1	58.5	135	22.3	305	160	40.7
4,4'-DDD	0	0	1.24	0	0	0	6.29	1.66	6.34
4,4'-DDE	133	19.2	75.2	41.8	59.6	21.5	189	238	119
ΣDDTs	133	19.2	76.5	41.8	59.6	21.5	195	240	125
% lipid	51.7	70.7	74.8	76.3	57.7	60.6	73.9	76.0	50.7

[a] Values shown as zero were below the limit of detection.

Appendix Table A11. Concentrations of organochlorine contaminants (ng/g wet mass) in brown adipose tissue of individual Kemp's ridley sea turtles.[a]

	Lk-99-794	Lk-99-754	Lk-99-759	Lk-99-770	Lk-99-771	Lk-99-780	Lk-99-789	Lk-99-798	Lk1	Lk2
PCB 18	0	0	0	0	0	0	1.64	0	0	4.89
PCB 28	0	0	0	0	0	0	1.77	2.40	0	0
PCB 29	0	0	0	0	0	1.55	0	0	0	0
PCB 45	2.50	0	0	0	0	1.86	2.92	0	0	0
PCB 49	0	0	0	0	0	0	0	4.27	0	0
PCB 52	0	0	0	0	0	0	0	5.28	0	0
PCB 56	7.30	0	0	2.63	2.17	5.28	2.65	22.9	7.22	2.23
PCB 63	1.74	0	0	1.83	1.42	1.34	1.20	2.22	0	0
PCB 66	5.10	0	0	5.96	3.09	17.7	4.61	69.8	32.4	7.65
PCB 70	0	0	0	0	0	0	0	0	0	0
PCB 74	2.66	0	1.18	5.41	3.64	5.70	6.78	16.3	1.23	1.70
PCB 92	6.72	0	0	3.86	2.19	7.62	4.70	24.7	6.27	2.78
PCB 95	1.22	0	0	0	0	5.81	1.13	8.17	10.0	0
PCB 99	84.0	3.06	1.40	17.6	17.5	67.7	13.3	124	107	29.7
PCB 104	0	0	0	0	0	0	0	0	0	1.41
PCB 105	16.3	0	0	3.42	3.85	15.7	2.40	62.8	30.0	8.71
PCB 107	5.73	0	0	1.43	0	0	1.35	16.3	9.19	3.06
PCB 110	0	0	0	1.18	0	0	1.89	43.0	1.68	2.59
PCB 118	66.4	5.45	3.31	20.8	16.0	82.7	14.7	200	133	36.1
PCB 128	17.6	0	0	3.63	2.45	12.2	1.19	40.2	26.0	10.1
PCB 132	0	0	0	0	0	0	0	9.22	0	0
PCB 138	103	6.59	1.64	25.7	17.9	50.0	13.1	163	105	41.1
PCB 146	37.3	2.04	0	9.46	8.10	26.3	5.60	57.7	50.3	16.3
PCB 149	5.73	0	0	6.65	4.15	4.35	4.64	57.6	3.25	6.18
PCB 151+82	5.40	0	0	2.17	1.11	2.91	14.9	34.2	8.62	4.23
PCB 153	245	16.8	9.12	54.3	48.5	167	38.4	348	313	105
PCB 154	2.57	0	0	0	0	2.46	0	2.92	9.21	5.20
PCB 156	4.68	0	0	0	0	3.63	0	16.6	7.96	3.72
PCB 157	1.13	0	0	0	0	0	0	4.43	1.84	1.39
PCB 158	8.40	0	0	0	0	3.28	0	23.6	8.68	3.80
PCB 163	33.1	1.16	0	6.83	5.68	23.1	2.43	67.1	38.2	14.1
PCB 170	18.3	0	0	1.78	1.95	5.17	0	24.6	15.2	10.6
PCB 174	1.32	0	0	1.09	0	0	0	7.60	0	2.07
PCB 180	63.1	3.14	0	10.6	11.2	19.5	4.82	82.8	60.4	33.1
PCB 183	29.7	1.12	0	5.95	4.79	11.3	2.29	39.3	29.8	11.3
PCB 187	60.5	3.52	0	15.6	12.2	31.5	7.94	84.7	90.3	32.5
PCB 193	1.74	0	0	0	0	0	0	2.34	2.19	1.43
PCB 194	3.61	0	0	0	0	0	0	5.80	6.35	6.19
PCB 201	1.46	0	0	0	0	0	0	1.83	5.72	1.51
PCB 206	0	0	0	0	0	0	0	1.60	8.15	4.99
PCB 209	0	0	0	0	0	0	0	0	5.03	1.24
ΣPCBs	844	42.9	16.6	208	168	576	156	1680	1130	426
α-HCH	28.8	1.95	0	0	7.02	9.87	8.72	3.88	3.60	0
β-HCH	14.5	3.94	0	18.4	4.97	7.91	143	107	2.65	0
γ-HCH	4.54	0	0	120	36.0	34.9	18.1	16.1	0	0
HCB	17.7	4.77	0	21.3	10.2	16.5	25.5	4.95	2.33	1.14
mirex	10.9	0	0	2.54	2.26	2.58	0	5.07	4.41	4.97
dieldrin	26.3	4.50	1.20	15.6	8.53	30.2	10.4	13.1	40.4	32.9
heptachlor epoxide	43.8	1.28	0	91.5	23.7	25.9	13.3	14.7	0	3.80
t-chlordane	5.05	0	0	7.52	1.04	4.28	0	1.09	0	0
c-chlordane	3.00	0	0	0	1.72	0	0	0	0	1.56
t-nonachlor	81.3	4.52	3.05	21.4	17.0	39.8	12.0	46.7	84.3	21.9
c-nonachlor	10.9	0	0	3.55	2.55	4.54	1.47	13.0	15.9	10.3
oxychlordane	59.8	4.85	2.88	22.6	19.6	96.1	20.3	74.8	106	15.1
Σchlordanes	160	9.37	5.92	55.1	41.9	145	33.8	136	206	48.9
4,4'-DDD	0	0	0	1.38	1.15	0	0	6.70	1.51	7.18
4,4'-DDE	136	21.2	6.00	82.6	42.5	68.7	24.9	174	219	134
ΣDDTs	136	21.2	6.00	84.0	43.7	68.7	24.9	181	221	141
% lipid	54.8	76.6	0.521	80.3	72.2	66.2	74.1	74.3	73.4	47.2

[a] Values shown as zero were below the limit of detection.

Appendix Table A12. Health data for individual loggerhead sea turtles from inshore waters of North Carolina.[a]

	3-25	3-26	3-27	3-28	3-29	3-30	3-31	3-32	3-33
Body condition[b]	15.1	14.6	15.0	13.4	11.4	14.2	14.9	14.1	13.7
RBC (10^6/ul)	-	-	-	0.380	-	-	-	-	0.280
HGB (g/dL)	-	-	-	8.5	-	-	-	-	7.5
HCT (%)	-	-	-	29	-	-	-	-	23
PCV Estimated (%)[b]	33.4	36.8	35.0	40.3	41.6	32.1	35.2	32.3	25.7
WBC (10^3/ul)	-	-	-	10.24	-	-	-	-	10.90
WBC Estimated[b]	-	-	-	-	-	-	-	-	-
Heterophil (%)	-	-	-	34	-	-	-	-	12
Lymphocyte (%)	-	-	-	45	-	-	-	-	75
Monocyte (%)	-	-	-	0	-	-	-	-	0
Eosinophil (%)	-	-	-	17	-	-	-	-	10
Basophil (%)	-	-	-	0	-	-	-	-	0
Azurophil (%)	-	-	-	4	-	-	-	-	3
Heterophil (10^3/ul)	-	-	-	3.5	-	-	-	-	1.3
Lymphocyte (10^3/ul)	-	-	-	4.6	-	-	-	-	8.2
Monocyte (10^3/ul)	-	-	-	0.0	-	-	-	-	0.0
Eosinophil (10^3/ul)	-	-	-	1.7	-	-	-	-	1.1
Basophil (10^3/ul)	-	-	-	0.0	-	-	-	-	0.0
Azurophil (10^3/ul)	-	-	-	1.4	-	-	-	-	0.4
H:L Ratio	-	-	-	0.8	-	-	-	-	0.2
G:L Ratio	-	-	-	1.4	-	-	-	-	0.3
Glucose	-	-	-	76	-	-	-	-	98
Protein (g/dL)	-	-	-	4.0	-	-	-	-	3.2
Protein (mg/ml)[b]	19.0	27.3	27.1	32.6	34.9	27.9	26.2	34.3	24.5
Albumin (g/dL)	-	-	-	1.0	-	-	-	-	1.0
Globulin (g/dL)	-	-	-	3.0	-	-	-	-	2.2
Albumin:Globulin	-	-	-	0.33	-	-	-	-	0.45
BUN (mg/dL)	-	-	-	89	-	-	-	-	109
Uric acid (mg/dL)	-	-	-	3.4	-	-	-	-	0.7
Creatinine (mg/dL)	-	-	-	<0.1	-	-	-	-	<0.1
Bilirubin (mg/dL)	-	-	-	0.1	-	-	-	-	0
AST (U/L)	-	-	-	312	-	-	-	-	225
ALP (U/L)	-	-	-	12	-	-	-	-	17
LDH (U/L)	-	-	-	135	-	-	-	-	77
CPK (U/L)	-	-	-	-	-	-	-	-	-
GGT (U/L)	-	-	-	<3	-	-	-	-	<3
Osmolality (mOsm)	316	310	234	293	294	301	256	289	368
Ca (mg/dL)	-	-	-	9.8	-	-	-	-	9.2
P (mg/dL)	-	-	-	5.8	-	-	-	-	6.5
Ca:P	-	-	-	1.69	-	-	-	-	1.42
Na (mmol/L)	-	-	-	158	-	-	-	-	164
K (mmol/L)	-	-	-	5.6	-	-	-	-	4.4
Na:K	-	-	-	28.2	-	-	-	-	37.3
Cl (mmol/L)	-	-	-	121	-	-	-	-	124
Mg (mg/dL)	-	-	-	5.4	-	-	-	-	4.1
Anion gap	-	-	-	12.6	-	-	-	-	17.4

Appendix Table A12. Con't

	3-34	3-35	3-36	3-37	3-38	3-39	3-40	3-41	3-42
Body condition[b]	14.0	15.7	14.0	14.8	13.7	17.4	13.7	14.0	13.7
RBC (10^6/ul)	0.445	0.390	0.380	0.380	0.475	0.400	0.295	0.380	0.275
HGB (g/dL)	9.5	12.0	10.5	10.0	9.5	9.5	9.5	10.5	7.0
HCT (%)	31	35	34	32	29	33	31	31	24
PCV Estimated (%)[b]	38.6	39.3	33.2	33.5	31.7	30.2	35.1	30.5	29.3
WBC (10^3/ul)	5.80	14.18	20.72	15.80	16.36	16.31	14.67	15.02	19.54
WBC Estimated[b]	-	-	-	13.00	13.60	12.00	9.00	-	15.20
Heterophil (%)	-	21	37	27	34	15	56	16	42
Lymphocyte (%)	-	71	49	58	57	65	39	75	51
Monocyte (%)	-	0	0	0	0	0	0	0	0
Eosinophil (%)	-	1	13	13	6	15	3	4	3
Basophil (%)	-	0	0	0	0	0	0	0	0
Azurophil (%)	-	7	1	2	3	5	2	5	3
Heterophil (10^3/ul)	-	3.0	7.7	4.3	5.6	2.4	8.2	2.4	8.2
Lymphocyte (10^3/ul)	-	10.1	10.2	9.2	9.3	10.6	5.7	11.3	10.0
Monocyte (10^3/ul)	-	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Eosinophil (10^3/ul)	-	0.1	2.7	2.1	1.0	2.4	0.4	0.6	0.6
Basophil (10^3/ul)	-	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Azurophil (10^3/ul)	-	1.5	0.4	0.5	1.0	0.8	1.1	0.8	1.3
H:L Ratio	-	0.3	0.8	0.5	0.6	0.2	1.4	0.2	0.8
G:L Ratio	-	0.5	1.1	0.7	0.8	0.5	1.7	0.3	1.0
Glucose	98	125	91	96	101	102	97	85	-
Protein (g/dL)	3.6	4.4	3.5	2.8	3.6	4.1	4.1	5.2	5.1
Protein (mg/ml)[b]	30.6	34.1	22.4	18.4	25.0	28.9	29.8	30.6	38.3
Albumin (g/dL)	1.2	1.3	1.0	<1.0	<1.0	1.1	1.2	1.3	1.0
Globulin (g/dL)	2.4	3.1	2.5	1.9	2.7	3.0	2.9	3.9	4.1
Albumin:Globulin	0.50	0.42	0.40	0.47	0.33	0.37	0.41	0.33	0.24
BUN (mg/dL)	30	143	80	150	65	134	72	66	108
Uric acid (mg/dL)	0.7	0.8	1.9	0.8	0.8	1.5	1.3	0.9	0.6
Creatinine (mg/dL)	<0.1	0.10	<0.1	<0.1	<0.1	0.10	0.10	<0.1	<0.1
Bilirubin (mg/dL)	0.1	0.20	0.1	0.1	0	0	0	0	0.1
AST (U/L)	148	174	147	160	206	272	208	355	214
ALP (U/L)	16	74	23	18	42	38	54	63	15
LDH (U/L)	125	335	180	97	398	465	310	445	334
CPK (U/L)	-	-	-	-	-	-	-	-	-
GGT (U/L)	<3	<3	<3	<3	<3	<3	<3	<3	<3
Osmolality (mOsm)	696	374	330	403	333	347	288	334	339
Ca (mg/dL)	8.4	11.4	8.8	10.6	8.0	7.7	9.1	8.1	7.5
P (mg/dL)	6.5	5.6	6.0	7.5	6.1	7.9	7.1	6.3	7.0
Ca:P	1.29	2.04	1.47	1.41	1.31	0.97	1.28	1.29	1.07
Na (mmol/L)	154	159	154	159	163	159	158	160	158
K (mmol/L)	4.3	4.3	4.6	4.5	4.8	4.6	4.2	4.2	4.9
Na:K	35.8	37.0	33.5	35.3	34.0	34.6	37.6	38.1	32.2
Cl (mmol/L)	118	113	113	120	125	117	111	120	120
Mg (mg/dL)	5.6	3.9	4.8	5.2	5.8	5.0	4.9	6.3	5.1
Anion gap	17.3	20.3	25.3	17.5	20.8	26.6	30.2	24.2	14.9

Appendix Table A12. Con't

	3-43	3-44	3-45	490	772	839/1377	923	956	982
Body condition[b]	20.9	13.5	15.7	18.1	16.1	-	14.3	15.2	16.7
RBC (10^6/ul)	0.505	0.535	0.615	-	-	-	-	-	-
HGB (g/dL)	11.0	12.0	10.5	-	-	-	-	-	-
HCT (%)	36	38	35	-	-	-	-	-	-
PCV Estimated (%)[b]	39.8	43.7	42.6	37.4	39.3	39.8	32.8	-	44.9
WBC (10^3/ul)	16.54	12.65	18.79	-	-	-	-	-	-
WBC Estimated[b]	14.11	13.26	14.07	8.50	22.10	-	-	-	15.10
Heterophil (%)	33	10	17	20	24	-	-	-	15
Lymphocyte (%)	60	77	80	64	63	-	-	-	66
Monocyte (%)	0	0	0	-	-	-	-	-	-
Eosinophil (%)	6	2	2	15	5	-	-	-	19
Basophil (%)	0	1	0	-	-	-	-	-	-
Azurophil (%)	1	10	1	1	8	-	-	-	0
Heterophil (10^3/ul)	5.5	1.3	3.2	-	-	-	-	-	-
Lymphocyte (10^3/ul)	9.9	9.7	15.0	-	-	-	-	-	-
Monocyte (10^3/ul)	0.0	0.0	0.0	-	-	-	-	-	-
Eosinophil (10^3/ul)	1.0	0.3	0.4	-	-	-	-	-	-
Basophil (10^3/ul)	0.0	0.4	0.0	-	-	-	-	-	-
Azurophil (10^3/ul)	0.3	1.0	0.2	-	-	-	-	-	-
H:L Ratio	0.6	0.1	0.2	0.3	0.4	-	-	-	0.2
G:L Ratio	0.7	0.3	0.2	-	-	-	-	-	-
Glucose	101	143	103	104	113	133	121	109	141
Protein (g/dL)	4.2	4.5	3.3	4.2	4.3	5.9	4.9	4.0	5.1
Protein (mg/ml)[b]	36.1	33.3	22.6	31.8	35.6	47.6	34.0	-	40.7
Albumin (g/dL)	1.4	1.3	1.1	1.1	1.0	1.4	<1.0	1.0	1.5
Globulin (g/dL)	2.8	3.2	2.2	3.1	3.3	4.5	4.0	3.0	3.6
Albumin:Globulin	0.50	0.41	0.50	0.35	0.30	0.31	0.23	0.33	0.42
BUN (mg/dL)	94	25	63	145	99	124	79	56	150
Uric acid (mg/dL)	0.5	0.4	0.5	2.0	0.7	0.8	0.4	0.8	2.7
Creatinine (mg/dL)	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1
Bilirubin (mg/dL)	0.1	0	0.1	0.1	0.2	0.1	<0.1	<0.1	0.1
AST (U/L)	244	128	130	188	301	342	288	250	265
ALP (U/L)	21	25	19	19	15	24	11	9	30
LDH (U/L)	252	172	148	136	169	233	234	60	99
CPK (U/L)	-	-	-	899	1118	2648	3420	551	589
GGT (U/L)	<3	<3	<3	3	<3	<3	4	3	3
Osmolality (mOsm)	285	291	335	371	353	361	337	325	361
Ca (mg/dL)	10.1	7.6	7.7	9.6	9.9	9.9	5.5	6.7	9.8
P (mg/dL)	7.7	8.5	8.3	7.3	8.4	8.0	7.9	9.1	8.7
Ca:P	1.31	0.89	0.93	1.32	1.18	1.24	0.70	0.74	1.13
Na (mmol/L)	157	155	158	157	160	155	160	157	156
K (mmol/L)	4.0	3.1	4.5	4.4	4.7	4.8	3.8	4.6	4.2
Na:K	39.3	50.0	35.1	35.7	34.0	32.3	42.1	34.1	37.1
Cl (mmol/L)	116	113	119	115	120	110	121	113	111
Mg (mg/dL)	4.9	6.5	5.3	5.6	4.9	4.9	6.1	4.6	5.3
Anion gap	13.0	15.1	16.5	13.4	15.7	13.8	8.8	12.6	11.2

Appendix Table A12. Con't

	1057	1110	1165	1180	1235	1269	1301	1304	1307
Body condition[b]	14.5	15.0	15.9	14.6	13.5	15.1	16.0	16.4	14.1
RBC (10^6/ul)	-	-	-	-	-	-	-	-	-
HGB (g/dL)	-	-	-	-	-	-	-	-	-
HCT (%)	-	-	-	-	-	-	-	-	-
PCV Estimated (%)[b]	35.1	40.2	38.2	40.7	18.1	37.9	38.0	37.4	40.4
WBC (10^3/ul)	-	-	-	-	-	-	-	-	-
WBC Estimated[b]	-	13.60	12.80	13.60	16.40	25.50	17.00	-	0.00
Heterophil (%)	-	39	41	14	30	51	21	-	31
Lymphocyte (%)	-	56	54	66	65	46	68	-	65
Monocyte (%)	-	-	-	-	-	-	-	-	-
Eosinophil (%)	-	5	0	18	1	2	10	-	1
Basophil (%)	-	-	-	-	-	-	-	-	-
Azurophil (%)	-	0	5	2	4	1	1	-	3
Heterophil (10^3/ul)	-	-	-	-	-	-	-	-	-
Lymphocyte (10^3/ul)	-	-	-	-	-	-	-	-	-
Monocyte (10^3/ul)	-	-	-	-	-	-	-	-	-
Eosinophil (10^3/ul)	-	-	-	-	-	-	-	-	-
Basophil (10^3/ul)	-	-	-	-	-	-	-	-	-
Azurophil (10^3/ul)	-	-	-	-	-	-	-	-	-
H:L Ratio	-	0.7	0.8	0.2	0.5	1.1	0.3	-	-
G:L Ratio	-	-	-	-	-	-	-	-	-
Glucose	105	88	133	118	111	88	137	111	122
Protein (g/dL)	4.3	4.5	4.4	4.6	3.4	4.5	4.1	3.5	3.2
Protein (mg/ml)[b]	34.6	38.2	36.8	35.4	28.5	36.2	36.5	31.0	31.4
Albumin (g/dL)	1.0	1.3	1.3	1.1	1.0	1.2	1.1	1.2	1.1
Globulin (g/dL)	3.3	3.2	3.1	3.5	2.4	3.3	3.0	2.3	2.1
Albumin:Globulin	0.30	0.41	0.42	0.31	0.42	0.36	0.37	0.52	0.52
BUN (mg/dL)	152	115	125	132	167	82	60	132	54
Uric acid (mg/dL)	1.5	0.3	0.5	0.6	0.8	0.6	0.4	0.8	1.0
Creatinine (mg/dL)	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1
Bilirubin (mg/dL)	0.1	0.1	0.1	<0.1	<0.1	<0.1	<0.1	0.1	0.1
AST (U/L)	260	228	252	210	261	195	259	210	161
ALP (U/L)	19	16	18	22	11	21	11	20	13
LDH (U/L)	90	157	119	158	157	310	141	95	127
CPK (U/L)	574	1346	456	455	1095	1608	341	1437	1338
GGT (U/L)	3	<3	3	<3	3	3	<3	<3	3
Osmolality (mOsm)	373	340	358	359	369	331	334	356	330
Ca (mg/dL)	8.0	7.1	7.9	8.1	8.0	7.3	7.0	9.0	8.5
P (mg/dL)	7.0	6.1	6.3	6.8	6.8	6.8	7.0	6.7	7.3
Ca:P	1.14	1.16	1.25	1.19	1.18	1.07	1.00	1.34	1.16
Na (mmol/L)	162	160	160	159	160	158	161	157	157
K (mmol/L)	4.6	4.4	4.6	4.8	4.7	4.9	4.6	4.0	5.5
Na:K	35.2	36.4	34.8	33.1	34.0	32.2	35.0	39.3	28.5
Cl (mmol/L)	120	122	114	115	113	121	119	117	117
Mg (mg/dL)	5.2	4.9	4.3	4.0	4.8	6.5	5.5	5.9	6.3
Anion gap	12.6	12.4	17.6	14.8	14.7	15.9	22.6	10.0	19.5

Appendix Table A12. Con't

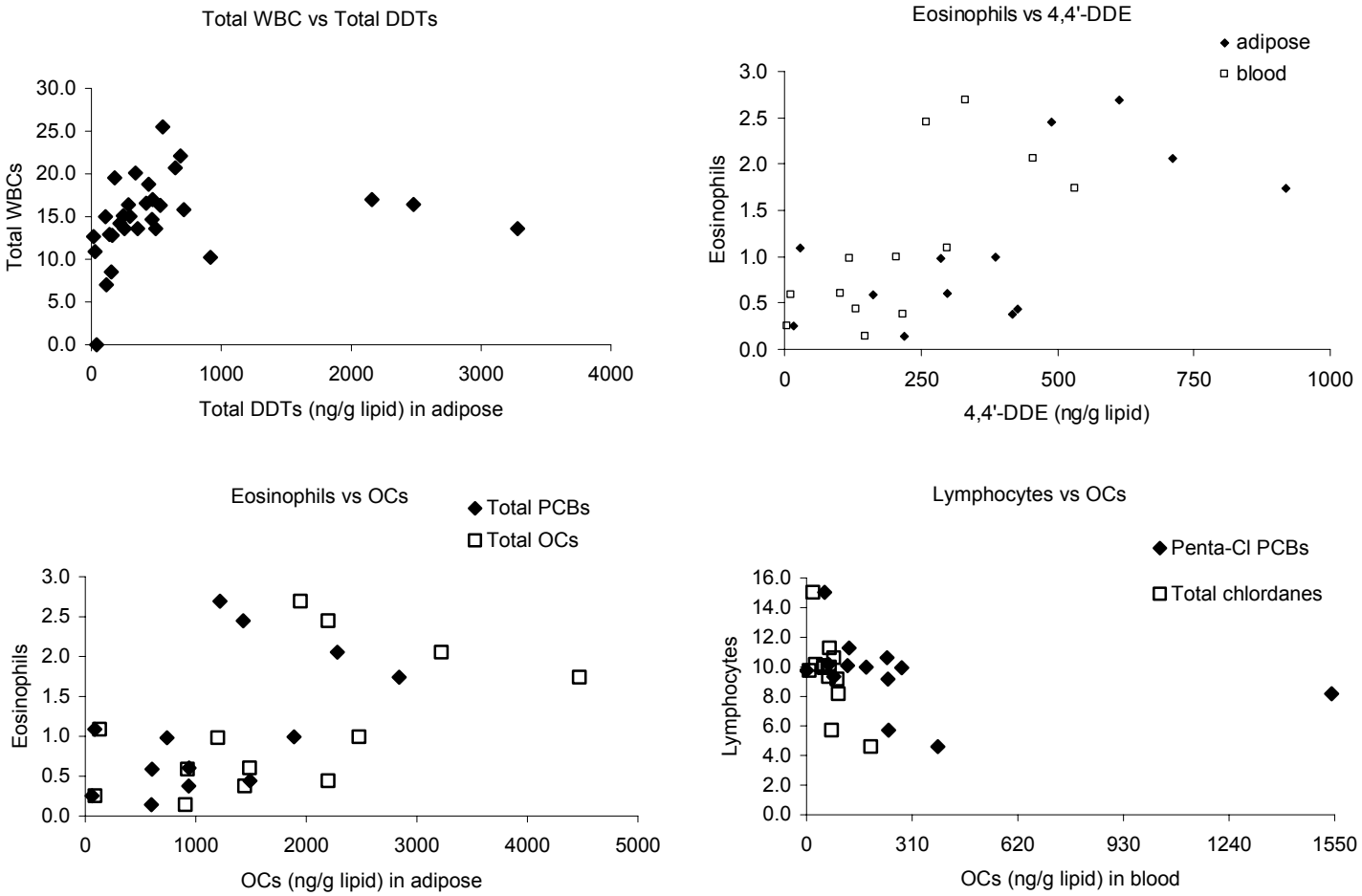
	1308	1310	1311	1325	1328	1332	1349	1377	1379
Body condition[b]	14.0	14.8	14.5	15.4	12.0	15.9	-	14.7	15.7
RBC (10^6/ul)	-	-	-	-	-	-	-	-	-
HGB (g/dL)	-	-	-	-	-	-	-	-	-
HCT (%)	-	-	-	-	22	-	-	-	-
PCV Estimated (%)[b]	33.9	45.0	32.8	41.6	40.7	22.7	-	39.6	42.6
WBC (10^3/ul)	-	-	-	-	-	-	-	-	-
WBC Estimated[b]	20.10	0.00	-	15.00	13.60	17.00	11.80	7.00	12.90
Heterophil (%)	37	26	-	9	29	48	30	73	35
Lymphocyte (%)	57	61	-	84	66	43	63	13	54
Monocyte (%)	-	-	-	-	-	-	-	-	-
Eosinophil (%)	6	11	-	2	1	7.5	1	2	6
Basophil (%)	-	-	-	-	-	-	-	-	-
Azurophil (%)	0	2	-	5	4	1.5	6	12	5
Heterophil (10^3/ul)	-	-	-	-	-	-	-	-	-
Lymphocyte (10^3/ul)	-	-	-	-	-	-	-	-	-
Monocyte (10^3/ul)	-	-	-	-	-	-	-	-	-
Eosinophil (10^3/ul)	-	-	-	-	-	-	-	-	-
Basophil (10^3/ul)	-	-	-	-	-	-	-	-	-
Azurophil (10^3/ul)	-	-	-	-	-	-	-	-	-
H:L Ratio	0.6	-	-	0.1	0.4	1.1	0.5	5.6	0.6
G:L Ratio	-	-	-	-	-	-	-	-	-
Glucose	119	114	96	122	93	82	-	124	139
Protein (g/dL)	3.4	3.4	4.0	3.1	2.4	2.8	-	3.7	4.3
Protein (mg/ml)[b]	29.3	33.6	32.5	27.4	17.2	21.6	38.7	31.2	35.4
Albumin (g/dL)	<1.0	1.0	1.0	<1.0	<1.0	<1.0	-	1.0	1.3
Globulin (g/dL)	2.5	2.4	3.0	2.2	1.5	1.9	-	2.7	3.0
Albumin:Globulin	0.36	0.42	0.33	0.41	0.60	0.47	-	0.37	0.43
BUN (mg/dL)	197	43	142	79	83	94	-	90	124
Uric acid (mg/dL)	0.3	0.5	0.4	0.5	0.4	0.4	-	0.5	0.6
Creatinine (mg/dL)	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	-	<0.1	<0.1
Bilirubin (mg/dL)	<0.1	0.1	0.1	0.1	0.2	0.2	-	0.1	<0.1
AST (U/L)	314	193	258	157	234	267	-	235	264
ALP (U/L)	29	36	17	14	14	11	-	22	17
LDH (U/L)	89	148	182	103	84	109	-	221	91
CPK (U/L)	281	516	1844	373	737	403	-	5667	716
GGT (U/L)	<3	<3	3	<3	<3	<3	-	<3	<3
Osmolality (mOsm)	380	320	363	334	335	342	-	353	354
Ca (mg/dL)	8.7	8.3	8.2	8.2	7.7	7.1	-	6.9	8.4
P (mg/dL)	5.6	5.8	6.2	6.7	5.2	5.6	-	6.2	7.3
Ca:P	1.55	1.43	1.32	1.22	1.48	1.27	-	1.11	1.15
Na (mmol/L)	159	157	160	154	156	161	-	159	157
K (mmol/L)	4.6	3.9	5.2	4.1	4.2	4.4	-	4.2	4.1
Na:K	34.6	40.3	30.8	37.6	37.1	36.6	-	37.9	38.3
Cl (mmol/L)	117	115	118	113	115	122	-	112	114
Mg (mg/dL)	4.9	5.4	5.5	5.4	4.8	5.9	-	6.6	4.3
Anion gap	15.6	7.9	21.2	11.1	6.2	6.4	-	13.2	12.1

Appendix Table A12. Con't

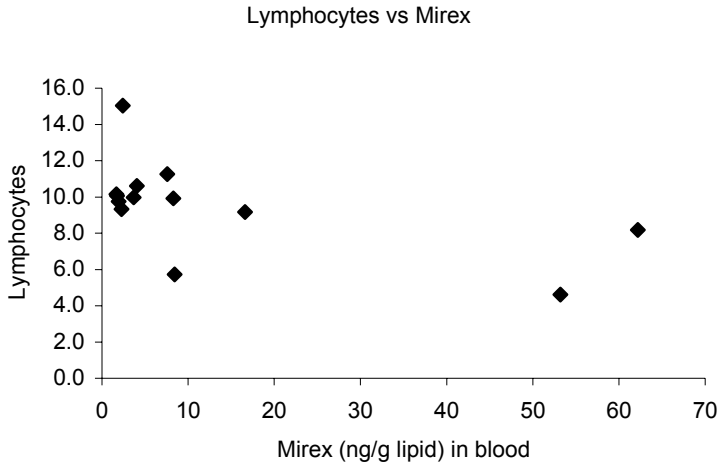
	1385	1392	1393	2-24	5-9
Body condition[b]	-	14.1	13.6	14.2	11.5
RBC (10^6/ul)	-	-	-	-	-
HGB (g/dL)	-	-	-	-	-
HCT (%)	-	-	-	8	-
PCV Estimated (%)[b]	47.8	42.9	49.3	-	21.2
WBC (10^3/ul)	-	-	-	-	-
WBC Estimated[b]	12.40	-	13.60	-	-
Heterophil (%)	24	-	30	-	-
Lymphocyte (%)	61	-	53	-	-
Monocyte (%)	-	-	-	-	-
Eosinophil (%)	15	-	13	-	-
Basophil (%)	-	-	-	-	-
Azurophil (%)	0	-	4	-	-
Heterophil (10^3/ul)	-	-	-	-	-
Lymphocyte (10^3/ul)	-	-	-	-	-
Monocyte (10^3/ul)	-	-	-	-	-
Eosinophil (10^3/ul)	-	-	-	-	-
Basophil (10^3/ul)	-	-	-	-	-
Azurophil (10^3/ul)	-	-	-	-	-
H:L Ratio	0.4	-	0.6	-	-
G:L Ratio	-	-	-	-	-
Glucose	143	92	88	78	55
Protein (g/dL)	5.2	3.2	3.7	2.6	3.3
Protein (mg/ml)[b]	44.2	31.7	33.0	-	48.8
Albumin (g/dL)	1.4	1.1	1.0	<1.0	<1.0
Globulin (g/dL)	3.8	2.1	2.7	1.7	2.4
Albumin:Globulin	0.37	0.52	0.37	0.5	0.4
BUN (mg/dL)	135	62	71	70	66
Uric acid (mg/dL)	0.5	0.3	0.5	0.4	1.4
Creatinine (mg/dL)	0.1	<0.1	<0.1	<0.1	<0.1
Bilirubin (mg/dL)	<0.1	0.1	0.1	0.1	<0.1
AST (U/L)	315	169	160	179	251
ALP (U/L)	19	15	22	3	27
LDH (U/L)	111	172	228	25	55
CPK (U/L)	977	1321	1597	263	5934
GGT (U/L)	<3	3	<3	<3	<3
Osmolality (mOsm)	364	339	339	-	-
Ca (mg/dL)	9.5	7.8	7.9	6.5	7.0
P (mg/dL)	7.6	7.4	7.8	4.4	7.6
Ca:P	1.25	1.05	1.01	1.48	0.92
Na (mmol/L)	158	162	158	142	149
K (mmol/L)	4.6	5.0	4.9	3.6	3.2
Na:K	34.3	32.4	32.2	39.7	46.6
Cl (mmol/L)	112	118	117	104	109
Mg (mg/dL)	4.6	5.6	6.7	3.9	4.7
Anion gap	23.6	22.0	18.9	8.6	10.2

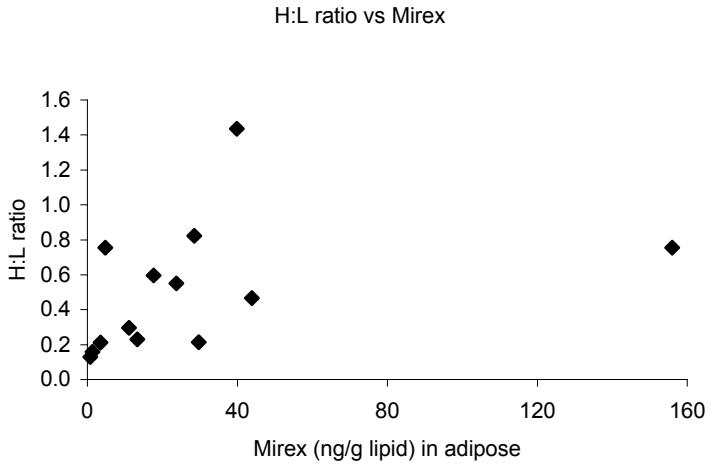
[a] See "Abreviations" section or Chapter 4 for explanation of abbreviations. Tests that were not performed are indicated with a dash (-).

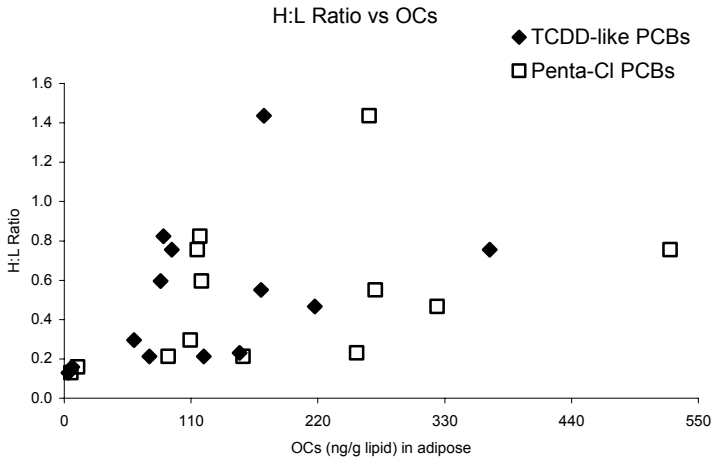
[b] Body condition = turtle mass in kg/straight carapace length from nuchal notch to marginal posterior notch x 100,000. PCV estimated = packed cell volume estimated as in Chapter 2 by measuring the volume of RBC and whole blood from a marked centrifuged blood collection tube. WBC estimated = total WBC count from a blood smear. Protein in mg/ml was determined by Bradford (1976) from plasma samples collected for VTG analysis.

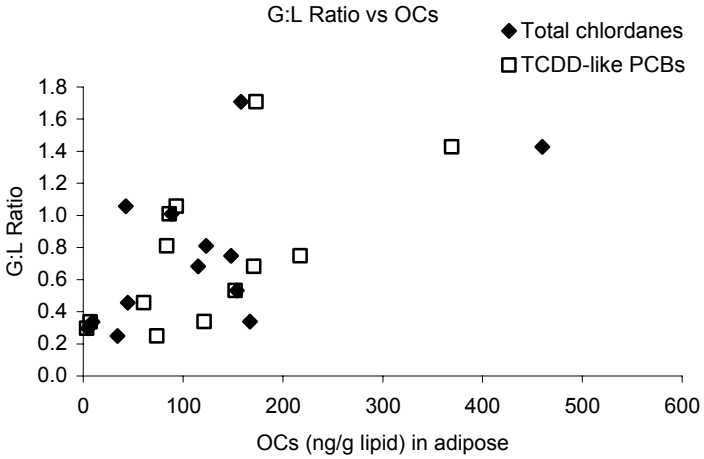


Appendix Figure A1. Plots of the statistically significant correlations between health indicators and organochlorine (OC) contaminants in fat biopsies or blood of juvenile loggerhead sea turtles.









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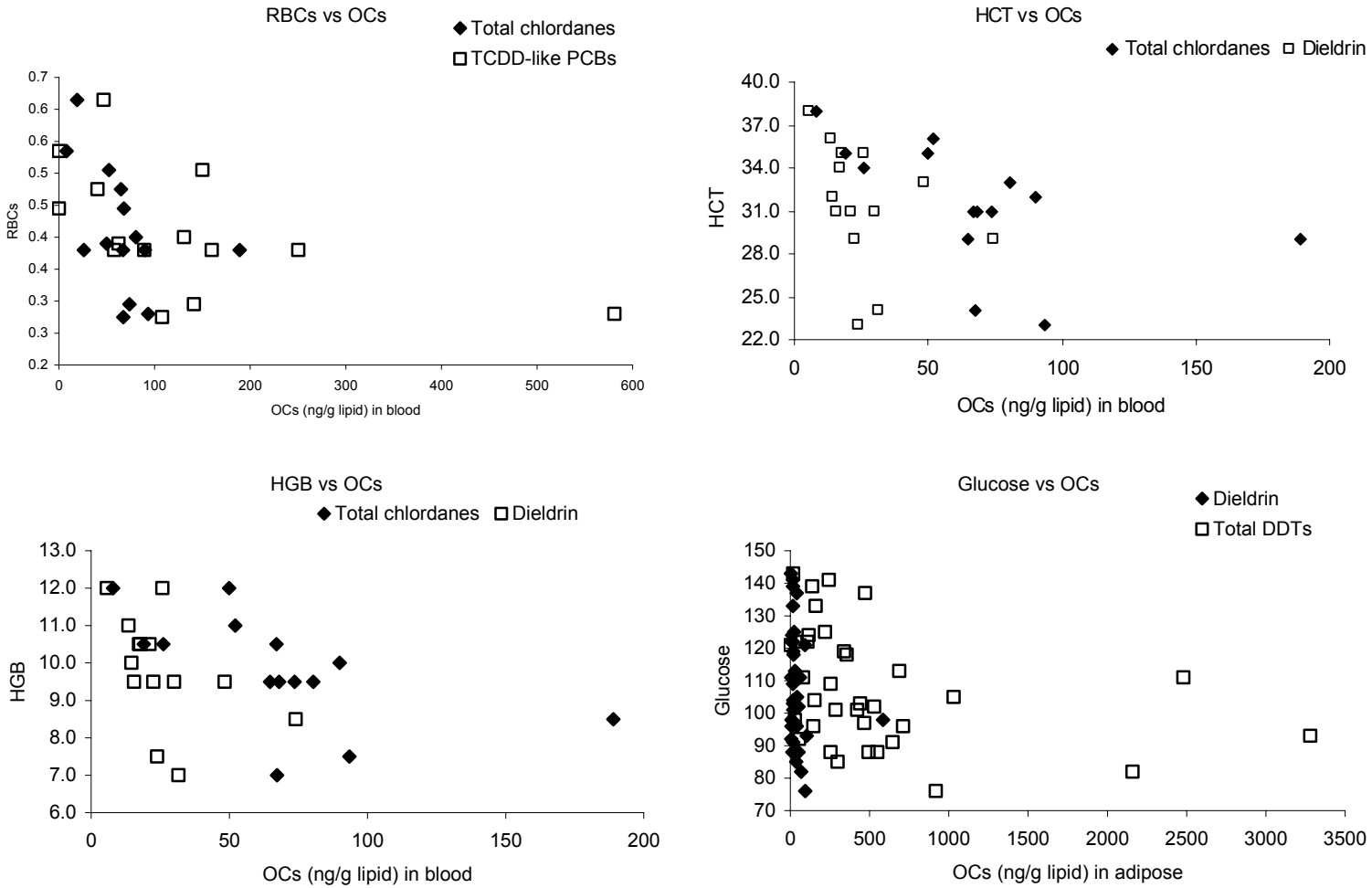

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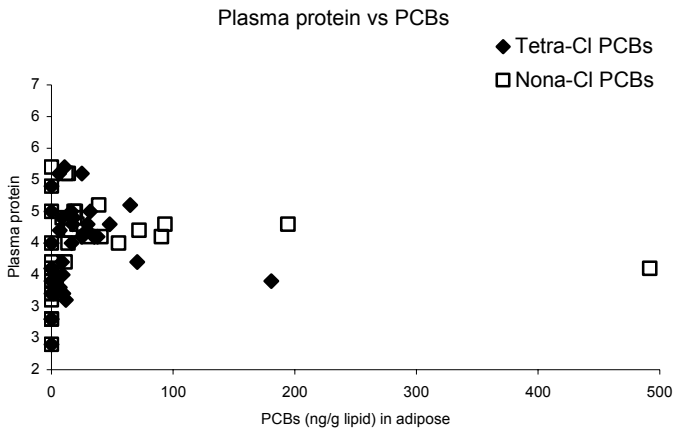

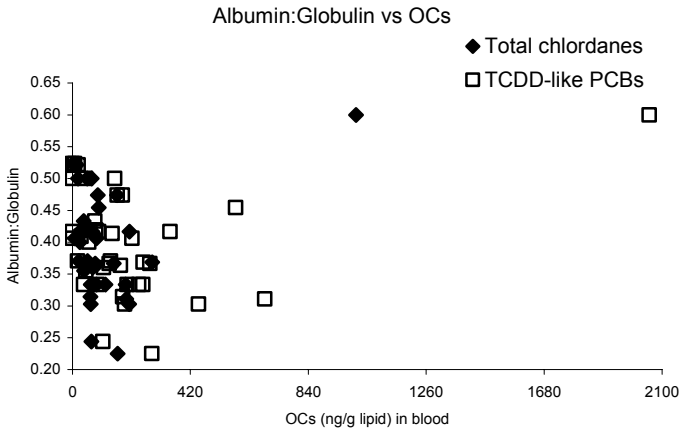

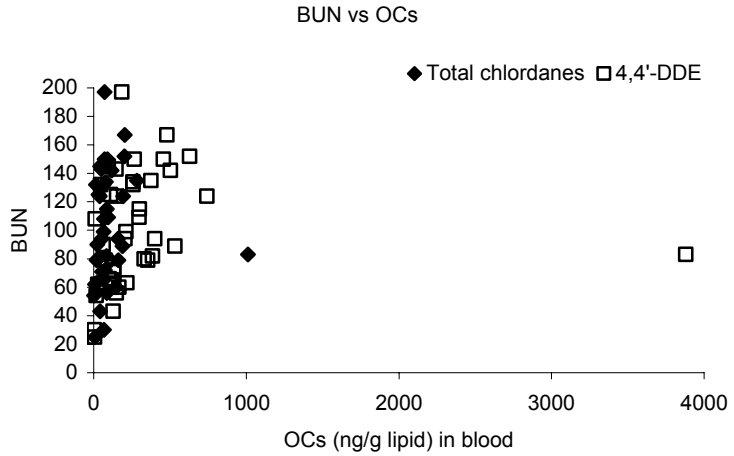

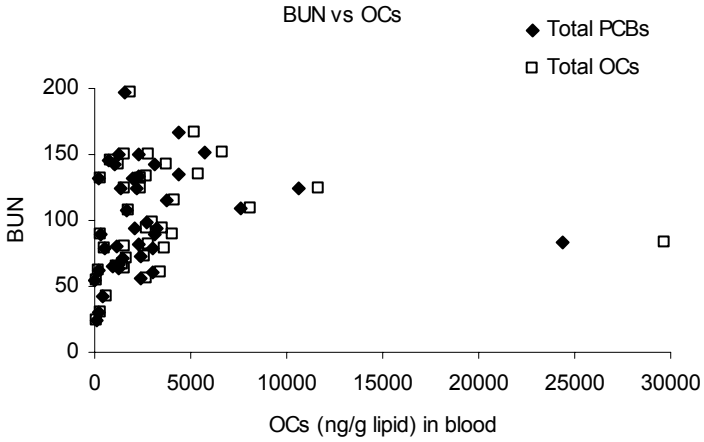

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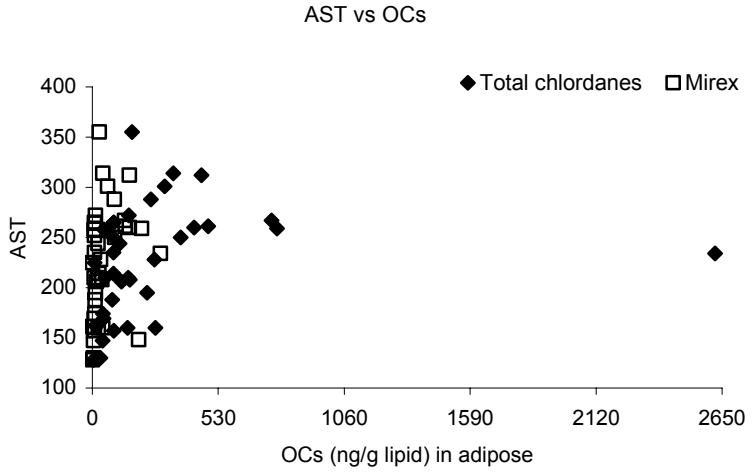

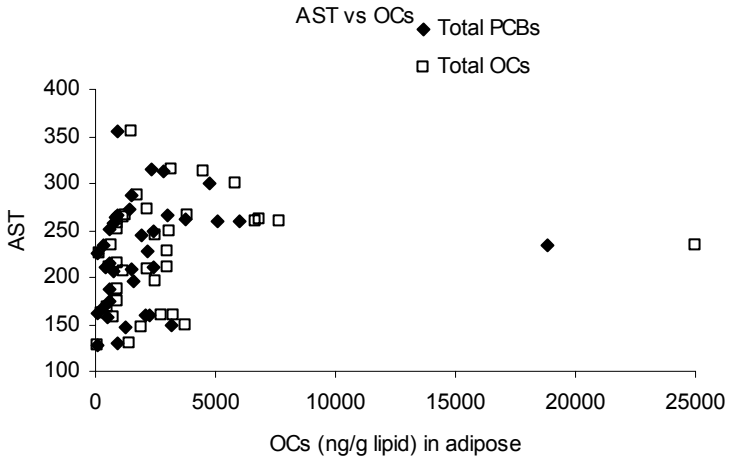

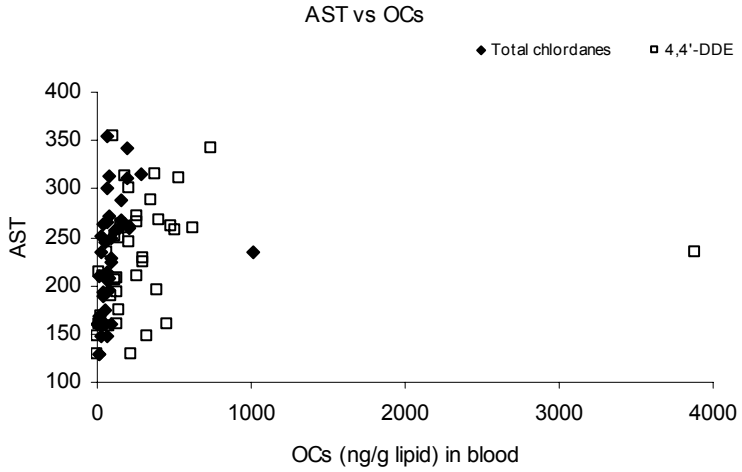

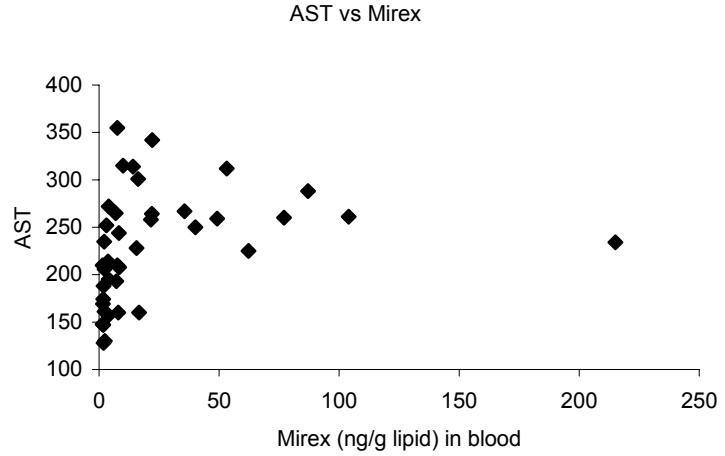

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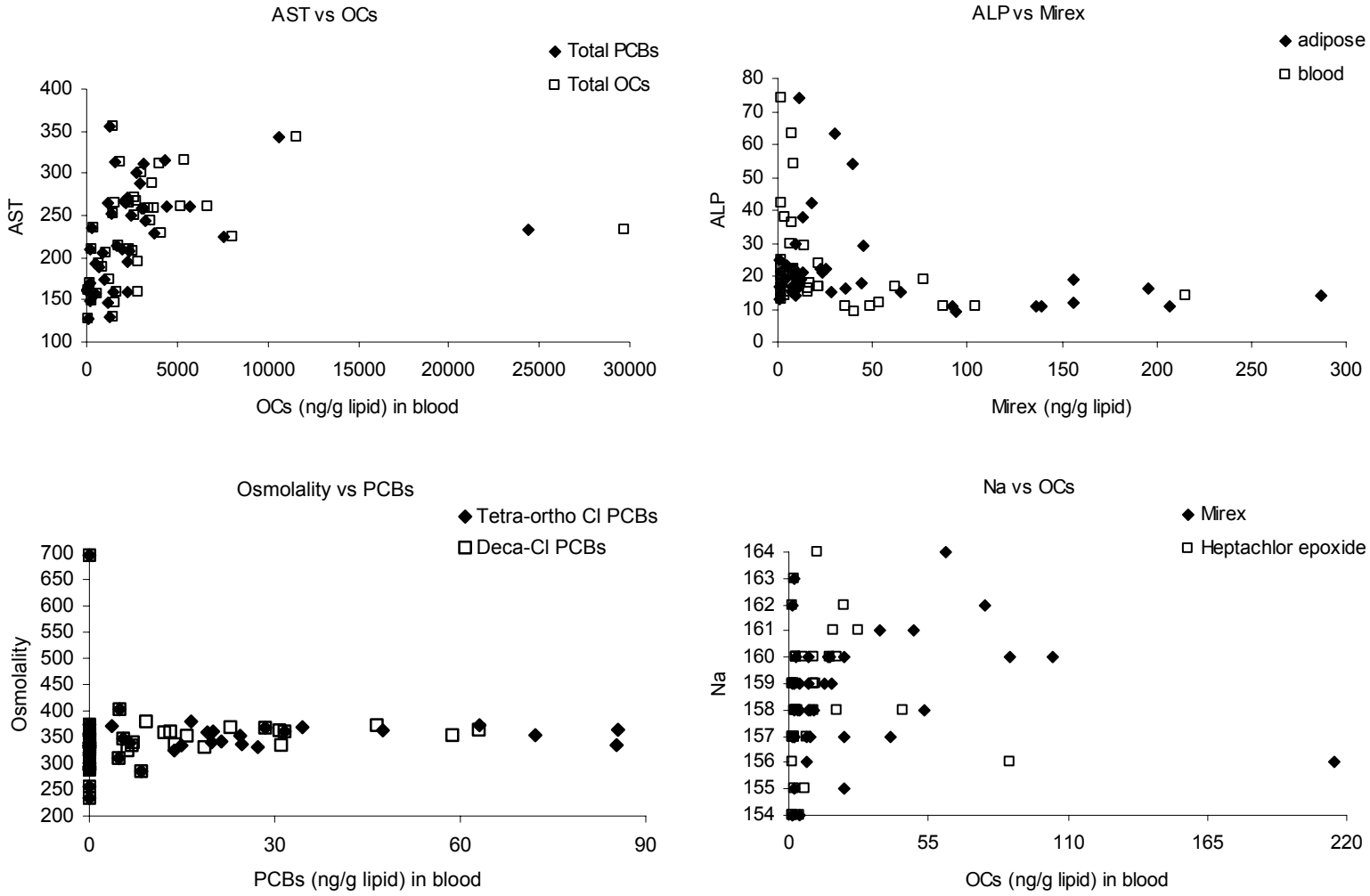

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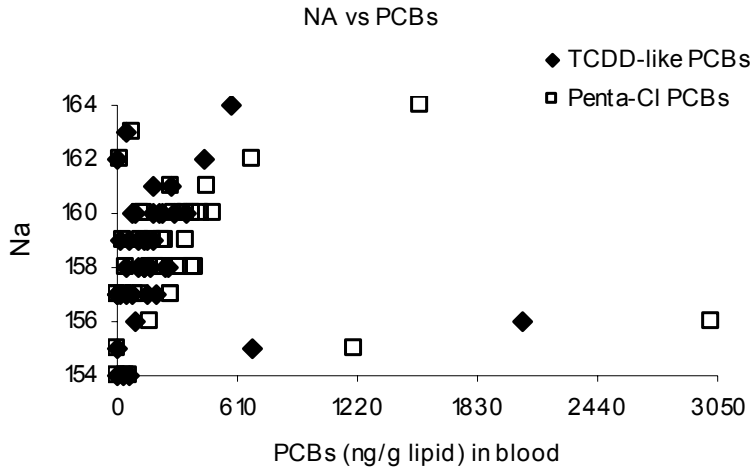

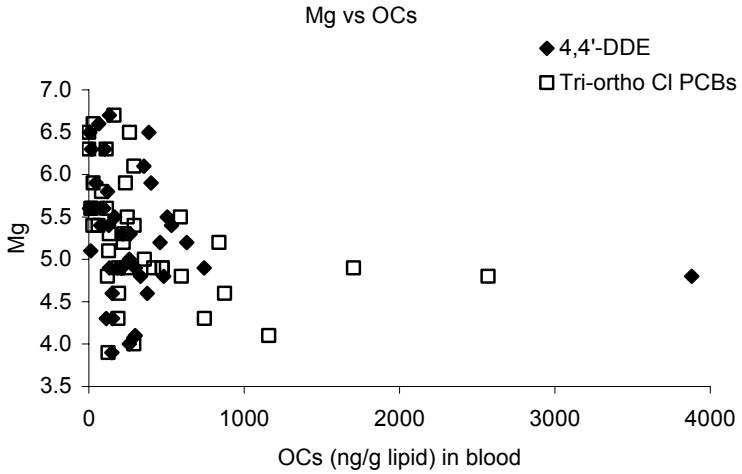

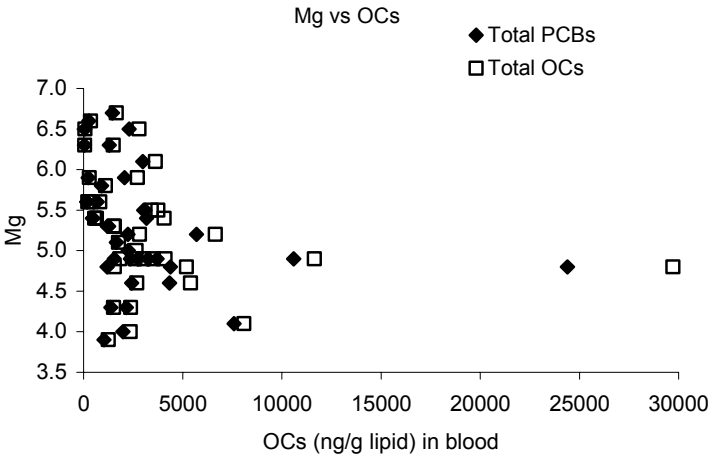

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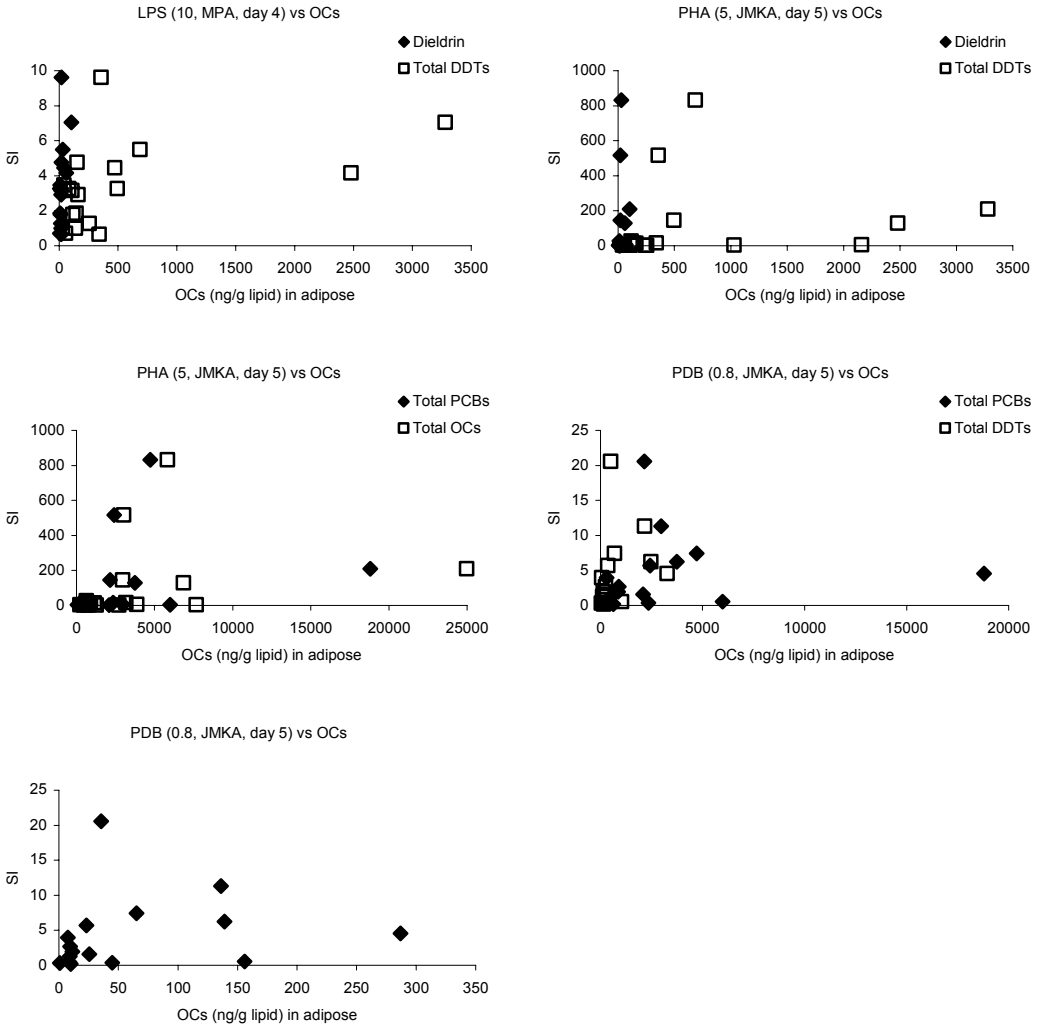
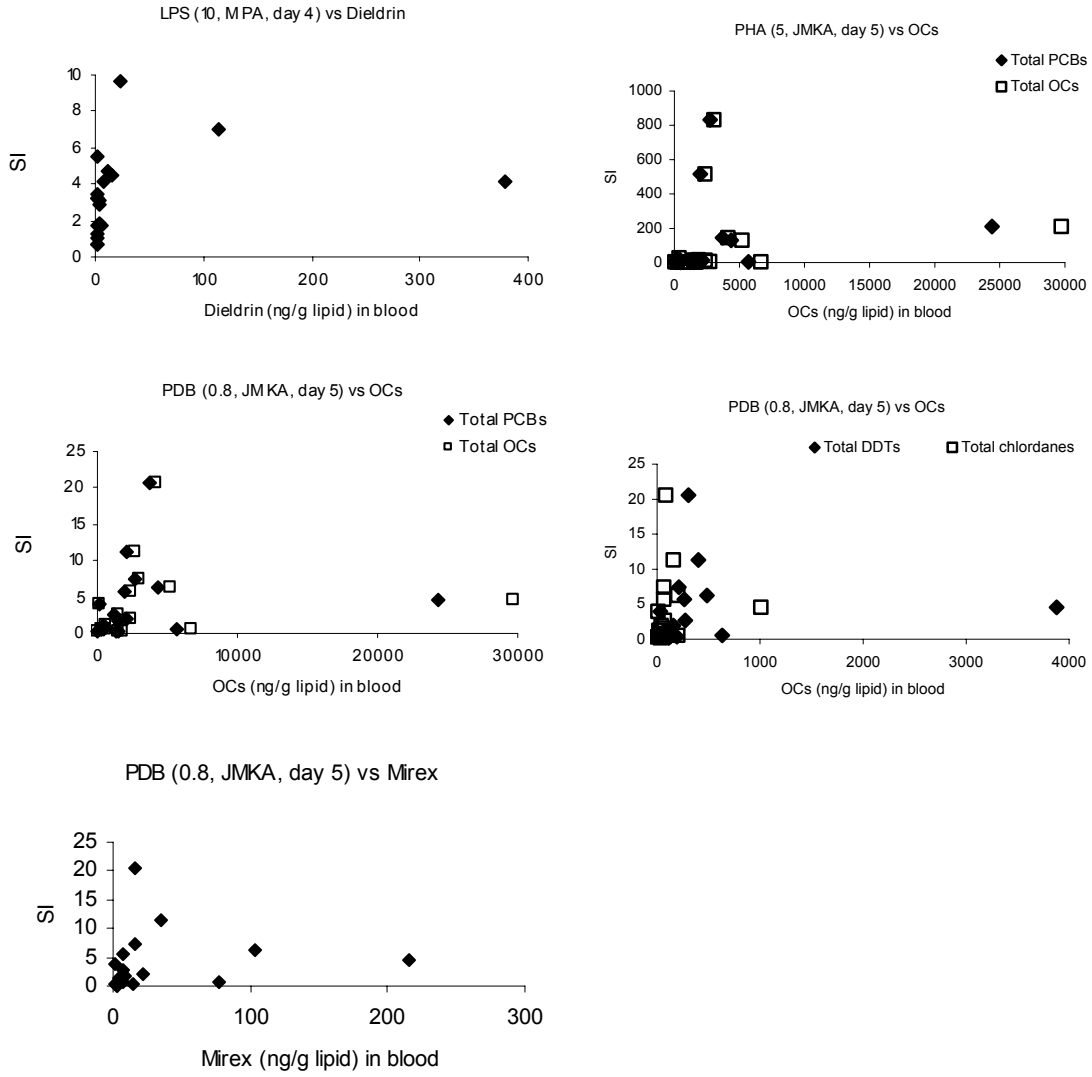


Appendix Figure A2. Plots of the statistically significant correlations between mitogen-induced lymphoproliferative responses and organochlorine (OC) contaminants concentrations in fat biopsies of juvenile loggerhead sea turtles. Mitogens used are listed above each graph (LPS = lipopolysaccharide; PHA = phytohemagglutinin; PDB = phorbol 12,13-dibutyrate). The conditions that were used are listed in parentheses, including the mitogen concentration in ug/ml of culture, the media type, and the day that proliferation was tested. SI = stimulation index = cpm stimulated cells/cpm unstimulated cells).



Appendix Figure A3. Plots of the statistically significant correlations between mitogen-induced lymphoproliferative responses and organochlorine (OC) contaminants concentrations in blood of juvenile loggerhead sea turtles. Mitogens used are listed above each graph (LPS = lipopolysaccharide; PHA = phytohemagglutinin; PDB = phorbol 12,13-dibutyrate). The conditions that were used are listed in parentheses, including the mitogen concentration in ug/ml of culture, the media type, and the day that proliferation was tested. SI = stimulation index = cpm stimulated cells/cpm unstimulated cells).

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BIOGRAPHY

Jennifer Michelle Keller

Birthdate: April 9, 1975 in Franklin, Indiana, USA

Education: 2003 Ph.D. Duke University, Durham, North Carolina
Advisor: Dr. Patricia McClellan-Green

1997 B.S. Indiana University, Bloomington, Indiana
Major: Biology Minor: Environmental Science

Publications:

Submitted Keller JM, Kucklick JR, McClellan-Green P. Organochlorine contaminants in loggerhead sea turtle blood: extraction techniques and distribution among plasma and red blood cells. Archives of Environmental Contamination and Toxicology.

2000 Harms C, Keller JM, Kennedy-Stoskopf S. Use of a two-step percoll gradient for separation of loggerhead sea turtle (*Caretta caretta*) peripheral blood mononuclear cells. Journal of Wildlife Diseases. 36:535-540.

2000 Schlezinger JJ, Keller J, Verbrugge LA, Stegeman JJ. 3,3',4,4'-Tetrachlorobiphenyl oxidation in fish, bird and reptile species: relationship to cytochrome P450 1A inactivation and reactive oxygen production. Comparative Biochemistry and Physiology Part C. 125:273-286.

1999/2000 Keller JM, Meyer J, Rau M, Mattie M, Augsperger T, Dong J, Levin E. Assessment of immunotoxicology in wild populations: Review and recommendations. Reviews in Toxicology. 3:167-212.

Awards for presentations:

2002 Keller JM, Peden-Adams M, Stamper MA, Kucklick JR, McClellan-Green P. Are contaminants affecting loggerhead health? 22nd Annual Symposium on Sea Turtle Biology and Conservation. April 4-7, 2002. Miami, FL. Awarded the Archie Carr Best Student Presentation Award for Best Biology Paper.

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2002 Keller JM, Peden-Adams M, Stamper MA, Kucklick JR, McClellan-Green P. Are organochlorine contaminants affecting loggerhead sea

turtle health? Carolina's Society of Environmental Toxicology and Chemistry Annual Meeting. April 25-27, 2002. Raleigh, NC. Awarded First Place for Student Paper Presentation.

Scholarships and Awards:

2002 Duke Graduate School Conference Travel Fellowship

2002 Nicholas School of the Environment Professional Development Travel Funds

2002 Named an e.hormone Fellow

2002 Gordon Research Conference Travel Grant

2002 Named a Morris Animal Foundation Fellow

2002 22nd Annual Sea Turtle Symposium Travel Grant

2002 Duke Graduate School Conference Travel Fellowship

2002 Nicholas School of the Environment Professional Development Travel Funds

2001 Duke University Marine Laboratory Fellowship – McCurdy Fellowship (for school year 2001-2002 and fall 2002)

2001 21st Annual Sea Turtle Symposium Travel Grant

2001 Nicholas School of the Environment Professional Development Travel Funds

2000 20th Annual Sea Turtle Symposium Travel Grant

2000 Nicholas School of the Environment Professional Development Travel Funds

2000 Duke University Marine/Freshwater Biomedical Center Feasibility Study Grant

1999 Duke University Marine/Freshwater Biomedical Center Feasibility Study Grant