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Fate and Persistence of DDT and its Metabolites in the Okavango Delta, Botswana

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For Jowitt, Neo, Wedu and Tashata

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Abstract

Dichlorodiphenyltrichloroethane (DDT) had been used in the Okavango delta, Botswana, since the 1950's for the control of malaria and sleeping sickness disease vectors. Concentrations of DDT were determined in water, plant material, invertebrates and fish samples. Isotopes of nitrogen ($\delta^{15}N$) were further used to infer organisms' trophic position and the level of biomagnification of total DDT (DDT and it's metabolites). Average concentrations of total DDT ranged from 0.04 ng.L-1 in water to 8.33 ng.g-1 (wet weight) in fish from the Okavango delta. As predicted from global distillation models, these concentrations are about 2 orders of a magnitude lower than the levels in fish from temperate and arctic regions of Canada, where DDT was banned in the early 1970s. The DDE metabolite was the most abundant fraction of total DDT. Although total DDT concentrations were higher in areas treated for malaria control than areas treated for tsetse control, these concentrations were driven by factors other than the historic application of the pesticide. Gaborone dam, an area where DDT had not been used, was sampled in order to compare total DDT levels to treated areas. Here, levels in fish were 3 times higher than in the Okavango delta but consistent with predictions from $\delta^{15}N$ values. Lipid was also a significant predictor of DDT concentrations. Fattier and larger fish such *as Hydrocynus vittatus* and *Synodontis sp.* contained higher levels of DDT than those with less fat.

Résumé

Le Dichlorodiphenyltrichloroethane (DDT) a été employé dans le delta du Okavango, Botswana, depuis les années 1950 pour contrôler les vecteurs de la malaria et de la maladie du sommeil. Des concentrations de DDT ont été mesurées à partir d'échantillons d'eau, de plantes aquatiques, d'invertébrés et de poissons. Les isotopes d'azote ($\delta^{15}N$) ont, par la suite, été employés pour identifier la relation entre le niveau trophique d'un organisme et le degré de biomagnification du DDT et ses dérivés. Les concentrations moyennes du DDT total s'étendaient de 0.04 ng.L-1 dans l'eau à 8.33 ng.g-1 (poids humide) chez les poissons du delta Okavango. Comme prédites par les modèles de distillation globale, ces concentrations sont environ deux ordres de grandeur plus faibles que les niveaux chez les poissons de régions tempérées et arctiques du Canada où le DDT est banni depuis le début des années 1970. Le métabolite DDE s'est révélé la fraction la plus abondante du DDT total. Bien que les concentrations de DDT soient significativement plus élevées dans les régions de malaria relativement aux régions des mouches tsé-tsé, ces concentrations sont influencées par des facteurs autres que l'historique de l'arrosage. Le barrage de Gaborone, site sans utilisation de DDT a été échantilloné pour comparer les concentrations par rapport aux régions arrosées. Ici, les concentrations étaient trois fois plus élevées que dans le delta Okavango. Ces résultats demeurent tout de même en ligne avec les prédictions des valeurs de $\delta^{15}N$. Le pourcentage de lipide chez les poissons était également un bon indicateur de la concentration de DDT dans le delta Okavango. Les plus grands et plus gras poissons tels l' *Hydrocynus vittatus* et *Synodontis sp.* possédaient des concentrations plus élevées de DDT que ceux ayant moins de gras.

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Chapter 1

Fate, Persistence and Effects of DDT and its Metabolites in the Environment

1.1 Introduction

For decades, DDT has played a major role in the global effort to control insects and other vectors that cause malaria and other tropical diseases. Currently, around 2.5 billion people in over 90 countries are at risk of contracting malaria, a disease that continues to be a global menace (WWF 1998, UNEP 1996). Each year 1-2 million children under the age of five die in Africa due to malaria (KemI 1996). According to WWF (1998), malaria in developing countries continues to be one of the leading causes of illness and death, contributing up to 3 million deaths and up to 500 million clinical cases every year. Most of the deaths occur in Sub-Saharan Africa.

In Botswana, the use of DDT dates as far back as the 1950's for malaria (WHO 1984), and in the 1960's for sleeping sickness and other agricultural uses (Davies 1980, Merron 1992). Both malaria and sleeping sickness are predominant in the Okavango delta (WHO 1984, Meron 1992, Fig. 1.1). The *anopheles* mosquitoes transmit malaria and the tsetse fly *(Glossina morsitans centralis)* transmits trypanosomes that cause trypanosomiasis or African "sleeping sickness" in humans and "nagana" in cattle. Some of these trypanosomes inhabit the blood and tissue of humans and animals. Although information on the extent of DDT use in the Okavango delta is scanty, WHO (1984) reported that DDT (75% wettable powder or DDT emulsion) was applied to homes in the area from 1950-1970. In some parts of the Okavango, Fenitrothion (50% emulsion concentrate) was tried in 1972 to replace DDT, but was abandoned because of the short residual effect in 1973 (WHO 1984). Since 1973 up to

1999 (Ministry of Health 1999), DDT (75% wettable powder) has been used for malaria control in the form of indoor residual spraying at the rate of 2g of technical grade DDT/m^2 once a year (WHO 1984). Although the amounts used specifically in the Okavango delta can not be quantified, the Ministry of Agriculture (1992, 1994) showed an increase of DDT use for malaria control per year ranging from 5 tons in 1993 to 10 tons in 1994.

Pesticide use with regard to tsetse control in the Okavango delta began in the early 1960's with DDT ground spraying (Davies 1980). According to Davies (1980), around 1975, eradication strategies entailed the use of ultra low volumes of the insecticide endosulfan. The success of ground spraying, was however, limited to accessible areas, hence in the mid-1970's large-scale aerial spraying in the Okavango with endosulfan was initiated (Davies 1980). Although aerial spraying had the advantage of covering large areas and the rapid mortality of tsetse flies, it had the disadvantage of reaching non-target organisms due to its blanket application nature (Merron 1992).

Extensive research (Douthwaite *et al.* 1981, Merron 1986, 1989, 1990, 1991, 1992, Merron and Bruton 1990) on residue levels, the effects, and impact of endosulfan for tsetse control has been done in the Okavango delta. However, there is currently no data on DDT residues, impact and effects. This has therefore prompted my interest to study the fate of DDT in the delta considering its long-term use in Botswana for both the control of *anopheles* mosquitoes and tsetse fly. I hypothesized that DDT concentrations will be different among locations. DDT concentrations are expected to be higher in areas sprayed for both malaria and tsetse control in comparison with areas sprayed for tsetse alone. I also hypothesized that DDT levels will increase with trophic position in the Okavango delta food chain.

In this chapter, I describe the Okavango delta with emphasis on the areas studied. I then discuss the literature on physical and chemical properties of DDT, which are relevant to the environmental fate and toxicity of DDT and its metabolites. The use of stable isotopes of carbon and nitrogen in food web relationships is also introduced so that in Chapter 2 the food web relationships in the delta can be established. This is further supported using fish stomach content analysis. The distribution of DDT residue levels in the delta are also determined and discussed in Chapter 2 and comparisons made between concentrations of DDT in the delta, other lakes in Southern Africa, and with temperate and sub-arctic regions. Chapter 3 provides a summary of the findings of this research and areas of future research interests.

1.2 Study site

The Okavango "Delta" is an alluvial fan located between 19° and 20° S and 22° and 24° E in the northern part of Botswana (Figure 1.1). The fan has an approximate surface area of 25 000 km^2 (Thomas and Shaw 1991). The area of the wetland is approximately 12 000 km^2 (McCarthy *et al.* 1998). It lies on the edge of the Kalahari Desert and is formed as a result of the discharge of the Okavango River at the southern extremity of the East African Rift system (Scholz *et al.* 1976). The entire Okavango River system lies within the Kalahari Basin partly filled with sediments of various types notably, wind-blown brown and white desert sands (Hutchins *et al.* 1976, McCarthy 1992, Ellery *et al.* 1991). Maximun and minimum average temperatures in ambient air during the summer range from 30.5° C to 33.7° C and 14.8° C to 19.2° C (Ellery *et al.* 1991). Water temperatures may vary between 17° C in Winter (July) up to 35° C in mid rainy season (January–February) (Cronberg *et al.* 1995). About 95% of the water inflow is lost by evapotranspiration (Dincer *et al.* 1981). The

delta consists of the panhandle and delta proper (McCarthy *et al.* 1993). The panhandle and the perennial swamp areas are interspersed with lagoons of various sizes (McCarthy *et al.* 1993) formed within the reed swamp, while in the seasonal swamps, persistent lagoons mainly consist of broader and deeper portions of the channels (Cronberg *et al.* 1995).

Extensive research exists on the hydrology, sedimentology and macroflora of the delta (Wilson and Dincer, 1976, McCarthy *et al.* 1991a, 1991b, McCarthy *et al.* 1993, McCarthy and Ellery 1994, McCarthy *et al.* 1998, Garstang *et al.* 1998) and a hydrological model has been developed (Dincer *et al.* 1987). Potential annual evapotranspiration calculated by Wilson and Dincer (1976) is 1860 mm while the annual rainfall is 500mm. The rainy season in the delta occurs in April and the flood wave has been observed to pass down the delta very slowly (Garstang *et al.* 1998). Evaporation is reported to exceed rainfall during all months of the year (Sutcliffe & Parkes 1989). These findings are a reflection of the semi-arid nature of northern Botswana, where evaporation is 3 to 4 times the precipitation (McCarthy 1992). Due to the high transpiration compared with evaporation, the occurrence of saline surface water in the Okavango delta is rare (Ellery and McCarthy 1994).

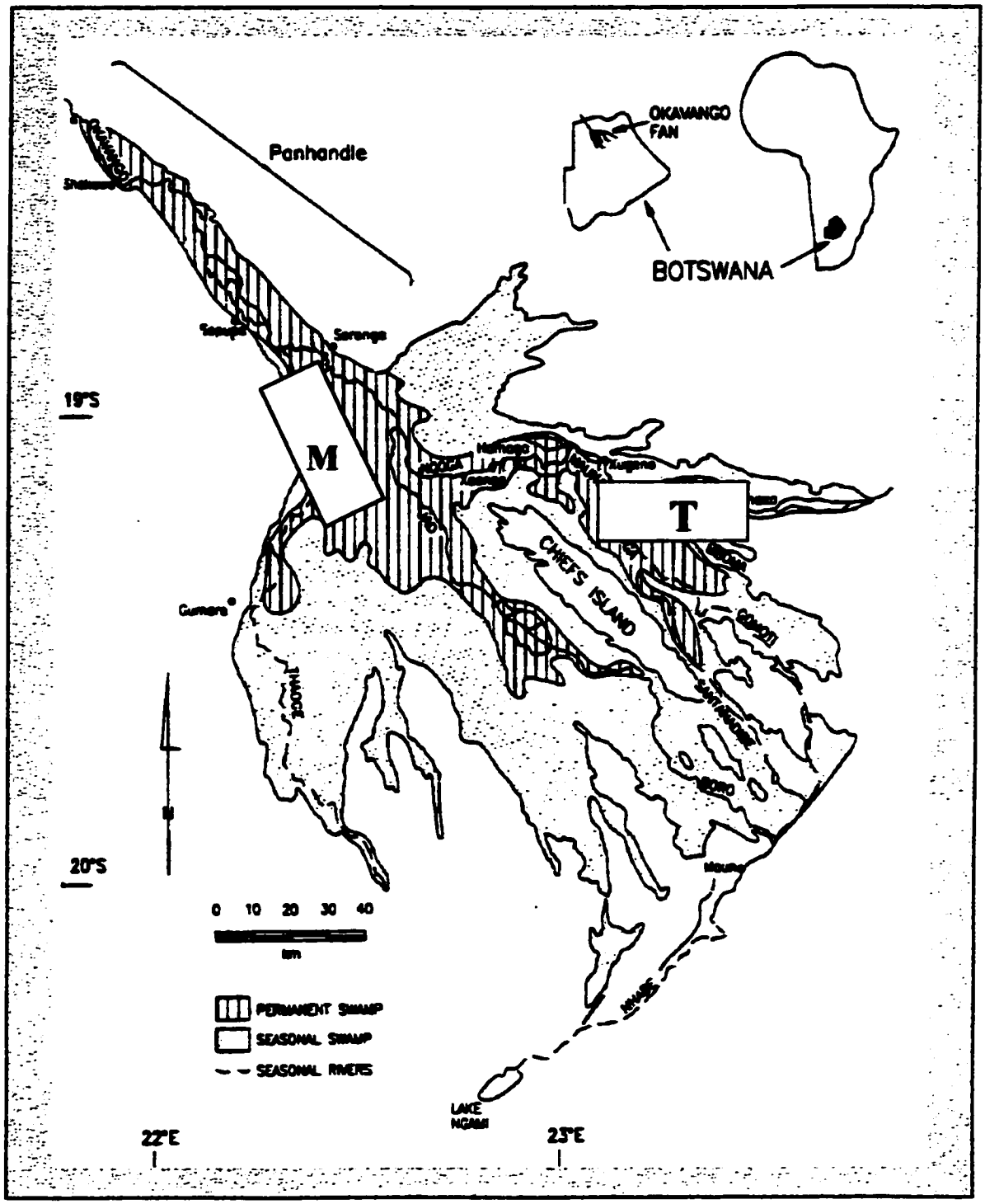
Water is distributed through the delta by a channel system - the Thaoge in the West, and the Jao/Boro in the central delta and the Maunachira-Mboroga-Santantadibe in the East (McCarthy 1992). The channels are flanked by dense swamp vegetation, which is rooted in peat varying in thickness (McCarthy *et al.* 1989). Water is transferred from one channel to another through the sandy soils rather than by direct channel flow (Wilson 1973). Many lagoons exist along these channels and in the present study only three were sampled. These are Ikoga and Guma located in the Thaoge channel in the west of the delta and Xakanaka

situated in the Maunachira-Mboroga channel in the east. Fish samples were also collected from Samuchima channel located near the Thaoge main channel.

Hydrobiological studies in the delta show that it is a hyperoligotrophic ecosystem (Cronberg *et al.* 1995). The low nutrient concentrations in the delta are due to its location on a substratum of eolian sand with inherently low nutrient status (Cronberg *et al.* 1995). Furthermore, the Okavango River, which supplies water to the delta, lies on the Kalahari sand (Garstang *et al.* 1998) which is also low in nutrients. Compared to other wetlands, nutrient species have been found to occur at extremely low concentrations - nitrogen (as nitrate and ammonia) is typically 0.08 ppm, phosphorus 0.04 ppm (Cronberg *et al.* 1995) and potassium 2.9 ppm (Cronberg *et al* 1995, this study). Detailed analysis of nutrient budgets in one community (*Papyrus sp.*) by Garstang and his colleagues (1998) show that "while large reservoirs of nutrients, especially N and P are present, mineralization rates are very low and the contained N and P are largely unavailable." The authors conclude that nutrients, and in particular phosphorus, are transported through the atmosphere, and contribute to the productivity of the delta ecosystem.

The Okavango delta plays a significant role in the cultural structure of Botswana. It is historically important, having served as a refuge during politically troubled times in past centuries and as a guaranteed source of water in years of drought (McCarthy 1992).

Figure1.1: Map of the Okavango delta showing approximate locations of the sampled areas in malaria (M = Ikoga, Samuchima, Guma) and tsetse (T = Xakanaka) control areas. (adapted from Smith *et al.* 1997).



It is ecologically important and a significant habitat for Botswana's wildlife (NCSA 1997, McCarthy 1992, Merron 1993). Socio-economically, it supports the traditional lifestyles of many local communities, providing reeds for thatching, fish and other livelihood resources (Ramberg 1997). The Delta is also important economically as a source of tourist revenue (McCarthy 1992, Ministry of Finance & Development Planning 1997, Ramberg 1997). Not only does it provide water for north central Botswana but also for the rest of the country, which is generally dry.

The following section reviews relevant literature on DDT and its metabolites in order to provide a framework for determining its fate and persistence in different environmental compartments and how this relates to its use in the Okavango delta.

1.3 Dichlorodiphenyltrichloroethane (DDT) and its Metabolites

DDT is a chlorinated hydrocarbon manufactured specifically for its insecticidal properties. It is one of the designated Persistent Organic Pollutant (POP) pesticides (UNEP 1991). In general, DDTs (including DDT, DDE, DDD) are chemically stable under ambient environmental conditions and persistent in the environment. While this persistence decreases the need for repeated use of the pesticide at the application site for the control of unwanted vectors, it also, unfortunately, increases the potential for effects on non-target organisms. The physicochemical properties of DDT and its metabolites, such as low solubility in water or high solubility in lipids, results in their tendency to partition out of air and water (Mackay 1991), thus, accumulating in tissues of aquatic organisms and biomagnifying in food webs (WHO 1989, Jensen *et al.* 1997).

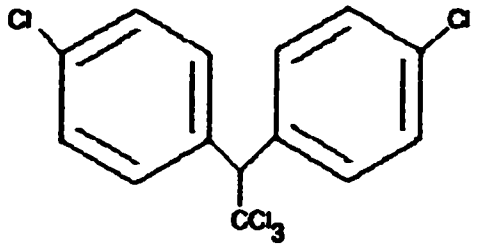
Evidence from a variety of studies has linked the bioaccumulation of DDTs to reproductive failure, eggshell thinning (Noble 1990, Bishop and Weseloh 1990, De Vault *et al.* 1995) cancer (Krieger *et al.* 1994), and immunosuppressive effects (WHO 1989, ATSDR 1994). As a result, DDT use is banned or severely restricted in developed countries. Nonetheless, DDT is still manufactured and used heavily in many areas of the world, particularly in the middle and far East, Africa and Latin America (UNEP 1996). Compared to many alternative pesticides, DDT is not acutely toxic to people working with it (UNEP 1991, 1996), and is relatively cheaper (WWF 1998). For these reasons, many developing nations continue to use DDT for the control of agricultural and public health pests (UNEP 1996, WWF 1998). On the other hand, the uptake of DDTs through diet poses a threat to human health and represents a major exposure route for predatory wildlife species (Edwards 1973a, 1973b, Lambert 1992, 1997, WWF 1998). DDT use also presents an added risk to residents in homes where it is sprayed in the interior of homes for the control of malaria-causing mosquitoes (Bouwman *et al.* 1990a, 1990b, Bouwman 1991).

1.3.1 Identity, physical and chemical properties

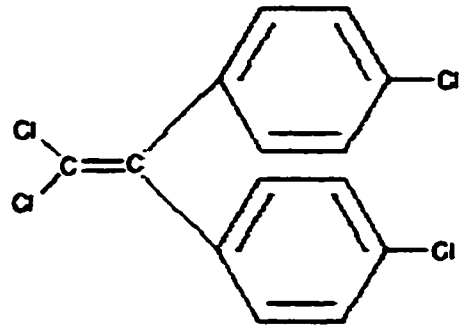
DDT refers to *p,p'*-DDT (1,1,1-trichloro-22-*bis*-(*p*-chlorophenyl) ethane(WHO 1989). There are several isomers of the DDT molecule and these vary in the positions of the chlorine atoms on the two phenyl rings (Figure 1.2). Technical grade DDT (TG- DDT), which is used in many formulated DDT pesticides, is comprised of three principal isomers, *para-para'*-DDT (77.1%), *ortho-para'* -DDT (14.9%) and para-*para'* -DDE (4.0%), *ortho-para'*- DDE (0.1%), *para-para'*-DDD (0.3%), *ortho-para'*-DDD (0.1%)

Figure 1.2: The molecular configuration of the commonest isomer found in technical DDT, *para-para* DDT, and its primary metabolites, *p, p'*-DDE and p, p'- DDD.

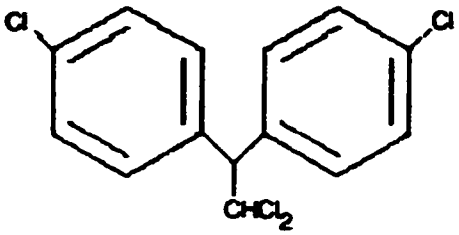
DDT



DDE



DDD



and a number of unidentified products (USEPA 1980, WHO 1989). In the environment, *p,p'*-DDT and *o,p'*-DDT are transformed into a number of compounds with similar chemical structures. Of these *o,p'*-DDE and *p,p'*-DDE [1,1-dichloro-2, 2-bis (p-chlorophenyl)ethylene] tend to be the most persistent and are therefore detected at highest concentrations. The two DDD isomers, *o, p'*-DDD and *p, p'*-DDD [1,1-dichloro-2, 2-bis (p-chlorophenyl) ethylene] are also formed when DDTs are degraded or metabolized. DDD, also called Rothane, was manufactured and used as an insecticide for several years in the United States (USEPA 1980). Several other metabolites and degradation products have also been identified in various environmental compartments (USEPA 1980). Table 1.1 shows basic chemical and physical properties of DDT isomers.

Technical grade DDT is nonflammable, odorless to slightly fragrant, colorless crystals or white powder at room temperature (WHO 1989, Worthing and Hance 1991). It is comprised primarily of the *p,p'*- DDT and *o,p'* DDT isomers, both of which have a molecular mass of 354.5 and a molecular formula of $CH_4H_9Cl_5$ (Table 1.1). At standard temperature and pressure, these isomers form colorless crystals that are sparingly soluble in water, strongly lipophilic (log K_{OW} of 6.0), and highly soluble in organic solvents such as acetone and ethanol (WHO 1989, Budavari *et al.* 1989).

The DDE isomers have a slightly lower molecular mass of 138.0 (Suntio *et al.* 1988). The melting point and the vapor pressure (0.8 to 1.0 mPa) are similar to those of DDT isomers. However, *p, p'*-DDE and *o, p'*-DDE are more soluble in water (40μg/L and 10μg/L respectively), slightly less hydrophobic (log K_{ows} of 5.7and 5.8 respectively) the o, p'-DDD isomer is slightly more hydrophobic (log K_{ow} 6.1) (Suntio et al. 1988).

Table 1.1: Physical and Chemical Properties of DDTs

Property	*p,p'*-DDT	*o,p'*-DDT	*o,p'*-DDE	*p,p'*-DDE	*o,p'*-DDD	*p,p'*-DDD
Molecular Formula	$C_{14}H_9Cl_5$	$C_{14}H_9Cl_5$	$C_{14}H_8Cl_4$	$C_{14}H_8Cl_4$	$C_{14}H_{10}Cl_4$	$C_{14}H_{10}Cl_4$
Molecular Weight	354.5[1]	354.5[1]	318.0[4]	318.0[4]	320.1[3]	320.1[3]
Melting Point	108.5°C[1]	74-74.5°C[2]	88-90°C[4]	88-90°C[4]	76-78°C[3]	109-110°C[3]
Boiling Point	185°C[2]	N/A	N/A	N/A	N/A	N/A
Vapor pressure @ 20°C	0.02 mPa[4]	0.02 mPa[4]	0.8 mPa[4]	1 mPa[4]	0.2 mPa[4]	0.1 mPa[4]
Henry's Law Constant @ 20°C[5]	2.36 Pa m^3/mol	0.35 Pa m^3/mol	6.2 Pa m^3/mol	7.9 Pa m^3/mol		0.64 Pa m^3/mol
Solubility - water @ 20° ethanol Acetate	3 µg/L[4] 20 g/L[3] 580 g/L[3]	3 µg/L[4]	100µg/l[4]	40µg/L[4]	50 µg/L[4]	100 µg/L[4]
Log K_{ow}	6.0[4]	6.0[4]	5.8[4]	5.7[4]	6.1[4]	5.5[4]

[1]Worthing and Hance 1991
[2]USEPA 1980
[3]Budavari *et al.* 1989
[4]Suntio *et al.* 1988
[5]Mackay *et al.* 2000

In this thesis, unless otherwise stated, the term total DDT (tDDT) refers to the sum of the concentrations of compounds including *p,p'*- DDT, *o,p'*-DDT, *o.p'*-DDE, *p.p'*-DDE, *o.p'*-DDD and *p.p'*-DDD. The term ΣDDT refers to the sum of the concentrations of *p,p'*- DDT, and *o,p'*-DDT isomers. The term ΣDDE refers to the sum of concentrations of *o,p'*-DDE isomers, and ΣDDD refers to the sum of concentrations of *o,p'*-DDD and *p,p'*-DDD isomers.

1.3.2 Environmental fate and distribution

The cumulative, global annual production of DDT is estimated at 30 000 tons (WWF 1998). Russia, Mexico, India and China still produce DDT for export and for domestic use in public health programs. Other countries such as South Korea and the former Soviet Union States may also produce and export DDT as well (UNEP 1996). In general developing countries still use DDT for the control of malaria. Due to this widespread use and release into the environment, DDTs are distributed in the atmosphere (Mackay 1991, Mackay and Wania 1995), water, sediments, soils and biota worldwide (Edwards 1973a, 1973b, UNEP 1996).

DDT in the atmosphere

The moderate vapor pressure, low solubility and low reactivity of DDT and its metabolites account for its wide distribution throughout the biosphere (Mackay 1991, UNEP 1996, Mackay and Wania 1995, Simonich and Hites 1995). Applications in agriculture, forestry and disease vector control represent the primary sources of DDTs to the atmosphere both during spraying operations, and subsequently through volatilization from water, plants

and soils. Simonich and Hites (1995) undertook a study to determine the global distribution of organochlorine compounds including DDT and its metabolites. Tree bark samples were collected from 90 sites worldwide. The results showed that *p,p'*-DDE was among the highest organochlorine compounds. *p,p'*-DDT was found in high concentrations relative to its degradation product *p,p'*-DDE in samples from India, Iran, Romania, Russia and the West Coast of Australia. Simonich and Hites (1995) also observed that the median ratio of *p,p'*-DDT to *p,p'*-DDE concentrations in tree bark was greater than 2 in countries which indicated recent DDT use.

A previous study by Calamari *et al.* (1991) had shown high concentrations of tDDT in the atmosphere in tropical and sub-tropical areas as compared to temperate ones. The highest values of ΣDDT (*o,p'*- DDT and *p,p'*-DDT) concentrations, as compared to ΣDDE (*p,p'*-DDE and *o,p'*-DDE) were found in countries such as India where DDT is still used in large amounts. Low levels of ΣDDT corresponded in general to high DDE percentages, and this could indicate long range, indirect contamination (Calamari *et al.* 1991). They also observed that tDDT (*o,p'*- DDT and *p,p'*-DDT *p,p'*-DDE and *o,p'*-DDE *o,p'*- DDD and *p,p'*-DDT) was not related to temperature. Nonetheless, for *o,p'*-DDT, a relationship with temperature was observed. This was attributed to the physicochemical properties of the molecule (Table 1.1), and in particular Henry's law constant of *o,p'*-DDT.

In another study by Larson *et al.* (1995), tDDT levels in the atmosphere were significantly higher in Zimbabwe compared to southern Sweden or over the Baltic Sea. The mean levels were 1050 pg/m^3 in the Kariba region, Zimbabwe, while levels were 130 times lower in southern Sweden (mean 8 pg/m^3). Larson *et al.* (1995) found that the DDT levels in the atmosphere of the two European stations were consistent with results from other studies;

in 1985, levels of tDDT were observed to reach 40pg/m^3 for 16 sampling stations in Sweden. In another study, 7 pg/m^3 was recorded in the atmosphere in Sweden (1983-1985) and 5 pg/m^3 from Southern Germany (1987) (Larson *et al.* 1995). Larson *et al.* (1995) also reports that in Congo, central Africa, the level of tDDT was 2800 pg/m^3 in 1989. This was comparable to the results from Zimbabwe (Larson *et al.* 1995). The high temperatures in warmer countries contribute to volatilization of DDT and its metabolites, hence the high concentrations of DDT in the atmosphere (Simonich and Hites 1995; Larson *et al.* 1995; Calamari *et al.* 1991). In all the three studies cited, evidence exists that because of its semi-volatility, DDT is not effectively distilled and would tend to remain in the area of use. Because it is sprayed as wettable powder on tsetse fly roosting sites and along rivers and human dwellings in developing countries, highest atmospheric values are detected in these areas as compared to temperate regions (Simonich *et al.* 1995, Ockenden *et al.* 1998).

Although there are difficulties in developing models that describe chemical fate and concentrations of persistent pollutants in the entire global environment due to the lack of data on the use of these chemicals, the present models developed by Wania and Mackay (1993, 1995) provide insights into the predicted concentrations of DDTs and other pesticides in various environmental compartments. These models show that atmospheric concentrations of contaminants will decrease with distance from source and studies from tropical and temperate regions show the same trend (Larson *et al.* 1995).

Once airborne, DDTs undergo a number of processes that can result in their transformation and / or removal from the atmosphere (Mackay 1991). Both DDT and DDD are subject to photo-oxidation under simulated atmospheric conditions, potentially decomposing to carbon dioxide and hydrochloric acid (WHO 1979). While the photo-

oxidation half-life of DDT has been estimated at 2 days (ATSDR 1994), the presence of DDTs in the atmosphere suggests that this reaction may occur more slowly under environmental conditions. Hence, precipitation (wet and dry deposition) and dust-fall are an important environmental process for DDTs released into the atmosphere (Mackay and Wania 1995).

Fate and persistence in water

Adsorption of DDTs to suspended particulate matter and subsequent deposition to aquatic sediments plays an important role in its environmental fate (WHO 1989, Mackay 1991). Several studies have shown that the presence of dissolved organic carbon (DOC) in the aqueous phase reduces the sorption of DDTs to sediments (Caron *et al.* 1985, Larson *et al.* 1992, Berg *et al.* 1992). In a study by Larson *et al.* (1992), humic compounds were shown to affect the uptake of persistent pollutants in fish. The humic substances adsorb lipophilic compounds such as DDT, and function as "carriers", thereby increasing their solubility. However, because the pollutants are firmly adsorbed to the carriers, their availability for uptake by fish is reduced (Larson *et al.* 1992). Levels of *o,p'*-DDE decreased as the amount of humic substances (water color) increased. Losses of DDTs from water may occur due to volatilization (Mackay 1991). Volatilization has been found to be even more important in marine systems, as laboratory studies suggest that DDE is likely to volatilize from seawater 10 to 20 times faster than it would from fresh water (Atlas *et al.* 1982).

The transformation of DDT in surface water has been observed to be a slow process (Howard *et al* 1991). It is estimated that the half-life for the hydrolysis of DDT in water is 20 - 22 years (Howard *et al.* 1991). In the absence of light, the hydrolysis half-life is about eight

years at 27°C and pH 7 (Wolfe *et al* 1977), while direct photolysis half-life of DDT in aqueous media has been estimated to be more than 150 years (Zepp *et al.* 1976). The half-lives are affected by natural substances in the aquatic environments such as humic acids, which increase the degradation rate of DDT. Observed half-life estimates for photo-oxidation in natural waters are lower, ranging from 7-350 days (USEPA 1979).

DDT may reach surface waters by runoff, atmospheric transport, drift, or by direct application (e.g. ground or aerial spraying for the control of tsetse fly or malaria mosquitoes) (ATSDR 1994). The reported half-life for DDT in water is 56 days in lake water, and approximately 28 days in river water (USEPA 1979). The main pathways for loss are volatilization, photodegradation, adsorption to water-borne particulates and sedimentation (ATSDR 1994). Field and laboratory studies in the United Kingdom demonstrated that very little breakdown of DDT occurred in estuary sediments over the course of 46 days (USEPA 1979). DDT has been widely detected in ambient surface water sampling in the United States at a median level of 1 ng. L^{-1} (part per trillion) (ATSDR 1994).

Biodegradation is considered a minor process for water-borne DDTs (ATSDR 1994). Nonetheless biodegradation can occur in surface waters provided that organisms with the capability of degrading DDTs (i.e. certain algae, bacteria, and fungi) (Jungest and Alexandra 1975) are present.

Fate and Persistence in Soil

Due to its extremely low solubility in water, DDT will be retained to a greater degree by soils and soil fractions with higher proportions of soil organic matter (WHO 1989, Alexander 1995, Mackay 1991). It may accumulate in topsoil layer situations where heavy applications

are, or were made annually (Liechtenstein and Schulz 1959). Generally, soil organic matter tightly sorbs DDT, but it (along with its metabolites) has been detected in many locations in soil and ground water where it may be available to organisms (USEPA 1989, WHO 1989, Alexander 1995). This is mainly due to its persistence; although it is immobile or only very slightly mobile over long periods of time, it may be able to eventually leach into ground water, especially in soils with little organic matter. Residues at the surface of the soil are much more likely to be broken down or otherwise dissipated than those below several inches (Edwards 1973a).

Half-life measurements under field conditions for aerobic degradation of DDT in soil range from 2 years (Liechtenstein and Shulz 1959) to greater than 15 years (Keller 1970, Stewart Chisolm 1971). According to Castro and Yoshida (1971), the biodegradation rate of DDT is faster in soil, with half-lives estimated at 16 to 100 days.

Soil type, climatic conditions, the amount of pesticide applied and its biological and physical properties have been found to influence the persistence of DDT in the soil (Liechtenstein and Schulz 1959, Edwards 1973a). In their study to test the persistence of DDT in muck and loam soil, Liechtenstein and Schulz (1959) found that the amount recovered from muck soil was 1.4 times more than that recovered from a Miami silt loam 6 months after soil treatment. The same ratio was obtained when both soil types were analyzed for DDT during the following three years. Temperature was also found to be an important factor in the persistence of DDT (Liechtenstein and Schulz 1959, Larson, *et al.* 1995). Studies in Arizona have also shown that volatilization losses may be significant and rapid in soils with very low organic matter (desert soils) and high irradiance of sunlight, with volatilization losses reported as high as 50% in 5 months (Edwards 1973a).

Several processes can result in migration of DDT in contaminated soils to other environmental compartments (i.e. sediment, water, atmosphere, and biota). These include temperature (which facilitates volatilization), sunlight (photo-degradation), transport of soil particles from the soil surface, ground water or surface water transport and bioaccumulation in biota (Edwards 1973a).

Fate and Persistence in Sediments

DDTs tend to form strong associations with sediment particles and this is related to their organic carbon partition coefficients (ATSDR 1994, Mackay 1991). Many processes can result in the movement of sediment-associated DDTs. For example, fine sediments, as is the case in the Okavango delta, can be re-suspended by currents in surface waters and then be transported long distances (Gillis *et al.* 1995). DDT can also be lost in sediments through volatilization (Edwards 1973b), especially in methanogenic sediments or those that are periodically dewatered as in the case of wetlands or intertidal area (Misch 1993). Because they are persistent and highly lipid soluble, as reflected by their high K_{ow}s, sediment-associated DDTs have a tendency to accumulate in biological tissues and some transported to other areas via fish, marine mammals, and terrestrial wildlife (Edwards 1973a, Environment Canada 1997).

Laboratory studies have shown that biodegradation half-lives of DDT in sediments ranged from roughly 3 weeks to more than 24 weeks, depending on the type of sediment considered (Jungest and Alexander 1975). Jungest and Alexander. (1975) found that DDTs biodegrade most rapidly in sandy sediments, while slower rates were observed in coarse

sands and fine sandy loam. Sediment cores have been used from Lake Ontario to estimate the half-life of DDT in sediments to be 14 to 21 years (Oliver *et al.* 1989).

Fate and Persistence in Aquatic Organisms

Aquatic organisms may be exposed to DDTs through the consumption of contaminated food, and, organisms at a higher trophic level in the food web are at greater risk (Webber *et al.* 1989; WHO 1989; Larsson *et al.* 1991, Kidd 1995b, 1998). In fish and amphibians, the uptake of DDT is influenced by exposure duration (age), lipid content, growth rate, trophic status, water temperature and sex (Licht 1976, Thomann 1989, Larson *et al.* 1991, Rowan and Rasmussen 1992). Laboratory studies have shown that DDTs can be bioconcentrated directly from the water column (Derr and Zabik 1972, Metcalfe *et al.* 1988, Larson *et al.* 1992). Contaminated sediments are also a source of DDT to aquatic organisms. Detritivorous macroinvertebrates have been found to accumulate DDTs to higher levels than predators or herbivores at 5 out of 6 sample sites (Webber *et al.* 1989). This study showed the importance of contaminated sediment as a principal reservoir for DDT. On the other hand, other studies have found that predators in aquatic habitats have high levels of persistent pollutants because they obtain the pollutants from both their food and through partitioning from breathing medium (Larson *et al.* 1992).

Predators generally live longer than their prey, resulting in longer periods of exposure to contaminants (Larson *et al.* 1991). In their study to determine the uptake of persistent pollutants in eel population (*Anguilla anguila*), Larsson *et al.* (1991) concluded that concentrations of PCBs and DDT and its metabolites increased with age. Studies have also shown that uptake of pollutants from water is a faster process (a matter of hours or days

depending on the chemical property of the pollutant) (Pizza and O'Connor 1983). Bengtsson (1980) has however observed that uptake of food is a slower process and hydrophobic pollutants may therefore, accumulate over years in fish. The age of the fish is therefore, a governing factor in the uptake process (Larson *et al.* 1991).

In fish, the highest DDT concentrations have been detected in tissues with the highest lipid content, particularly liver, red muscle, and gonads (Muir *et al.* 1992, Larsson *et al.*1991, 1992, Kidd 1996). Lower DDT concentrations are therefore, expected to accumulate in tissues with lower lipid levels, such as blood, viscera, skin, brain, kidney, gills and white muscle (Licht 1976, Larson *et al.* 1993). Other factors influencing the uptake of DDT in fish include the concentration of humic substances, water temperature, pH, the calcium concentration and ionic strength (Larson *et al* 1992). Larson *et al.* (1992) found that the levels of persistent pollutants in pike were lower in humic lakes than in clear-water lakes. Since the humic compounds should also reduce availability of lipophilic pollutants for gill breathing prey species, uptake from food by predator fish is also expected to be reduced compared with the aquatic environments with less colored water (Larson *et al.* 1992).

Once aquatic organisms are contaminated with DDTs, their capacity to metabolize these substances differs substantially (Environment Canada 1997). In a study by Burnett (1972) none of the radiolabeled DDT absorbed by marine phytoplankton, *Dumaliella sp.* and *Phaeodactylum sp.* was transformed either to DDE or to DDD. He however found that, both DDT and DDE, but not DDD, were detected in the copepods (*Tigriopus* sp.) that consumed these algal species. Bunett (1972) concluded that the copepods have the capability of aerobically metabolizing DDT in their tissue. Licht (1976) also found that fish and

amphibians appear to have the metabolic pathways necessary to degrade DDT to DDE and, to a lesser extent, DDD.

Bioaccumulation of DDTs in Aquatic and Terrestrial Organisms

The lipophilic and persistent character of many environmental pollutants causes them to occur in higher concentrations in consumers than in their respective foods (WHO 1989,Kidd *et al.* 1995b Kidd 1996). Successive consumption in a food web can expose top predators to pollutant concentrations in the tissues that may be orders of magnitude higher than those of the primary producers in the base of a food web (Kidd *et al.* 1998). DDTs have been found to readily bioaccumulate in humans, terrestrial and aquatic organisms (WHO 1989, Connolly 1988, Bouwman 1990 a, 1990b, Larson *et al.* 1991, Evans *et al.* 1991, UNEP 1991). The biomagnification of bioaccumulated chemicals occurs when concentrations of the chemical increases as the chemical passes up through two or more trophic levels, resulting in the transfer of the chemical from food to consumer (Kidd *et al* 1995a, 1998, Kidd 1996, Kucklick *et al.* 1998).

Several studies have shown that fish accumulate contaminants such as DDT and mercury mainly through diet (Kidd *et al.* 1995b, Kidd 1996). Studies conducted in cold water lakes have shown that piscivorous fish have higher concentrations of these pollutants than fish that feed upon insects or other invertebrates (Kidd *et al.* 1995bKidd 1996, Rassmusen *et al.* 1990). These studies have shown that DDT and other organochlorine pesticides biomagnify in fish. Older and slower-growing fish have also been observed to have much higher levels of pollutants than fish that have much shorter lifespan and those that grow quickly (Connolly *et al.* 1988, Larson *et al.* 1991, Evans *et al.*1991).

Rowan and Rasmussen (1992) concluded in their study that fish lipid content, trophic status and trophic structure of the food web accounted for 59% of the variation in contaminant concentrations of 25 species in the Great Lakes. They also observed that physical characteristics of the system could also influence the bioaccumulation in fish. In particular, an increase in water temperature can significantly enhance DDT uptake by fish (Reinhert *et al.* 1974, Larsson *et al.* 1992). Larson *et al.* (1992) also observed that, as lake productivity (measured as chlorophyll α and phosphorus concentrations, as well as secci depth) increases, levels of DDT in northern pike (*Esox lucius*) decreased

Bioaccumulation of DDTs by avian species has been confirmed by the results of long-term monitoring programs conducted in the field. According to WHO (1989), field data show that the highest levels of DDTs are generally observed in terrestrial predators that feed primarily on other birds or aquatic predators that feed largely on fish. Species such as peregrine falcons (*Falco peregrimus*), kestrels, herons, kingfishers, grebes and bald eagles (*Haliaeetus leucocephalus*) tend to have the highest DDT residues (WHO 1989, Fasola *et al.* 1998).

Factors which influence bioaccumulation of DDTs in mammals include sex, duration of exposure, parental body burden (passed on to young through lactation), metabolic capacity to degrade DDT and lipid content (Woodwell and Craig 1971, Borrel and Aquilar 1987, WHO 1989, Larsson *et al.* 1992, Tenabe *et al.* 1994).

Humans can be exposed to DDT, DDE, and DDD primarily by eating food that contains small amounts of these compounds. In the United States, the average amount of DDT and DDE eaten daily in food in 1981 was 2.24 μg/day (0.000032 mg/kg/day) with root and leafy vegetables containing the highest amount (Gunderson 1995). Gunderson found that

meat, fish and poultry also contained very low levels of these compounds. In a study done by Kannan *et al.* (1992) the estimated daily intake of DDT and its metabolites in Vietnam was 19μg/person/day. This study found that fish, shellfish, prawn and crab were the primary route of DDTs to humans.

In areas where DDT is used for indoor spraying, as is the case in Botswana and many other developing countries, there is an added risk of increasing residue levels through inhalation. According to WWF (1998), an adult male resident is estimated to take up DDT from indoor application in the order of 1 μg per hour or 20 μg per day by inhalation. Bouwman *et al.* (1991) found that serum from household members living in DDT treated dwellings had significantly higher levels of DDT and its metabolite (140 μg/l) in Kwazulu, South Africa.

1.4 Toxicological effects

1.4.1 Acute toxicity

DDT is toxic to fish and other aquatic organisms (WWF 1998). Moderately severe poisoning through ingestion has been found to cause cardiac and respiratory failure, brain and nerve damage, and death (WHO 1989, UNEP 1991, ATSDR 1994). Other studies have shown that DDT causes liver damage and degeneration of the central nervous system (WHO 1989). DDT is readily absorbed through the gastrointestinal tract, with increased absorption in the presence of fat (ATSDR 1994). A one time administration of DDT to rats at doses of 50 mg/kg led to decreased thyroid function, a single dose of 150 mg/kg led to increased blood levels of liver-produced enzymes and changes in the cellular chemistry in the central nervous system of monkeys (ATSDR 1994).

Unless it is in solution, DDT is not readily absorbed through the skin (ATSDR 1994). Because of this, it has been found to be slightly to practically non-toxic to test animals through the skin, with reported dermal LD50s of 2500-3000 mg/kg in female rats (ATSDR 1994). Current thinking is that inhalation would result with DDT trapped in mucus secretions and swallowed by exposed individuals following tracheo-bronchial clearance of secretions by cilia (ATSDR 1994).

1.4.2 Chronic Toxicity

Studies done on experimental animals have shown that DDT causes chronic effects on the nervous system, liver, kidneys and the immune system (WHO 1979, ATSDR 1994). According to ATSDR (1994) effects on the nervous system observed in test animals include tremors in rats at doses of 16-32 mg/kg over 26 weeks, and tremors in mice at doses of 6.5-13 mg/kg/day over 80-140 weeks (ATSDR 1994). In mice, doses of 8.33 mg/kg/day over 28 days caused increased liver weight and increased liver enzyme activity (ATSDR 1994). Liver enzymes are commonly involved in detoxification of foreign compounds, so it is not clear whether increased liver enzyme activity in itself would constitute an adverse effect (ATSDR 1994). Immunological effects observed in test animals include reduced antibody formation in mice following administration of 13 mg /kg/day (ATSDR 1994). No immune system effects were observed in mice at doses of 1mg /kg/day (ATSDR 1994).

There is evidence that DDT causes reproductive effects in animals (Faber *et al.* 1973, Fry and Toone 1981, Johnstone 1996). Widespread declines in some predatory and fish-eating bird populations first came to light in the 1960s, because the DDT metabolite DDE

reduced the eggshell thickness of species such as peregrine falcon, brown pelicans, bald eagles and osprey (Carson 1962, Faber *et al.* 1973, Newton 1974, WHO 1989).

DDT is an endocrine disrupting chemical (ECD) (Colborn 1997, WWF 1998). An ECD is defined as "an exogenous agent that interferes with the production, release, transport, metabolism, binding, action or elimination of natural hormones in the body responsible for the maintenance of homeostasis and the regulation of development process" (Kavlock and Ankely 1996). When absorbed into the body, the chemical either mimics or blocks hormones and disrupts the body's normal functions. The disruption can happen through altering normal hormone levels, halting or stimulating the production of hormones, or changing the way hormones travel through the body, thereby affecting the functions that hormones control (Colborn *et al.* 1997). Some of the effects are diminished reproductive organs, which eventually prevent successful reproduction (Colborn *et al.* 1997). Evidence from a variety of sources has linked the bioaccumulation of DDTs to reproductive failures and other adverse effects in many wildlife species (UNEP 1991). Fry and Toone (1981) found that concentrations of DDT as low as 2ppm could feminize male birds by developing female reproductive organs in male embryos.

Available epidemiological evidence from studies does not show that reproductive effects in humans have occurred as a result of DDT exposure (ATSDR 1994). Wassermann *et al.* (1982) reported a significant association between maternal DDT blood levels and miscarriages. However, the presence of other chemicals such as PCBs in maternal blood, which may also account for the effect, makes it impossible to attribute the effect solely to DDT and its metabolites (Berkowitz 1995).

DDT has also been associated with breast cancer, teratogenic and mutagenic effects (WHO, 1979) in both humans and animals. However, the relationship between DDT levels and breast cancer has not been consistently observed (Safe 1995). Krieger *et al.* (1994) observed that the results from a nested case-control study of women from San Francisco area showed no difference in serum DDE or PCB levels between breast cancer patients and control subjects. They concluded that exposure to DDE and PCBs does not contribute to breast cancer. On the other hand, other researchers have found that the concentration of DDE is high in women with breast cancer relative to women without breast cancer (Falck *et al.* 1992, Wolff *et al.* 1993). Other researchers have suggested that hormone responsive breast cancer (but not hormone –unresponsive breast cancer) may strongly be associated with high levels of DDE in adipose tissue (fat) and plasma (WHO 1989, ATSDR 1994, Safe 1995). These results suggest that the more the DDE present in a woman's body, the higher the chance of developing breast cancer.

Laboratory and field studies have proved beyond doubt that DDT and its metabolites persist in the different environmental compartments. These studies have also shown that several factors govern this persistence. Reliable and credible environmental measurements, which can be used to compare factors governing the fate and persistence of DDTs, are pertinent. The following section describes the use of stable nitrogen and carbon isotope ratios to quantify and contrast biomagnification of organochlorines in aquatic food webs.

1.5 The Use of stable nitrogen and carbon isotope ratios in food web studies

Stable isotopes of nitrogen ($\delta^{15}N$) and carbon ($\delta^{13}C$) are currently used by several researchers to describe distinctive food-web pathways leading to the top trophic position.

Kling and fry (1992) have used isotopes of C and N to define the food web structure in Arctic lakes, comparing that structure with results based on more traditional analysis such as observations of predator-prey relationships, feeding strategies, and trophic energetics often summarized in food-web diagrams. The diagrams, as Paine (1988) describe them, ".... are subjective constructs strongly biased by the inability to observe the relevant taxonomic, spatial and temporal variations in trophic interactions". Stable isotope analysis, therefore, provides a more accurate picture of food web structure and function (Kling and Fry 1992).

Organisms preferentially excrete the lighter nitrogen isotopes (^{14}N) resulting in a stepwise enrichment of the heavier isotope (^{15}N) from producers to consumers (Minagawa and Wada 1984). According to Peterson and Fry (1987), the enrichment of the heavier isotope of nitrogen from prey to predator can be 3-5‰. The relative distribution of nitrogen isotopes (^{15}N) in organisms is indicative of trophic classification and can be used as a continuous variable to quantify trophic position (Peterson and Fry 1987). Trophic position has been useful in measuring pelagic trophic structure and omnivory (Fry 1988, Gu *et al.* 1994) and to differentiate between realized and potential trophic structure (Kling and Fry 1992, Kidd 1995b, Kidd 1996, Kidd *et al* 1998). Other advantages associated with this method is that it does not require assumptions of prey trophic levels, and as such would account for complexity and omnivory at lower trophic levels (Zaden *et al.* 1996). Trophic position has been used by several researchers to describe food web relationships and biomagnification of organic contaminants in organisms (Broman *et al.*1992, Rolff *et al.* 1993, Cabana and Rasmussen 1994, Kidd *et al.* 1995a, Kidd 1995b). Kidd *et al.* (1995b) found a significant relationship between trophic position and organochlorine concentrations

in biota from Lake Laberge and concluded that trophic position could be used as a predictor of chlorinated hydrocarbons in aquatic organisms.

Isotopes of carbon ($\delta^{13}C$), reflect an organism's diet and have been used to trace the source of carbon from prey to predator in complex food webs (DeNiro and Epstein 1978). These authors found that the fractionation rate of carbon isotopes is relatively low (0-3‰) compared to that of nitrogen isotopes. Carbon source, as defined by $\delta^{13}C$, has the advantage of providing a time-integrated measure of assimilated diet and an independent means of assessing the diet of a consumer that supplements information from stomach contents (Fry and Sherr, 1984).

The unique nature of the Okavango delta, and the general lack of data on food web relationships, provides an opportunity to apply stable isotope analysis for describing the interactions between producers and consumers within its food web. This will also allow the assessment on the impact of such interactions on the bioaccumulation of DDT and its metabolites. Isotopes of nitrogen ($\delta^{15}N$) and carbon ($\delta^{13}C$) are used to achieve these objectives. Stomach content analysis is also used to supplement this information.

Chapter 2

Factors Influencing the Biomagnification of DDT and its Metabolites in the Okavango Delta, Botswana

2.1 Introduction

The accumulation of DDT and other organochlorine pesticides by biota is influenced by several factors. Fish and invertebrates may accumulate DDT from water (bioconcentration) and from food (biomagnification). The relative importance of these pathways is governed by several factors among which are the water solubility of the chemical of interest (Bruggeman *et al.* 1984, Oliver *et al.* 1988, Collony *et al.* 1988) and trophic position of the organism (Oliver *et al.* 1988, Rasmussen *et al.* 1990, Kidd *et al.* 1995b). A decrease in water solubility and increase in trophic position would therefore, imply greater accumulation from food.

The accumulation of bioaccumulative pollutants such as DDT can be influenced by factors such as the length of the aquatic food web and its structure (Rasmussen *et al.* 1990, Cabana and Rasmussen 1994). These relationships have in the past been investigated through stomach content analysis, which Hyslop (1980) describes as only providing a "snapshot" of the fish's present diet while excluding averages that would integrate temporal and spatial variations. Boisclair and Leggett (1988) have also observed that stomach content analysis would not account for the discrepancies between the contents themselves and the already assimilated materials.

The ratios of ^{15}N to ^{14}N, expressed as $\delta^{15}N$, are used to characterize an organism's trophic position (Spies *et al.* 1989, Kidd 1996, Kidd *et al.* 1998, Kiriluk *et al.* 1995). The

advantage of this technique, as opposed to the conventional stomach content analysis, is that the isotope signatures integrate dietary habits over a period of years for slower growing species (Hesslein *et al.* 1993). Peterson and Fry (1987), showed that $\delta^{15}N$ is enriched by a factor of 3 to 5 ‰ from prey to predator, thereby providing a continuous variable with which to quantify and contrast biomagnification of organochlorines in aquatic food webs. Other factors known to contribute to bioaccumulation of organic contaminants include lipid content (Bentzen *et al.* 1996, Rasmussen *et al.*1990) and body size of the organism (Rasmussen *et al.*1990, Berg *et al.*1992).

DDT has been used in Botswana for over 30 years for tsetse control (Davies 1980) and malaria control (WHO 1984, Ministry of Agriculture 1992, 1994). Although DDT use for malaria control, which was only stopped in 1999 (Ministry of Health 1999), is confined to residential homes, there is a great potential for indoor house spraying to contribute to increased environmental contamination (WWF 1998). Felmate *et al.* (1998) have developed a "mass balance" model which accounts for the fate of DDT and other pesticides used for indoor house spraying. The model uses the concept of fugacity- the tendency for chemicals to move from one or more components of the environment to others (Mackay 1991). It is assumed that, due to its crystalline form, DDT would eventually fall off the walls and ultimately be transported outdoors. DDT could also be removed from homes through wash water, as surveys in Mexico indicate (WWF 1998). The model also assumes that other losses of DDT into the environment would be through evaporation.

To study the fate and persistence of DDT in the Okavango, I have chosen Xakanaka lagoon, where DDT has been used exclusively for tsetse control from the 1960's to the late 1970s (Davies 1980). Two other lagoons, namely, Ikoga and Guma have been chosen due to

their location in areas where DDT has been used for malaria control since the 1950s up until 1999, where there was a switch to synthetic pyrethroids (WHO 1989, Ministry of Agriculture 1992, 1994, Ministry of Health 1999). Samuchima area, which is not a lagoon but a portion of the Thaoge channel, has also been sampled, and was closest to the village than the rest of the locations sampled. This location also falls within the malaria sprayed areas. A review of literature on DDT and other pesticides used in the delta suggests that malaria locations chosen for this study could also be affected by DDT used for tsetse control (Davies 1980) as well. It should therefore, be noted that concentrations of DDT in Xakanaka are the only ones that can be solely attributed to tsetse control activities while the other three locations may be affected by DDT used for both tsetse and malaria control activities. The term "malaria areas" in this study therefore, refers to areas where DDT has been used for both malaria and tsetse control purposes, while "tsetse control area", means areas where DDT was used exclusively for tsetse control activities.

For comparison purposes, fish and zooplankton samples have also been collected from the Gaborone dam, located in the capital city of Botswana. DDT has not been used for any of the above purposes in this location. Although the area has moderate industrial activity, which could contribute to increased pollution, it is a non-agricultural area, and therefore, excludes the possibility for the dam to be contaminated by DDT use for the control of agricultural pests.

The objectives of this study are, therefore, 1) to measure concentrations of DDT in the Okavango delta in order to determine whether some areas of the delta would be more contaminated than others. It is hypothesized that DDT concentrations would be highest in biota from malaria areas (Guma, Samuchima, and Ikoga), lower in the tsetse control area

(Xakanaka) and lowest in Gaborone (control area where DDT has not been sprayed). 2) To examine relationships between inferred trophic position ($\delta^{15}N$), carbon source ($\delta^{13}C$), lipid content, length and weight (fish only) with DDT concentration in biota of the Okavango delta, Botswana. Biomagnification factors, defined as the slope of log DDT concentration versus $\delta^{15}N$ (Kidd *et al* 1998), will be compared with those of published studies in temperate regions.

2.2 Materials and methods

2.2.1 Sampling

Sampling of biota was conducted in July, August and December 1999. The inaccessibility of the delta by both road and boat prevented the collection of one species of fish from all the sampled locations around the delta to allow for a more extensive assessment of site to site differences in DDT concentrations. The small sample size in this study has also been due to these factors.

Fish species used in this study have been well studied by Skelton (1993). They include highly piscivorous fish such as the tigerfish (*Hydrocynus vittatus*) the completely omnivorous catfish (*Clarias gariepinus*), and the redbreast tilapia (*tilapia rendalli*), which is a herbivore. Other species studied include threespot tilapia (*Oreochromis andersonii*, nembwe, brownspot largemouth (*Serranochromis robustus, thumbergi*), bulldog (*Marcusenius macrolepidotus*), largemouth and spotted squeakers (*Synodontis macrostoma; nigromaculatus*) and the striped robber (B*rycinus lateralis*). Most of the fish were bought from fishermen in the sampled locations. Zooplankton was collected by horizontally towing a zooplankton net.

Total body weight and standard length of the fish were measured in the field before dissection and freezing. Samples were then frozen in solvent rinsed- aluminum foil and sealed in Whirlpak bags. Water samples were collected in air-tight 19-liter stainless steel cans and extracted in Botswana through the use of a solid phase extraction technique using 200mg ENV+ cartridges (Chromatographic Specialties) and GF/F filters.

2.2.2 Stomach Contents analysis

Fish stomachs were removed on the field and were either frozen for stable isotope analyses or preserved in 4% formalin for taxanomic analyses. All contents were wrapped in solvent rinsed aluminum foil, stored in cooler boxes filled with ice in the field, and then stored at –60°c in the laboratory until analysis. Fish stomach contents were identified with the aid of a dissecting microscope.

2.2.3 Stable isotope analysis

Zooplankton, fish muscle (excluding skin) and plant tissue were freeze dried. Both wet weight and dry weight (freeze dry weight) were measured. These were then ground with a mortar and pestle, weighed into tin capsules (approximately 200 μg). All samples were analyzed by the Earth Sciences Laboratory (University of Ottawa), in dual isotope mode for carbon and nitrogen, with automated CE instrument EA - 110 (elemental C and N analyzer) coupled to a Finnighan Mat delta ^{PLUS} IRMS with a Conflow II interface. For each run of 10 samples, two NIST (National Institute of Standards and Technology) isotopic standards for carbon (USGS -24 and NBS-21) and two for nitrogen (USGS-25 and IAEA-N-2) were used to help correct for drift in values between runs. A precision of $\leq 0.3‰$ (based on analysis of

replicates) is obtained using this analytical procedure, generally within 0.2‰ for nitrogen and 0.31‰ for carbon.

Stable nitrogen and carbon isotopes are expressed in "delta" notation (δ) and have units of parts per thousand (per mil) difference from the standard. $\delta^{15}N$ and $\delta^{13}C$ values are calculated with Pee Dee belemnite (PDB) and atmospheric N_2 standards respectively as follows:

$$\delta^{15}N \text{ or } ^{13}C \text{ per mil} = [(R_{sample}/R_{standard})-1] \times 10^3$$

Where R is $^{15}N/^{14}N$ or $^{13}C/^{12}C$

2.2.4 Organochlorine analysis

Samples were prepared for gas chromatographic analysis for organochlorine compounds using the methods as described by Kidd *et al.* (1998). Briefly, the dorsal muscle (including skin) and whole small fish samples of 5-10 g were homogenized with sodium sulfate, placed on cellulose extraction thimbles (pre-cleaned by Soxhlet for 6 hours) and extracted with dichloromethane (DCM) on Soxhlet apparatus for 16 hours. Surrogate recovery standards of polychlorinated biphenyl 30 (PCB30) and octachloronaphthalene (OCN) were added to the samples prior to extraction. The extract was rotary evaporated to approximately 2ml. Extractable lipids were determined gravimetrically with 1/10th of the extract, and the remaining lipids removed by gel permeation chromatography (GPC) on a column of biobeads SX-3 with Hexane/DCM (55:45). The GPC eluant containing organochlorine pesticides was collected and separated into three sub-fractions on a Florisil column (8g, 1.2% deactivated with HPLC grade water). The first fraction was eluted with 37 ml of hexane, the second with 38 ml of hexane-DCM (85:15), and the third fraction with 50

ml of dichloromethane (DCM). The first fraction contained all PCBs and most of the DDE while fraction 2 contained all hexachlorocychlohexane, chlordane and DDT compounds.

Water samples (approximately 1meter depth) were collected in 19liter airtight containers. Prior to collection, the jars were rinsed 3 times with wash-grade acetone/hexane. Water was extracted in Botswana following the methods as described by Helm *et al.* (2000). The water was pushed through a 142 mm glass fibre (1μm) and an ENV+ cartridge (Chromatographic Specialties) using a multi-plate filtration system. Water was allowed to flow at about 250ml/min. The ENV+ cartridges (200mg) were cleaned at the University of Ottawa prior to shipment to Botswana by eluting them with 15ml of dichloromethane (allowed to flow by gravitation) and dried through a vacuum dessicator. Cartridges were then wrapped with aluminum foil (pre-cleaned with wash grade acetone/hexane solvents and baked overnight at 200°C) and sealed in Whirlpak bags. Prior to extraction, ENV+ cartridges were conditioned with 5ml of trace grade methanol followed by another elution of the column with deionized water (DIW) to get rid of methanol. Samples were then spiked with PCB30 and OCN surrogates. The ENV+ cartridges and the glass fibre filters (GF/F) were then re-wrapped in solvent rinsed aluminum foil, sealed in Whirlpack bags and frozen immediately until shipment to the University of Ottawa where they were extracted for analysis. Field and laboratory blanks of ENV+ cartridges were carried along with the extraction apparatus in the field but not used for sample collection.

To extract organochlorines (OCs), ENV+ cartridges were unfrozen first to remove excess water. They were then eluted with 15ml of DCM followed by another 10ml to remove any remaining compounds from the column. The eluant containing organochlorine pesticides was rotary evaporated down to 2ml and separated into three sub-fractions on a Florisil

column (8g, 1.2% deactivated with HPLC grade water). The glass fibre filters used for the collection of suspended particulate material from water were cut into small pieces, mixed with sodium sulfate and extracted with DCM on a Soxhlet Extractor. Extracts were then treated as described above.

2.2.5 Sample Analysis

All florisil eluates (fish, plant, zooplankton, particulates and water) were rotary evaporated down to approximately 2ml and solvent exchanged to iso-octane, which was then super concentrated to a final volume of 1ml under a steady stream of high purity nitrogen. The final eluate was then analyzed by capillary gas chromatography (GC) with ^{63}Ni electron capture detection on a 30 m x 0.25 mm DB-5 column using Hewlett Packard©) GC 6890 and helium carrier gas 1.3mL/min; initial flow of 2.0 ml/min; constant pressure of 22.5 psi; 1psi=6.895 kPa and N_2 makeup gas. The temperature program used held samples at 80°C for 2 minutes; 10°C/min ramp to 110°C; 3°C/min to 280°C with a 5-minute hold. PCB and OC standards were run once every 10 injections and reagent blanks every 10 samples.

Extraction efficiencies for all quantified PCB congeners and OCs were determined by analysis of spiked standards (Environment Canada, CCIW, Burlington, ON). Average (±SD) surrogate recoveries for zooplankton, fish and plant material were 86±16 and 73±13 percent for PCB30 and OCN respectively. Surrogate recoveries for water were 76 ±6.2 and 98 ±13 % for OCN and PCB30 respectively. All data are reported without correction of extraction efficiencies. Organic contaminant peaks were identified and quantified using Hewlett Packard© Chemstation (1990-98) software based on their appearance at the same retention time (± 0.3s) as the standard. Reagent blanks were generally clear for DDTs. Low

background interferences were however noted for PCB congeners for both water and biota and Hexachlorobenzene (HCB) for water only (data not shown). The limit of detection (LOD) was then calculated according to Long and Winefordner (1983). (1983). Because of the absence of distinguishable peaks in the DDT blanks, limits of detection (LODs) for DDT were not calculated.

2.2.6 Data treatment and statistical analysis

Concentrations of each compound were calculated on a wet weight and lipid weight basis. Data were analyzed using Systat 7.0.1 (Wilkinson 1997). All means were calculated using $\log_{10}$ transformed data to equalize variances and normalize residuals. Comparisons of means were conducted using ANOVA (with Bonferroni aposteriori). Non parametric ANOVA tests (Kruskall-Wallis) were performed when assumptions of normality, homoscedasticity and independence of residuals were not met.

Percent lipid, weight and length were $\log_{10}$ transformed. Analysis of covariance (ANCOVA) was used to predict the effect of lipid on DDT or $\delta^{15}N$ on DDT or body size on DDT. The purpose of this analysis was to assess whether the effect of the independent variables was equivalent among the four locations namely, Guma, Samuchima, Xakanaka and Ikoga. Homogeneity of slopes was first tested followed by that of the intercepts. If the slopes or intercepts were significantly different ($p< 0.05$) then separate regression analysis for each location was done. Data were only pooled across lagoons if the slopes among lagoons were similar. Single regression analysis was used to determine which variable (lipid, $\delta^{15}N$, body size) best predicted the concentrations of DDT and its metabolites in each species

across all lagoons. A stepwise multiple regression analysis was conducted to determine which combination of the variables stated above, best predicted DDT and its metabolites.

2.3 Quality control and assurance

Procedural blanks containing 10 g of sodium sulfate (pre-cleaned at 600°C for 16h) were extracted with DCM on a Soxhlet apparatus and subjected to the same extraction procedures as the samples. These were run every ten samples. Field and laboratory blanks were also run for ENV+ cartridges used for the extraction of water samples.

2.4 Results

2.4.1 DDT concentrations in fish and other aquatic organisms

Table 2.1 and 2.2 summarizes mean concentrations (±SD) of DDT and its metabolites ($ng.g^{-1}$ wet weight) in different fish species, zooplankton, plant material and water from the Okavango delta and Gaborone dam (a control area) respectively. Table 2.3 and 2.4 summarizes pooled concentrations of DDT and its metabolites ($ng.g^{-1}$ wet weight) in fish species from the malaria and tsetse control areas respectively.

Total DDT (tDDT) concentrations ranged from 0.004 ng. L^{-1} in water to 18.80 ($ng.g^{-1}$ wet weight) in fish from the Okavango delta (Table 2.1, Appendix 2). The results recorded for water represent both freely dissolved concentrations and concentrations bound to suspended particulates. When fish data for the same species in the malaria areas were pooled and compared with data from the tsetse control area, log tDDT and log DDE concentrations were significantly different ($p < 0.05$). For instance, mean concentrations (±SD) of tDDT concentrations ($ng.g^{-1}$) in *Clarias* species were 7.18±7.34 in malaria areas while it was 3.00 ±

2.33 in the tsetse control area. The same was observed with *Tilapia rendalli* where the mean concentration of tDDT in malaria and tsetse areas were 2.56 ± 2.00 and 0.1 ± 0.1 respectively. Analysis of individual locations showed that fish from Samuchima area had the highest mean concentrations of tDDT (Table 2.5), which were significantly different from Guma, Ikgoga and Xakanaka lagoons ($p<0.05$).

Concentrations of tDDT in Gaborone dam (the control area) ranged from 0.37 in zooplankton to 37.43 ($ng.g^{-1}$ wet weight) in fish (Table 2.2, Appendix 2). Pooled data showed no significant differences in mean concentrations of tDDT between Gaborone and the Okavango delta (ANOVA, $p > 0.05$). Separate analysis of the data by each location in the delta, however, showed significant differences (ANOVA, $p < 0.05$) in mean concentrations of all lagoons but Samuchima ($p>0.05$). Contrary to tDDT, DDE concentrations (pooled data), were significantly different between the Okavango and Gaborone ($p=0.04$, Figure 2.1).

Species of a higher trophic level (Figure 2.7) had higher concentrations of tDDT ($ng.g^{-1}$ wet weight) than those of a lower trophic level (Table 2.1, Figure 2.2). This was comparable to the stepwise increase in $\delta^{15}N$ observed from the lower to the upper trophic level of this food web (Table 2.1). There was a significant relationship between $\delta^{15}N$ and log wet weight and log lipid normalized concentrations of tDDT (see equations below, Table 2.6, Figure 2.3). The relationship between $\delta^{15}N$ and wet weight concentrations of tDDT was stronger ($r^2 = 0.49$, Figure 2.2) than the lipid normalized concentrations ($r^2 = 0.16$, Figure 2.3). $\delta^{15}N$ and log percent lipid were correlated ($r^2 = 0.31$, $p = 0.0001$) (Table 2.3, Figure 2.4).

Log tDDT (ng.g^{-1} wet weight) = 0.21 (±0.03) δ^{15}N -1.12 (±0.22), r^2 = 0.49, p= 0.0001

Log tDDT $_{\text{lipid normalized}}$ (ng.g^{-1} lipid) = 0.09 (±0.04) δ^{15}N +1.57 (±0.24), r^2 = 0.16, p= 0.01

Log % lipid = 0.13 (± 0.033) δ^{15}N - 0.91 (±0.219), r^2 = 0.31, p = 0.0001

Log percent lipid was significantly related to log tDDT (r^2 = 0.57, p=0.0001) and DDE concentration (r^2 = 0.53, p=0.0001, Table 2.3, Figure 2.5). Slopes of regression (ANCOVA) for log transformed tDDT and DDE concentrations on percent lipid were not significantly different in Ikoga, Xakanaka, Samuchima but Guma (p>0.05). There were however, differences in intercepts (p<0.05). Percent lipid was not a significant predictor of tDDT in Guma (p > 0.05). However, it was a significant predictor of log tDDT and log DDE (p<0.05) in Xakanaka, Ikoga and Samuchima. Figure 2.6 compares percent lipid and its relationship with tDDT concentration on a wet and lipid weight basis among locations of the Okavango delta. Although not statistically significant, Samuchima lagoon had fattier fish than the rest of the locations in the delta, hence had significantly higher tDDT concentrations (on a wet weight basis), than Guma (ANOVA, p=0.002), Xakanaka (ANOVA, p=0.014) and Ikoga (ANOVA, p= 0.05).

The relationship between log lipid and concentrations of tDDT with pooled fish data showed a similar relationship as shown in the following equation:

Log tDDT = 1.03 (0.17) log lipid +0.1.6 (0..9), r^2= 0.53, p = 0.0001

The r^2 was however reduced by 4% (see Table 2.6, where all data was included).

Body size as determined by length and weight of fish, was significantly related to

$\delta^{15}N$ (ANCOVA $p < 0.05$), Table 2.3, APPENDIX 6&7). Log length was significantly related to log DDE and log tDDT in fish muscle ($r^2 = 0.36$, $p = 0.0001$ and $r^2 = 0.37$, $p < 0.0001$ respectively, Appendix 7). Fish weight was also significantly related to tDDT and DDE concentration ($r^2 = 0.30$, $p < 0.05$ and $r^2 = 0.29$, $p < 0.05$ respectively, Table 2.6, APPENDIX 6). The larger the fish, the higher the concentration of tDDT and DDE (Table 2.3, 2.4). Slopes of regression and intercepts for log tDDT versus log length and log weight were not significantly different among all locations ($p > 0.05$).

An assessment of $\delta^{15}N$, percent lipid and body size (length and weight) on tDDT concentration using multiple regression analysis showed a stronger relationship of log percent lipid and $\delta^{15}N$ on tDDT as shown in the following equation:

Log tDDT (ng.g-1 ww) = 0.78 (±0.14) **log lipid** + 0.14 (±0.04) $\delta^{15}N$ - 0.78 (±0.22), $r^2 = 0.67$ $p = 0.0001$ (log % lipid) and $p = 0.001$ ($\delta^{15}N$).

2.4.2 Food web relationships and DDT biomagnification

Figure 2.7 shows the mean $\delta^{15}N$ and $\delta^{13}C$ signatures of biota from the Okavango delta. This figure also shows the relationship between $\delta^{13}C$ and $\delta^{15}N$ of the Okavango food web and illustrates the predator-prey relationships or consumption patterns of this food web. The range in $\delta^{15}N$ mean (±SD) is 1.2 ± 0.6 to 8.3 ± 0.72 ‰ (Table 2.1). The $\delta^{15}N$ signatures define trophic levels of *Hydrocynus vittatus*, *Clarias gariepinus* and *Synodontis sp.* as the top predators with the highest range of $\delta^{15}N$ values of 6.85 to 9.29. *Hydrocynus vittatus* has the highest range of $\delta^{15}N$ values from 7.5 to 9.3 ‰ followed by *Clarias gariepinus* (6.85 - 8.97 ‰) and *Synodontis sp.* (7.80 - 7.94 ‰) This suggests that these species feed mainly on other

fish such as *Brycynus lateralis* and *Oreochromis andersonii* (mean $\delta^{15}N$ of 6.4 and 6.8 ‰ respectively). *Serranochromis sp.* had a wider range of $\delta^{15}N$ values from 5.04 to 9.0 ‰, reflecting a wider range of prey items. For instance, *S. robustus* preys on *Synodontis sp.* (Skelton 1993) while *S. angusticeps* preys on *Brycinus sp*, and *thumbergi* on insects, snails and fish (Skelton 1993). The next level is occupied by species feeding mainly on invertebrates such as *Oreochromis andersonii, Brycinus lateralis, Mormyrus lacerda* and *Marcusenius macrolepidotus* with $\delta^{15}N$ values ranging from 5.3 to 7.8 ‰ (see APPENDIX 2). *Tilapia rendalli* was shown in this study to be a herbivore with mean (± SD) $\delta^{15}N$ of 3.1 ± 1.4 ‰ and it occupied about the same trophic level as zooplankton (3.21 ‰). Plant material and particulate organic matter, with mean (±SD) $\delta^{15}N$ values of 1.4 ± 0.3 and 1.2 ± 0.6 ‰ respectively, occupied the lowest trophic levels.

$\delta^{13}C$ signatures of biota from the Okavango delta ranged from -27.20 to -18.84 ‰ and, even though the range was narrow, it permitted some separation of fish species (Figure 2.7). $\delta^{13}C$ signature of *Hydrocynus vittatus* (-23.61± 0.94) was generally similar to that of *Brycinus lateralis* (-23.8 ± 2.9‰), and *Mormyrus lacerda* (-23.84‰). $\delta^{13}C$ of *Synodontis sp.* (-21.91 ±1.2 ‰) was similar to that of *Tilapia rendalli* (21.12 ± 2.0 ‰) and *Marcusenius macrolepidotus* (20.8 ‰), suggesting that *Hydrocynus* species fed mainly upon these species. This was also supported by the isotope values for fish prey items obtained from the stomach of *Hydrocynus sp.* where $\delta^{15}N$ values were 5.95‰ and $\delta^{13}C$ values -25.27 ‰ (Table 2.7). The $\delta^{13}C$ values for plant material (-23.7 ‰) was consistent with that of *Tilapia rendalli* (-21.2 ± 2.1) confirming plant material as a major diet for this species.

Table 2.7 shows $\delta^{15}N$ and $\delta^{13}C$ of predator and prey in selected fish stomachs in order to identify the prey items even further. Prey items and their percent occurrence in fish stomachs

are shown in APPENDIX 3 and 4 respectively. Stomach content items for *Tilapia rendalli*, were mostly dominated by aquatic plant stems, leaves, periphyton and algae (APPENDIX 4, Table 2.7). This was consistent with dietary studies done previously on this species (Skelton 1993). $\delta^{15}N$ values for this species ranged from 1.3 to 4.85 ‰. One fish from this species had aquatic insects, which explains the high $\delta^{15}N$ value in this particular fish (4.5 ‰). Stomach items for the catfish (*Clarias gariepinus*), a highly omnivorous fish, showed a diet dominated by a variety of items which included small fish, plant material, flies, and aquatic and terrestrial insects. $\delta^{15}N$ signatures for prey fish from the stomachs were 5.05 ‰ (Table 2.7), matching those of *Mormyrus lacerda* and *Marcusenius macrolepidotus*, and suggesting them as the prey species for the catfish in the Okavango. The tigerfish (*Hydrocynus vittatus*), known to become exclusive fish feeders when they attain a length of 90 to100 mm (Skelton 1993) showed these feeding characteristics in this study as well, with the smallest tigerfish having aquatic insects and fish diet and the largest and oldest exclusively feeding on fish. This study confirmed these feeding habits with the $\delta^{15}N$ values increasing from the smallest (7.57 ‰) to largest size (9.29 ‰) showing a shift in diet. $\delta^{15}N$ signatures for insect prey items ranged from 3.18 - 3.73 ‰ therefore, providing supportive evidence that the smallest of this species ($\delta^{15}N$ = 7.5‰) feeds on insects.

A comparison of mean $\delta^{15}N$ values for *Clarias gariepinus* between Gaborone and Okavango showed higher average (± SD) values from the latter (10.29 ± 3.01 compared to 8.1 ± 0.78 ‰) than in the delta (8.10 ± 0.78, Tables 2.2, 2. 5, Figure 2.8). *Oreochromis* species from Gaborone dam had mean $\delta^{15}N$ values even higher than the piscivorous *Hydrocynus* species in the Okavango delta (10.10 ± 0.2 and 8.3 ± 0.72 ‰ respectively).

2.5 Discussion

2.5.1 DDT concentrations in fish and other aquatic organisms

The occurrence of DDT and its metabolites in the Okavango delta is to be expected given its previous use for the control of malaria and sleeping sickness disease vectors (Davies 1980, WHO 1984, Merron 1992, Mosweunyane 1994 Ministry of Agriculture 1992, 1994). The low levels of these compounds in the delta compared to non-DDT use areas such as Gaborone, were however not expected.

Concentrations of DDT and its metabolites in water were much lower than the levels previously found in the neighboring Zimbabwean Lake McIlwaine (Mhlanga and Madziva 1990). The hydrophobic nature of DDT, with a solubility of 3.1μg. L^{-1} (Mackay 1991) suggests its rapid movement from water to other environmental compartments such as biota or the atmosphere (Mackay 1991, Wania and Mackay 1993). Levels of DDT in the particulate phase of the water were 4 times higher than in the dissolved phase. Because suspended particulates have a high sorptive capacity for DDTs (Moriarty 1975, WHO 1989, Mackay 1991), this behavior may therefore explain the difference in concentration between the dissolved and particulate phase of the water.

Concentrations of tDDT were significantly higher in one out of three locations in the malaria control areas (Samuchima) compared to Xakanaka (tsetse control). Although DDT used for malaria control may have contributed to the higher levels in Samuchima, compared to Xakanaka where DDT contributions can solely be attributable to DDT use for tsetse control alone, several other factors are of equal importance. Because DDT has been sprayed in this location for tsetse control as well, this could have contributed to the increase in DDT levels (Davies 1980).

Besides the high levels in Samuchima, the results in this study generally reflect that DDT concentrations were not related to area of use, but rather to biological factors. Fish from Samuchima were generally fattier hence the detection of high DDT concentrations in comparison with other lagoons in the delta (Table 2.1). It can also be seen from Table 2.4 and 2.5 that species such as the catfish, and tiger fish, both of which had higher $\delta^{15}N$ values, were present in Samuchima, and their $\delta^{15}N$ values were higher in Samuchima compared to Xakanaka.

Trophic level, expressed by $\delta^{15}N$ also explained 42 % of the variability of tDDT concentrations in biota from the Okavango (this study). Fish at a higher trophic position such as *Hydrocynus vittatus*, *Clarias sp.* and *Synodontis sp.*, also had high tDDT concentrations. Previous studies have also shown that trophic position was significantly related to contaminant concentrations (Berg *et al.* 1992, Douthwaite and Tingle 1994, Kidd *et al* 1995b, 1998).

DDT concentrations in fish collected from Gaborone (the control area), were significantly higher than concentrations in fish from the Okavango delta (Table 2.2, Figure 2.1). Interestingly, ΣDDT (*p,p'*-DDT, *o,p'*-DDT) in Gaborone was on average 29 times lower than the DDE metabolite reflecting an old source of DDT in this area. Log percent lipid and trophic position in Gaborone explained 84% ($p=0.004$) of the variability and 85% ($p=0.003$) respectively. Because DDT has not been used in the Gaborone area, and because of the higher DDE/DDT ratio, DDT in this area may be attributed to atmospheric deposition. The catchment area for Gaborone dam, which includes water from areas outside Gaborone, may also draw fish from locations where DDT may have been used and as such may contribute to the higher DDT residues. Factors such as lipid, body size and trophic level seem to have

influenced DDT biomagnification in the dam. Figure 2.8 shows that $\delta^{15}N$ values were higher in Gaborone than the rest of the locations in the Okavango delta. Because Gaborone is a moderately industrial area, the high $\delta^{15}N$ values are also suggestive of sewage inputs (Spies *et al.* 1989).

By comparison with DDT concentrations in other parts of the world, fish from the Okavango delta have very low residue values. DDT concentrations from other countries in Southern Africa show values that are higher than in the Okavango. Average tDDT values (wet weight basis) for Tilapia in lake Mcllwaine, Zimbabwe were 75 $ng.g^{-1}$ (Mhlanga and Madziva 1990), making them over 50 times higher than the same species in the Okavango. In the same study, concentrations of tDDT in catfish and tigerfish had average values of 97 and 128 ($ng.g^{-1}$wet weight) respectively, making their concentrations 19 and 17 times higher than the concentrations in the Okavango delta. Figure 2.9 shows a comparison of tDDT concentrations in fish ($ng.g^{-1}$ lipid weight) species from the Okavango, Lake Kariba and Lake Mcllwaine. It is evident from this Figure that the levels in the Okavango are very low. The low tDDT values observed in the Okavango delta are somewhat the same as the levels in Lakes Nakuru in Kenya (Greichus *et al.* 1978) and Malawi (Kidd *et al.* 2000). A recent study by Kidd *et al.* 2000 in Lake Malawi showed low average tDDT residue values in similar species. The *Clarias and oreochromis* species in Lake Malawi had average levels as low as 1. 4 and 0.5 $ng.g^{-1}$ wet weight respectively, making them 3 and 5 times lower than the levels in the Okavango delta. *Synodontis* species, however, had tDDT levels that were 7 times higher than the levels in the same species from the Okavango delta (Kidd *et al.* 2000).

Comparisons of DDT concentrations between the Okavango and lakes in colder regions also show the same trend. High levels of DDT in these areas are surprising

considering the lack of DDT use since the 1970's. In 1990, De Vault et al. (1996) showed that tDDT concentrations were on average 1900 $ng.g^{-1}$ in 1976 and 840 $ng.g^{-1}$in 1990 in Laurantian Great Lakes. Lakes in the Yukon Territory also have levels of tDDT that are higher than the Okavango delta (Kidd 1996, Kidd *et al.* 1998).

The low levels of DDT concentrations in the Okavango can be explained by several factors. Some of the factors include annual quantities of DDT used in the delta, soil and sediment structures, and most importantly the chemical properties of DDT and its metabolites. Quantities of DDT used in Botswana are relatively low compared to those used in other countries. In Zimbabwe for instance, 300 tons of DDT were sprayed annually in 1982, about 180 in 1984, 170 in 1986 and 80 tons in 1988 (Larson *et al.* 1995, Mattheissen 1985). These quantities are on average 18 times higher than quantities used for malaria control in Botswana. This may contribute to the low levels in fish from the Okavango delta. In colder regions quantities of DDT previously used are not comparable to those used in Botswana. In New Brunswick Canada, DDT quantities used for the control of spruce budworm from 1952 to 1967 were 5 746 422 kg (Environment Canada. 1997). These quantities are not comparable to the maximums of 10 tons of DDT used for malaria control in Botswana (Ministry of Agriculture 1992, 1994), hence the high levels of DDT which persist in Canadian environments could be due to this factor. It is however, acknowledged that, fish in arctic lakes tend to live longer and grow more slowly and as such may accumulate more contaminants due to the longer food chains than fish in tropical regions (Kidd *et al.* 1995b).

Partition coefficients, such as log octanol/water (K_{OW}), a measure of hydrophobicity, or the tendency of a chemical to partition out of the water, lipid-water partition coefficients

(K_{FW}), air/water partition coefficients and organic/carbon (K_{OC}) are but a few of the important factors which could be greatly influencing the fate of DDT in the Okavango (Mackay 1991). Chemicals with K_{ow} values greater than 5 are highly hydrophobic, and DDT is among them. Because of this hydrophobicity, DDT would easily partition into the soil, biota or the atmosphere. Studies on the hydrology and sedimentology of the Okavango delta showed that sediments in the delta are sandy with very low organic matter (Garstang *et al.* 1998). This, coupled with climatic conditions in the delta such as high annual temperature, and the amount of DDT applied, would have an influence on its persistence in soils or sediments (Liechtenstein *et al.* 1959) and availability of DDT to biota. Because of the sandy nature of the delta, some DDT would either volatilize into the atmosphere or be washed away to other locations, therefore reducing uptake by organisms in the immediate environment, which most often takes a long time (Larson *et al.* 1995).

2.5.2 *Food web relationships and DDT biomagnification*

A positive relationship was evident between $\delta^{15}N$ and tDDT concentrations. The use of stable isotopes of nitrogen in this study has helped to describe the biomagnification of DDT in the Okavango food web, and has provided a quantifiable measure of trophic level. An increase in $\delta^{15}N$ with increasing DDT concentrations indicated that trophic level plays an important role in the movement of contaminants through the Okavango food web. A rather weak relationship between lipid-normalized tDDT and $\delta^{15}N$ was however observed. Despite this weak relationship, this result is an indication that trophic position has a significant effect over and above that due to lipid alone.

Trophic level was also significantly related to log percent lipid in other related studies in temperate regions (Kiriluk *et al.* 1995, Kucklick 1980, Kidd *et al.* 1995a, 1995b, 1998), and in all cases, $\delta^{15}N$ versus contaminant concentration was weaker on a lipid-adjusted concentration basis relative to the wet-weight basis. Kiriluk *et al.* (1995) found a strong correlation between $\delta^{15}N$ and wet weight concentrations of DDE, mirex and total PCB than they did in the lipid normalized concentration of the contaminants. If lipid adjusted tDDT correlates to $\delta^{15}N$, then there is evidence for biomagnification to supercede equilibrium partitioning on lipid. Kidd *et al.* (1995a) found a significant relationship between $\delta^{15}N$ on both the log wet weight and lipid corrected concentrations of DDT, toxaphene and hexachlorocyclohexane in biota from Lake Laberge, Yukon Territory. They concluded that the slope of these regressions may be related to the biomagnification capability of specific lipophilic contaminants. Kidd *et al.* 1995a, also observed that the weaker relationship between trophic level and log lipid adjusted DDT concentrations relative to wet weight, signified that $\delta^{15}N$ explained a greater proportion of the variation in contaminant concentrations than percent lipid.

Species with high $\delta^{15}N$ values such as the *Hydrocynus vittatus* (tigerfish) and *Clarias gariepinus* (catfish) had higher mean tDDT concentrations than *Tilapia rendalli*. In Zimbabwe, Berg *et al.* 1992, found that the tigerfish in Pongolo Flood Plain had about 3 to 10 times higher DDT concentrations than the redbreast tilapia. A similar comparison can be made in this study where the mean concentrations of tDDT in redbreast tilapia are 6 times lower than in the tigerfish, and the catfish.

Figure 2.10 shows the relationship between $\delta^{15}N$ signatures of fish muscle and log tDDT concentrations ($ng.g^{-1}$ wet weight) between lakes in the Yukon Territory and lagoons

in the Okavango Delta. There were no significant differences in the slopes ($p>0.05$), indicating similar biomagnification factors for DDT concentrations in tropical and temperate regions.

In this study, percent lipid was also a significant predictor of tDDT levels in the invertebrates, plant material and fish of the delta. Fish with the highest percent lipid such as *Hydrocynus vittatus*, *Clarias gariepinus*, *Synodontis sp.*, *Mormyrus lacerda* (western bottlenose), and *Marcusenius macrolepidotus* (bull dog), had the highest tDDT concentrations which can be explained by the high percent lipid. Log percent lipid, successfully predicted tDDT levels in the Okavango delta as it has in several other studies undertaken in temperate (Rasmussen *et al.* 1990, Larsson *et al* 1991, 1993, Kidd 1996) and tropical regions (Mhlanga and Madziva 1990, Berg *et al.* 1992).

In Lakes Mcllwaine and Kariba, Zimbabwe, Southern Africa, fish with the highest lipid content, such as the tigerfish, had the highest tDDT concentrations (Mhlanga and Madziva 1990, Berg 1992, Douthwaite and Tingle 1994). Douthwaite *et al.* (1981) also found that fattier fish in the Okavango delta contained more residues of the pesticide endosulfan. It was observed that visceral adipose had both the highest fat and residue levels of the pesticide. *Brycinus lateralis* (striped robber), a relatively small fish (Table 2.2) was extracted whole due to its body size. This species was, however, excluded in the statistical analysis because of a very high percent lipid (relative to its body size), which interfered with the overall normal distribution of the data. The high percent lipid was believed to be due to the extraction of whole body as opposed to the dorsal muscle as was the case with other species.

Although this study did not focus on a single species and as such, the sample size on the fish species is small compared to similar studies, the results on the effects of lipid on the bioaccumulation of DDT are comparable. Larson *et al* (1996) found fat content to be significantly related to persistent pollutants in salmon population (*Salmo salar*) in the southern Baltic Sea. He found that tDDT and ΣHCH were significantly related to fat on muscle tissue ($p< 0.05$, $r^2 =0.53$). A significant relationship between percent lipid and tDDT was also observed by Benzen *et al.* (1996) in their study investigating the role of lipid and food web structure in lake trout ($p<0.05$, $r^2 = 0.62$). The relationship between percent lipid and DDT concentrations was shownn in fish from lakes of the Yukon Territory (Kidd 1996). The intercepts of these regression models are higher than those of the Okavango, reflecting higher DDT concentrations in these locations. Figure 2.11 compares regression slopes of log tDDT on log lipid concentrations between the Okavango lagoons and lakes in sub-arctic regions.

The findings from these studies show that percent lipid can be used to predict concentrations of DDT and other organochlorine pesticides irrespective of the geographic locations. The results however need to be interpreted with caution as several factors may influence the uptake of pollutants. These include contaminant loadings, food web structure, (Benzen *et al.* 1996), fat quality (Ewald and Larson 1994), and foraging strategies by individual fishes (Larson *et al.* 1996).

Body size as determined by length and weight of fish, were significantly related to $\delta^{15}N$. These results are corroborated by the findings of Bootsma *et al.*(1996), who attributed an increase of $\delta^{15}N$ with size in pelagic fish (*Engraulicyprus sardella*) in Lake Malawi to a shift in trophic level. Because larger individual fish tend to feed at higher trophic levels, they

have higher $\delta^{15}N$ values, and consequently higher tDDT levels. The ability of body size to predict DDT concentrations was however low compared to both lipid and trophic position. On the whole, the best model (multiple regression) for predicting tDDT in the Okavango Delta showed significant effects of trophic position and percent lipid.

Stable isotope results were consistent with known feeding habits of the species analyzed in this study. Tigerfish (*Hydrocynus vittatus*) is a top predator and is known to feed mainly on other fish (Skelton 1993, this study). Both stable isotope results and stomach content analyses confirmed this feeding habit. The sharptooth catfish (*Clarias gariepinus*) is well known for its scavenging habits, grabbing on any organic food from plants to fish and small mammals (Skelton 1993). This was confirmed in this study where stomach content analysis showed a variety of food items such as plants, insects, detritus, flies and small fish, reflecting a highly omnivorous feeding behavior. *Tilapia rendalli* relied heavily on plant material and algae. A few insects were also found in some of the stomachs of this species. These findings are consistent with other studies undertaken on the feeding habits of this species using limited gut content analysis (Merron and Bruton, 1990, Skelton 1993). *Oreochromis sp.* consumed invertebrates and small insects, while *Synodontis species* diet was mainly insects and fish (Skelton 1993, this study). *Serranochromis sp.* diet was dominated by fish and mollusks, these finding corroborate those of Merron and Bruton (1990) and Skelton (1993).

The $\delta^{13}C$ values in this study complemented the relationships observed from the stomach content analysis of the species studied from the Okavango delta. This information has helped to describe the significance of diet composition in relation to DDT concentrations among the different species (Borgmann and Whittle 1992).

2.6 Conclusions

The objectives of this study were; 1) to measure concentrations of DDT in biota from the Okavango delta, to determine whether some areas of the delta would be more contaminated than others as a result of previous DDT use and, 2) to examine relationships between trophic position ($\delta^{15}N$), carbon source ($\delta^{13}C$), lipid content, length and weight (fish only) with DDT concentration in biota of the Okavango delta, Botswana.

Concentrations of tDDT were highest in areas that have been treated for both malaria and tsetse control as it was hypothesized. Xakanaka lagoon, located in a tsetse control treatment area, had the lowest tDDT concentrations. Samuchima area had higher levels of tDDT concentrations compared to the rest of the sampled areas in the delta. Gaborone dam, located in a non-DDT use area, on the contrary had the highest DDT concentrations.

Fish samples from malaria areas had higher lipid content, body size and $\delta^{15}N$ values. All these could have influenced concentrations of DDT rather than the history of application and the amount of DDT applied.

DDT levels in fish and water from the Okavango were low compared to levels in nearby locations such as Lake Kariba in Zimbabwe and in colder regions. Factors such as quantities of DDT used, the high temperatures in Botswana, and the chemical properties of DDT may have influenced the low levels of the pesticide. Although concentrations of DDT in other compartments of the environment such as air and sediments have not been measured, there is greater possibility that DDT levels could be higher in the air, and therefore could be transported to other DDT non-use areas. DDT used in the delta could therefore contribute

significantly to contamination of areas far away from the sources of DDT. The detection of higher concentrations of DDT in Gaborone can be linked to this phenomenon.

This study demonstrates the extent to which $\delta^{15}N$, $\delta^{13}C$, percent lipid, and body size (fish length and weight) can be used to predict DDT concentrations in biota from the Okavango delta. Energy sources for the species studied, and how they relate to contamination transfer have been aided by the application of $\delta^{13}C$ and stomach contents analysis.

The use of both $\delta^{15}N$ and $\delta^{13}C$ in this study has helped to determine the relationship of the Okavango delta food web and DDT biomagnification. Trophic level, as described by $\delta^{15}N$, explained trophic position of the individual fish species studied in the delta, and provided a measure of biomagnification potential of DDT. On the other hand, $\delta^{13}C$, or carbon source, helped to differentiate between food sources that could potentially transfer DDT concentrations to top predators. For instance, fish that consume other fish, such as the *Hydrocynus vittatus*, *Clarias gariepinus* and *Synodontis sp.* had higher DDT concentrations than herbivores like the *Tilapia rendalli*.

Larger fish, with a higher percentage of lipid had the highest DDT concentrations. Although body size (length and weight) can also be used to predict tDDT concentrations, there are limitations with this variable in that DDT concentrations in smaller fish species may be underpredicted, while larger ones my be overpredicted. Hence for this data set, percent lipid and $\delta^{15}N$ served as the best predictors of concentrations of DDT and its metabolites. These findings are consistent with results of studies carried out in temperate and other tropical regions.

TABLE 2.1: Mean $\delta^{15}N$, $\delta^{13}C$ (‰), weight (g), length (mm), % lipid and concentrations DDT and its metabolites in water (ng. L^{-1}), in fish, zooplankton, plant material and particulate organic matter (ng.g^{-1} wet weight) from the Okavango delta. Data are reported as mean ± SD

SPECIES	*n*	$\delta^{15}N$	$\delta^{13}C$	WEIGHT (g)	LENGTH (mm)	% LIPID	*p,p'*-DDE	*p,p'*-DDD	*o,p'*-DDD	*p,p'*-DDT	ΣDDT	tDDT
Fish												
Serrachromis[1]	9	7.6 (± 1.1)	-24.2 (±1.7)	390 (± 190)	353 (± 73)	0.7 (± 0.4)	1.12 (±0.91)	0.42 (±0.39)	0.12 (±0.11)	0.47 (±0.47)	0.62 (± 0.35)	1.9 (± 1.7)
Tilapia rendalli	8	3.1 (± 1.4)	-21.2 (± 2.0)	284(±163)	205 (± 51)	0.5 (± 0.50)	0.89 (±1.17)	0.47 (± 0.72)	0.05 (±0.04)	0.41 (±0.64)	0.43 (± 0.67)	1.33 (± 1.85)
Clarias gariepinus	5	8.1 (±0.78)	-22.6 (± 2.0)	1316 (± 523)	472 (± 75)	3.1 (± 4.5)	3.11 (± 2.77)	1.57 (±0.88)	0.93	1.98 (± 2.57)	2.21 (± 3.03)	5.51 (± 5.80)
Marcusenius macrolepidotus	1	5.4	-20.8	220	240	10	3.7	0.98	0.45	0.99	1.44	6.17
Synondotis[2]	2	7.9 (± 0.10)	-21.9 (± 1.2)	193(±1.6)	207.57 (± 3.5)	4.1 (± 1.27)	5.12 (±0.52)	5.12 (±0.52)	0.67	1.89 (±0.40)	2.84 (±1.7)	8.04 (±1.7)
Oreochromis andersonii	5	6.8 (± 0.80)	-26.1 (± 1.4)	113 (± 90.9)	229 (± 20.7)	1.7 (±0.91)	1.56 (± 1.53)	0.31	0.20 (± 0.24)	1.08 (± 1.46)	1.09 (± 1.24)	2.78 (± 2.82)
Hydrocynus vittatus	4	8.3 (± 0.72)	-23.6 (± 0.94)	935 (± 996)	366 (± 103)	4.1 (±5.1)	3.79 (± 4.2.3)	0.95 (± 1.07)	0.53 (± 0.57)	3.43 (± 2.64)	3,78 (± 3.0)	7.50 (±8.07)
Brycinus lateralis	2	6.4 (± 0.12)	-23.8 (± 2.9)	12 (± 2.8)	83(± 3.5)	12.1 (± 2.5)	2.4 (± 2.9)	0.57(± 0.69)	ND	0.86 (± 1.10)	0.86 (± 1.10)	3.8 (± 4.7)
Mormyrus lacerda	1	5.3	-23.8	550	270	5.7	3.90	0.53	0.93	2.97	3.9	8.33
Zooplankton	1	3.2	-22.3			0.4	0.02	0.06	-	0.09	0.09	0.18
Plant material	2	1.4 (± 0.3)	-23.7		-	0.07 (± 0.04)	0.07 (± 0.01)	-	0.01	-	0.14 (± 0.05)	0.14 (± 0.05)
Water	4				-		0.03 (± 0.02)	0.001	0.002 (0.001)	0.04 (±0.04)	0.04 (± 0.04)	0.04 (± 0.05)
Suspended particulates	4	1.2 (± 0.6)	-25 (±1.23)	-	-	-	0.04 (± 0.04)		0.03 (± 0.03)	0.09 (± 0.07)	0.11 (± 1.0)	0.13 (± 014)

[1] *S.thumbergi, angusticeps and robustus jallae*
[2] *S. vanderwaal, and nigromaculatus*

TABLE 2.2: Mean $\delta^{15}N$, $\delta^{13}C$ (‰), length (mm), weight (g), % lipid and concentrations DDT and its metabolites (ng.g^{-1} wet weight) in fish and zooplankton from Gaborone Dam. Data are reported as mean ± SD.

SPECIES	*n*	$\delta^{15}N$	$\delta^{13}C$	LENGTH	WEIGHT	% LIPID	*p,p'*-DDE	p,p'-DDD	*o,p'*-DDD	*pp'*-DDT	ΣDDT	tDDT
Clarias gariepinus	3	13.93 (±0.33)	-20.15 (±1.47)	393 (±37)	815(±202)	2.3 (±0.987)	24.72 (±11.93)	0.28 (±0.16)	0.03	0.71 (± 1.14)	0.72 (1.26)	24.95 (±11.80)
Oreochromis andersonii	3	10.1 (±0.26)	-18.67 (±0.91)	227 (± 15)	302 (±64)	0.37 (±0.25)		0.18 (±0.14)		0.13 (±0.06)	0.15 (±0.09)	1.55 (±1.31)
Caprinus capio	1	19.9	-19.8	-21.8	520	2.1	7.44	0.06	-	-	-	7.50
Zooplankton	1					0.13	0.26	0.07	-	0.05	0.05	0.369

TABLE 2.3: Mean $\delta^{15}N$ (‰), length (mm), weight (g), percent lipid and concentrations of DDT and its metabolites ($ng.g^{-1}$ wet weight) in fish from malaria areas (pooled data from Ikoga, Samuchima and Guma). Data are reported as mean ± SD.

SPECIES	n	($\delta^{15}N$)	LENGTH	WEIGHT	% LIPID	*p,p'*-DDE	*p,p'*-DDD	*o,p'*-DDD	*p,p'*-DDT	ΣDDT	tDDT
Clarias gariepinus	3	8.4 (±0.5)	486 (±56)	1651 (±303)	3.77 (±5.84)	3.97 (±3.52)	0.95	0.93	2.60 (± 2.75)	2.91 (±3.29)	7.18 (±7.34)
Serrachromis[1]	7	7.98 (±0.64)	281 (52)	467 (±133)	0.77 (± 0.35)	1.399 (±0.833)	0.48 (±0.38)	0.11 (±0.11)	0.53 (±0.46)	0.58 (±0.52)	2.39 (±1.58)
Tilapia rendalli	4	4.38 (±0.34)	245 (±41)	406 (±144)	0.85 (±0.55)	1.71 (±1.17)	1.29	0.05 (±0.04)	0.67 (±0.76)	0.70 (±0.79)	2.56 (±2.00)
Hydrocynus vittatus	2	8.73 (±0.80)	395 (±148)	1300 (±1556)	6.75 (±6.86)	6.25 (±5.20)	1.71 (±1.02)	0.63	4.85 (±1.318)	6.71 (±5.32)	18 (±13.12)
Mormyrus lacerda	1	5.3	270	550	5.7	3.90	0.53	0.93	2.97	3.9	8.33

[1] *S.thumbergi, angusticeps and robustus jallae*

TABLE 2.4: Mean $\delta^{15}N$ (‰), length (mm), weight (g), percent lipid and concentrations DDT and its metabolites ($ng.g^{-1}$ wet weight) in fish from a tsetse control area (Xakanaka). Data are reported as mean ± SD.

SPECIES	*n*	WEIGHT	LENGTH	($\delta^{15}N$)	% LIPID	*p,p'*-DDE	*p,p'*-DDD	*o,p'*-DDD	*p,p'*-DDT	ΣDDT	tDDT
Clarias gariepinus	2	813 (±264)	450 (±120)	7.65 (±1.14)	2.1 (±2.35)	1.84 (±0.7)	2.19		0.12	0.12	3.000 (±2.33)
Serrachromis	2	12 (±21)	15 (± 46)	6.19 (±1.63)	03 (±0.14)	0.14 (± 0.28)	(±0.02)		(±0.04)		0.2
Tilapia rendalli	4	162 (±41)	164 (±8.08)	1.778 (±0.33)	0.23 (±0.13)	0.07 (±0.06)	0.04 (±0.04)	-	0.02 (±0.014)	0.02 (±0.14)	0.10 (±0.09)
Hydrocynus Vittatus	2	569.2 (±157)	337.5 (±81)	7.9 (±0.44)	1.4 (±1.56)	1.3 (±1.55)	0.19 (±0.21)	0.13	0.58	0.71	1.87 (±2.26)
Oreochromis andersonii	5	357 (±98)	229 (±27)	6.90 (±0.76)	1.70 (±0.94)	1.56 (±1.53)	0.31	0.2 (±0.24)	1.08 (±115)	1.16 (±117)	2.78 (±2.82)
Marcusenius macrolepidotus	1	220	240	5.3	10	3.7	0.98	0.45	0.99	1.44	6.17
Synondotis[2]	2	193 (±1.6)	208 (±3.5)	7.9 (±0.10)	4.1 (±1.27)	5.12 (±0.52)	0.70 (±0.33)	0.67	1.89	2.20 (±0.88)	8.04 (±1.73)

[1] *S.thumbergi, angusticeps and robustus jallae*
[2] *S. vanderwaal, and nigromaculatus*

TABLE 2.5: Average % lipid, $\delta^{15}N$ (‰), weight (g), length (mm) and concentrations of DDT and its metabolites ($ng.g^{-1}$ wet weight) by location. Means recorded with ±SD.

Location	n	% lipid	$\delta^{15}N$	Weight	Length	p,p' DDT	p,p' DDD	o,p' DDT	p,p' DDT	ΣDDT	ΣDDT
Xakanaka	13	2.1 (±2.92)	5.1 (±2.78)	328 (±278)	246 (±118)	1.61 (±1.97)	0.56 (±0.72)	0.42 (±0.27)	0.69 (±0.83)	0.85 (±1.03)	2.52 (±3.21)
Ikoga	11	2.46 (±3.1)	6.9 (±1.36)	760 (±601)	314 (±121)	2.12 (±2.34)	0.60 (±0.33)	0.56 (±0.44)	1.5 (±1.70)	1.67 (±2.07)	4.09 (±4.67)
Samuchima	7	2.5 (±4.07)	7.21 (±1.98)	717 (±752)	316 (±84)	3.44 (±2.89)	1.11 (±0.71)	0.22 (±0.35)	2.08 (±1.99)	2.27 (±2.26)	6.67 (±5.43)
Guma	4	0.9	7.23 (±2.09)	348 (±105)	235 (±38)	1.10 (±0.6)	0.23 (±0.16)		0.09 (±0.03)	0.09 (±0.03)	1.35 (±0.56)
Gaborone	7	1.3	12.2 (±2.00)	836 (±786)	340 (±117)	12.2 (±13.77)	0.11 (±0.12)	0.03	0.47 (±0.87)	0.43 (±0.86)	12.4 (±13.7)

TABLE 2.6: The linear relationship between $\delta^{15}N$, $\delta^{13}C$ (‰) and log % lipid, and between $\delta^{15}N$(‰), log % lipid, length (mm), log weight (g) and the log concentrations of *p,p'*-DDE and tDDT(ng.g^{-1} wet weight and lipid normalized) in biota from the Okavango Delta[1]. ($n = 48$)

Y variable	Slope	X variable	intercept (β_0)	SE_{est}	R^2	P
$Y = \beta X \pm \beta_0$						
% lipid	0.13 (± 0.03)	δ15N	- 0.91 (± 0.22)	0.49	0.31	< 0.0001
%lipid	-0.04 ± 0.05	δ13C	- 1.1(±1.04	0.59	0.03	>0.05
$LogY = \beta_1 X_1 \pm \beta_0$						
p,p'-DDE (ng/g wet weight)	0.24 (± 0.03)	$\delta^{15}N$	- 1.62(±0.21)	0.56	0.58	< 0.0001
tDDT(ng/g wet weight)	0.21(± 0.03)	$\delta^{15}N$	- 1.86 (± 0.22)	0.59	0.49	< 0.0001
p,p'-DDE(ng/g wet weight)	0.98(± 0.15)	Log lipid	- 0.07 (± 0.09)	0.55	0.53	< 0.0001
tDDT(ng/g wet weight)	1.01(± 0.15)	Log lipid	+ 0.15(± 0.09)	0.53	0.57	< 0.0001
DDE(ng/g wet weight)	2.83(± 0.65)	Log length	- 6.88(± 1.58)	0.60	0.36	< 0.0001
tDDT(ng/g wet weight)	2.98(± 0.68)	log length	-7.05(± 1.65)	0.63	0.37	< 0.0001
DDE(ng/g wet weight)	1.18(± 0.32)	Log weight	-3.12(± 0.85	0.64	0.29	< 0.00
tDDT(ng/g wet weight)	1.25(± 0.34)	Log weight	-3.10(± 0.88)	0.66	0.30	<0.05
tDDT(ng/g lipid)	0.09(±0.04)	$\delta^{15}N$	+ 1.57(±0.24)	0.55	0.16	<0.05
Log length (mm)	0.05 (±0.01)	$\delta^{15}N$	+ 2.12(±0.06)	0.12	0.42	<0.0001
Log weight (g)	0.09 (±0.02)	$\delta^{15}N$	+ 2.05 (±0.15)	0.28	0.31	<0.0001

[1]**Values given as means ± standard error**

TABLE 2.7: Comparison of $\delta^{15}N$ and $\delta^{13}C$ (‰) among individual fish species and their prey items obtained from fish stomachs

Fish Species	Fish number	$\delta^{15}N$	$\delta^{13}C$	Prey item	$\delta^{15}N$	$\delta^{13}C$
Hydrocynus vittatus	SA2	8.16	-24.2	fish	5.95	-25.27
Synodontis nigromaculatus	X11	7.94	-22.8	insects	3.18	-24.47
Serranochromis thumbergi	S7	7.84	-23.1	fish	7.76	-28.25
Serranochromis thumbergi	S4	8.35	-23.1	fish	7.87	-28.25
Serranochromis thumbergi	S4	8.35	-23.1	lamprey	7.23	-27.76
Serranochromis robustus	Gu5	7.94	-25.1	fish	7.0	-28.59
Clarias gariepinus	X6	8.46	-21.2	fish	5.03	-23.54
Clarias gariepinus	Gab2	13.9	-19.3	fish	7.57	-17.65
Mormyrus lacerda	Ik5	5.31	-23.8	insects	3.73	-17.65
Tilapia rendali	X5	1.83	-19.8	algae	1.51	-21.32
Tilapia rendali	Gu4	4.29	-24.7	periphyton	4.36	-25.29

Figure 2.1: Comparison of mean concentrations of DDE ($ng.g^{-1}$ wet weight ± SD) in fishes from Gaborone Dam and lagoons of the Okavango delta.

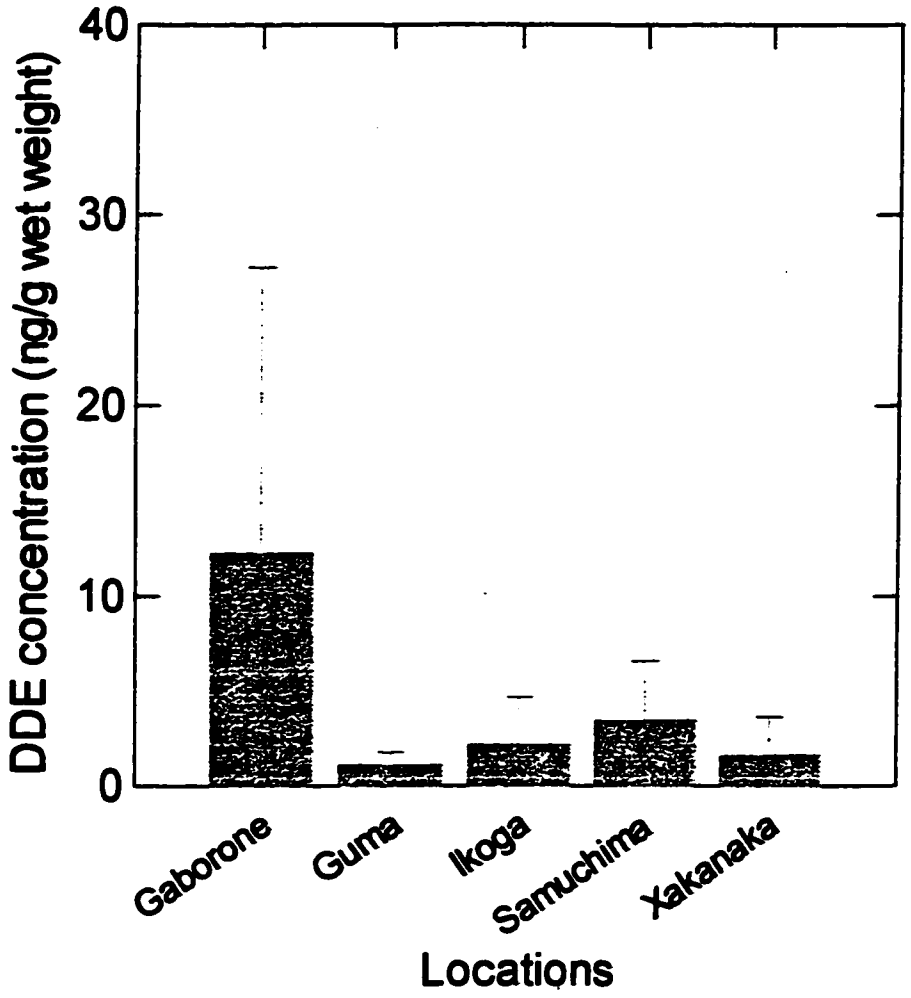


Figure 2.2: Relationship between log tDDT (ng.g^{-1} wet weight) and δ^{15}N for biota of the Okavango delta food web.

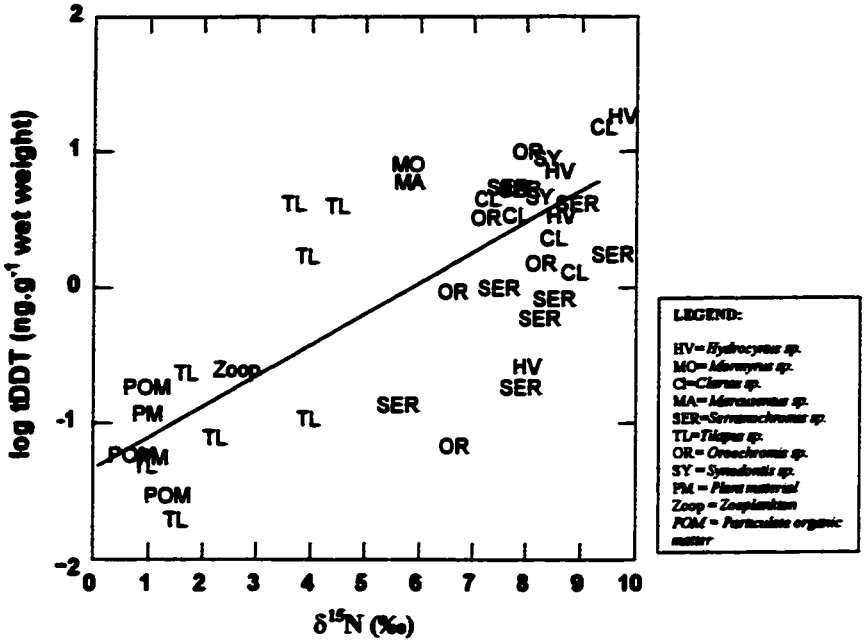


Figure 2.3 Relationship between log tDDT (ng.g^{-1} lipid) and δ^{15}N for biota of the Okavango delta food web.

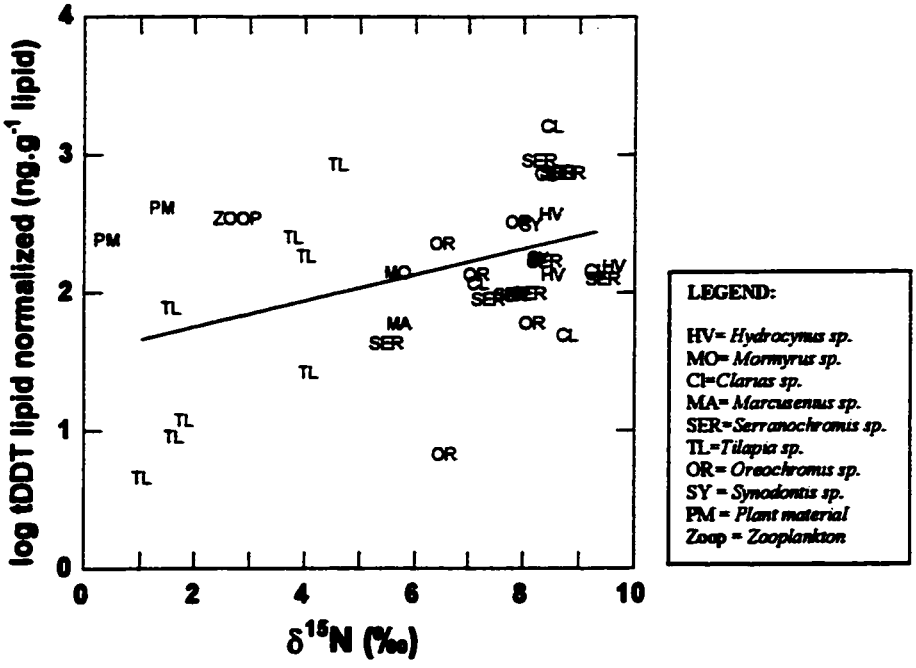


Figure 2.4: Relationship between log % lipid and isotopes of nitrogen ($\delta^{15}N$) for biota from the Okavango delta

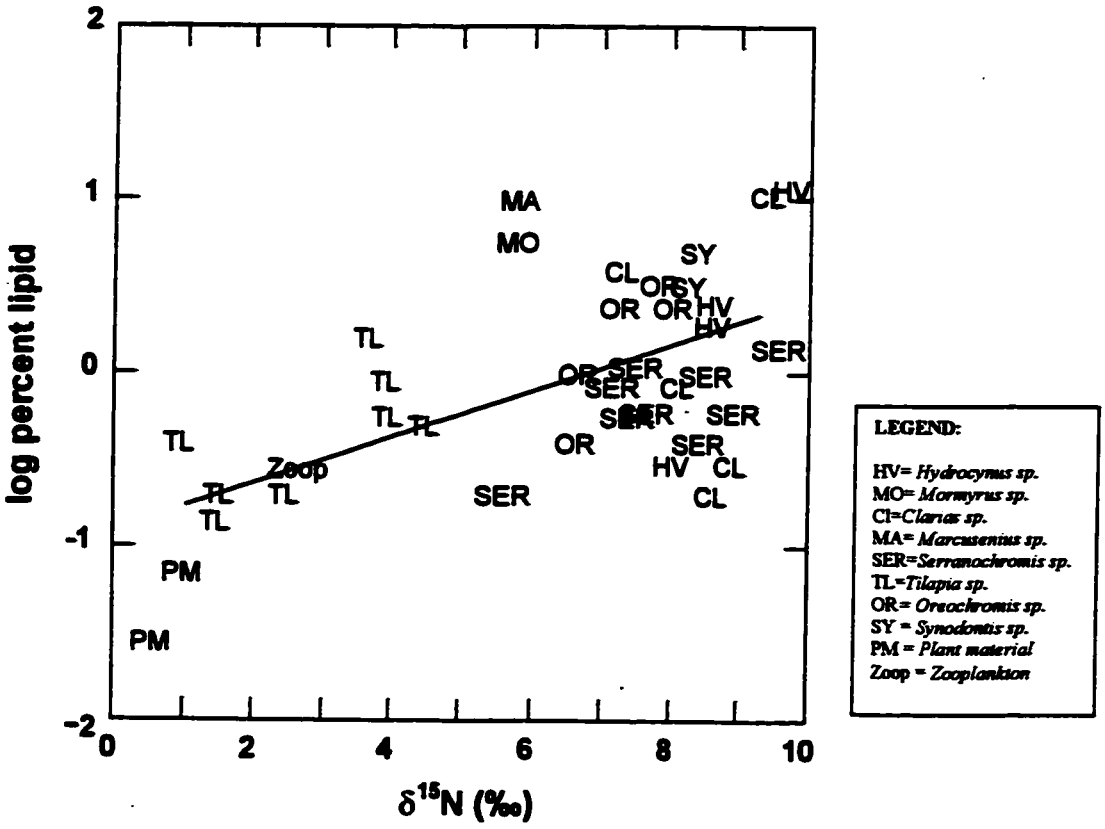


Figure 2.5: Relationship between log tDDT concentrations (ng.g-1 wet weight) and log percent lipid in biota of the Okavango delta.

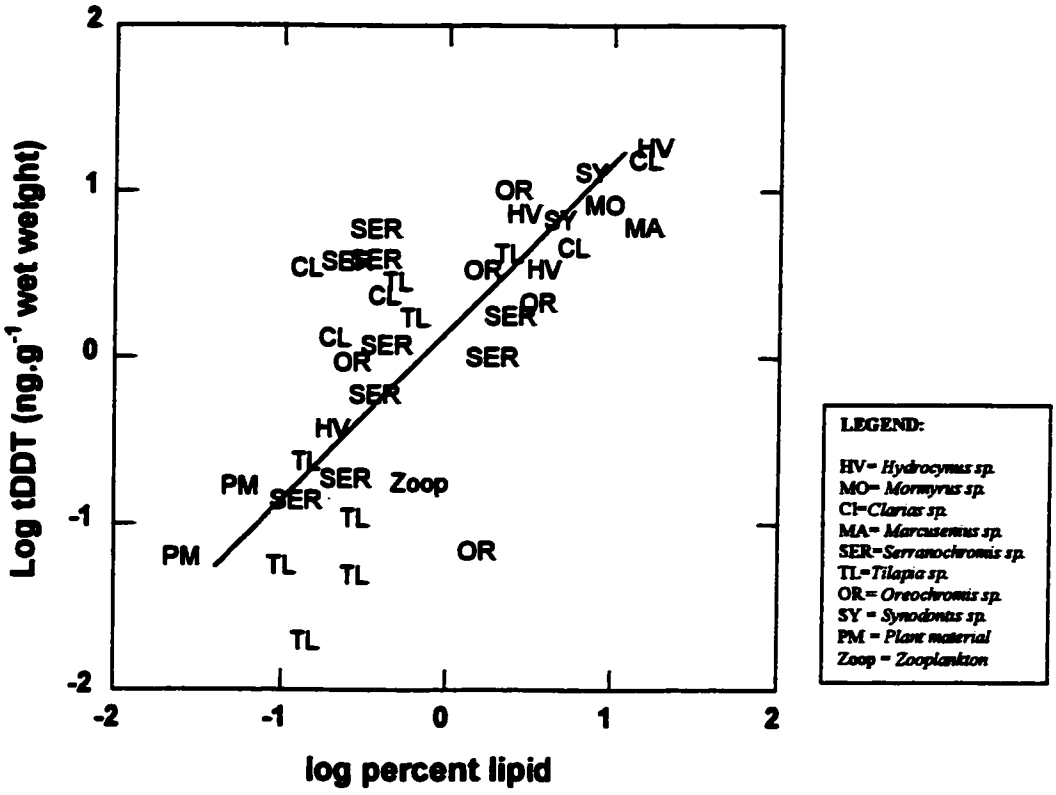


Figure 2.6: Comparison of percent lipid and tDDT ($ng.g^{-1}$ wet weight & lipid basis) among lagoons of the Okavango delta

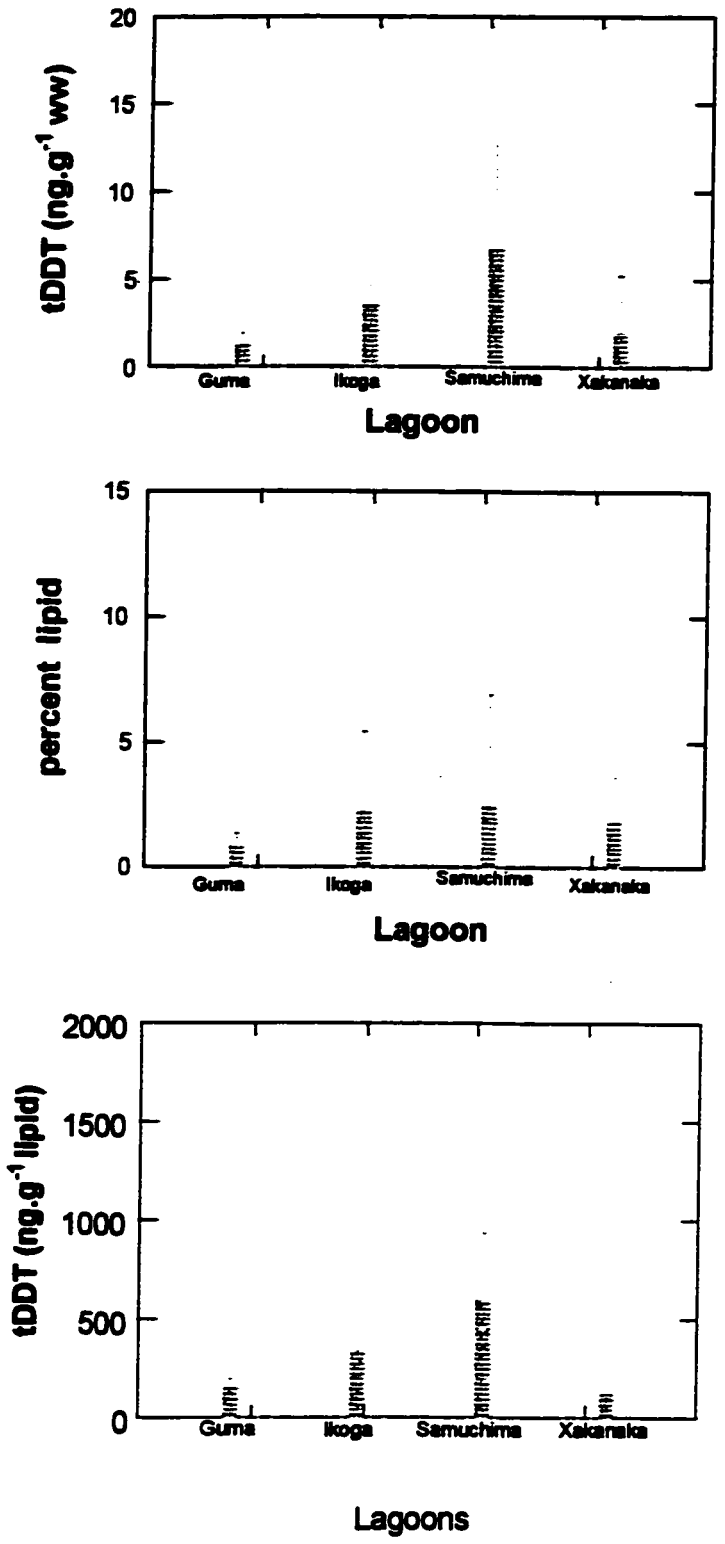


Figure 2.7: Mean (±SD) $\delta^{15}N$ and $\delta^{13}C$ (‰) of particulate organic matter, plant material, invertebrates, and fishes of the Okavango delta.

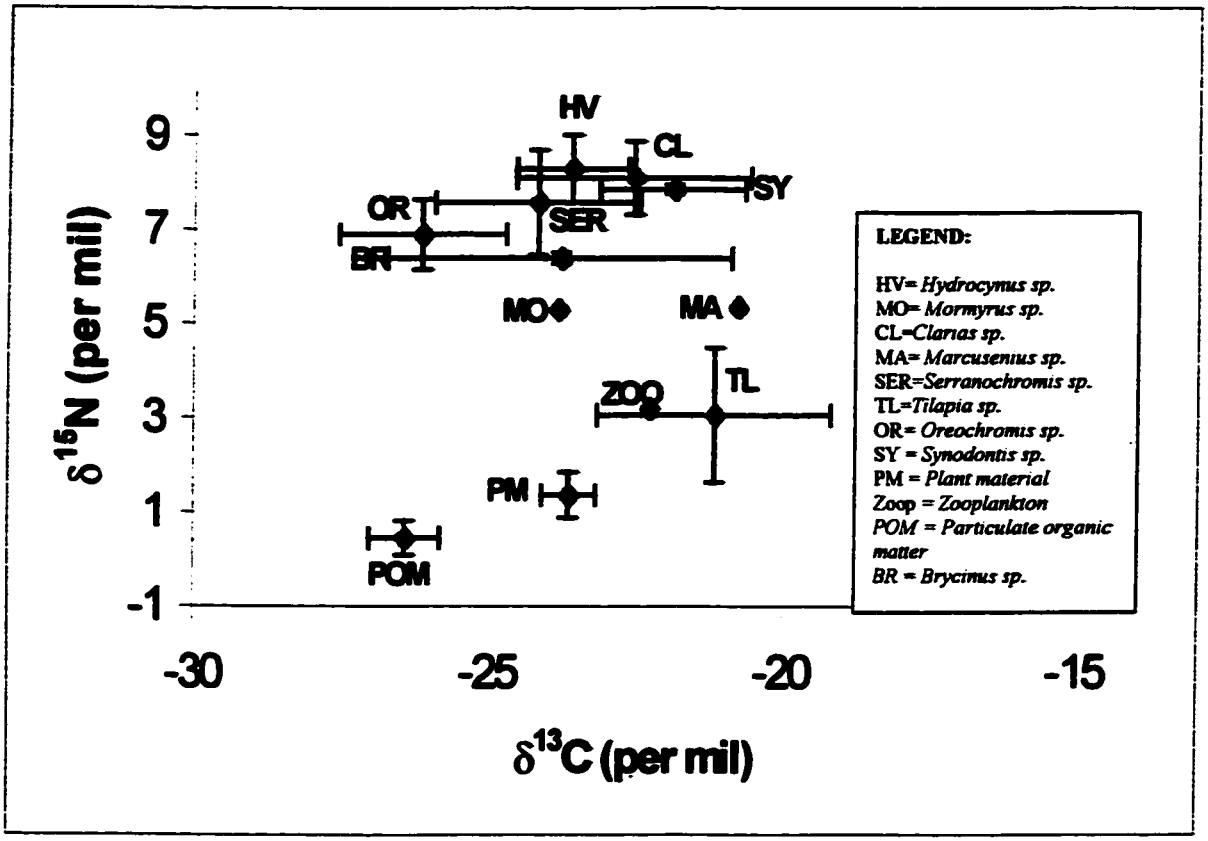


Figure 2.8: Mean $\delta^{15}N$ (‰) in fishes of the Okavango delta (malaria and tsetse areas) and the Gaborone dam.

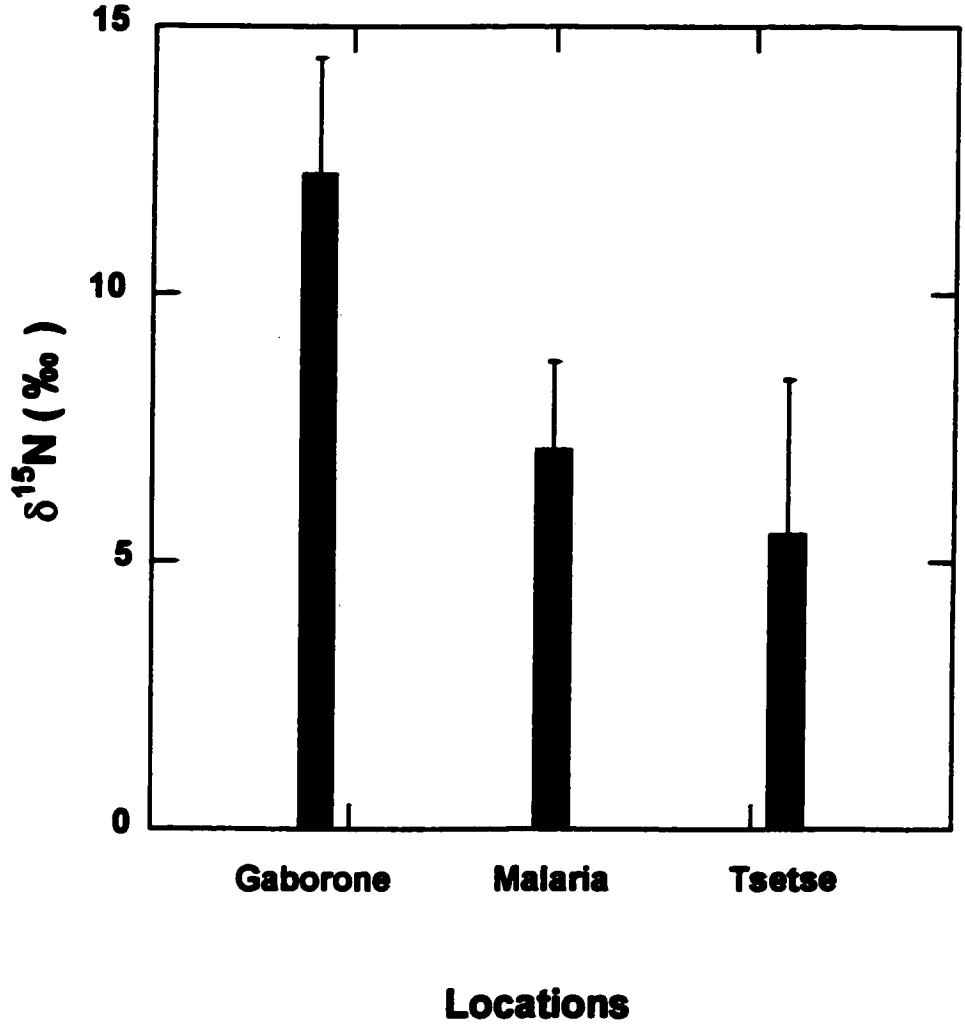


Figure 2.9: Mean Concentrations of tDDT (ng.g-1 lipid) in fish from the Okavango delta and two Zimbabwean lakes; Kariba and McIlwaine (this study, Berg *et al.* 1992)

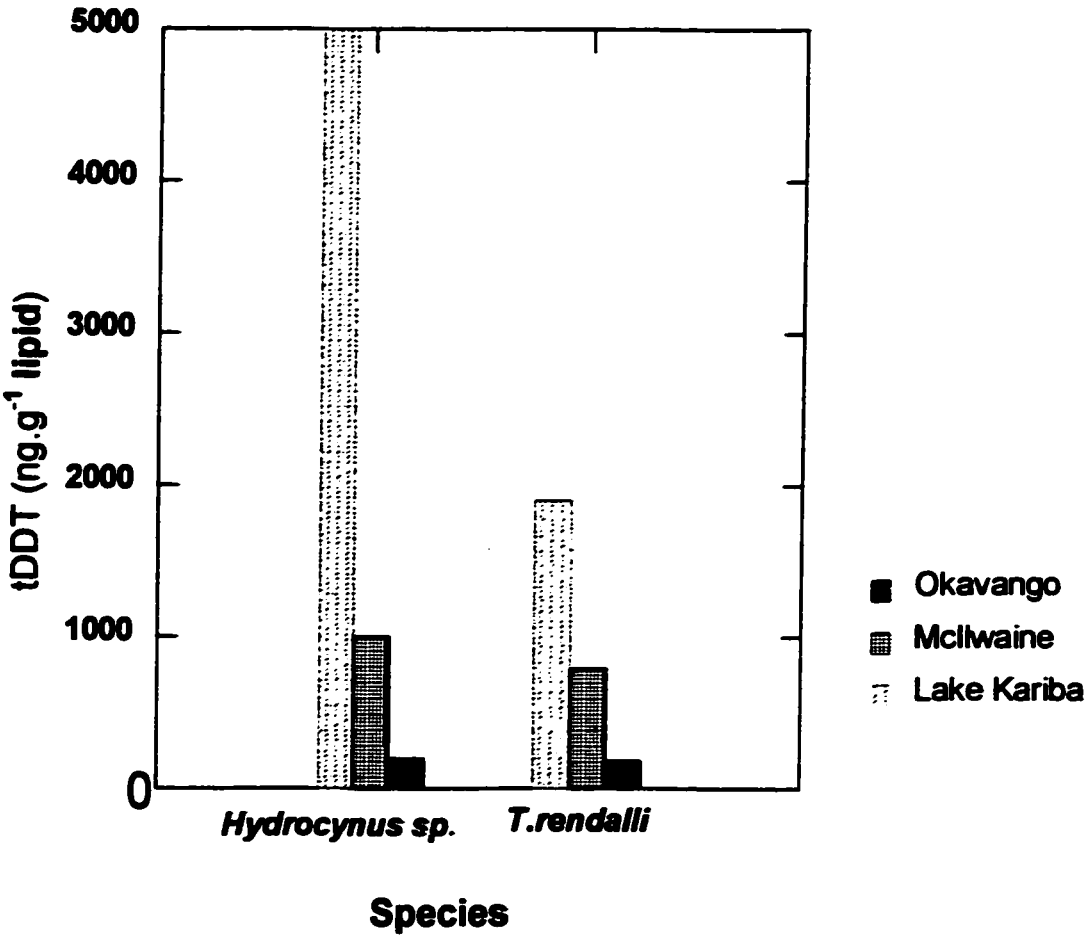


Figure 2.10: A comparison of the relationship between log tDDT concentrations ($ng.g^{-1}$ wet weight) and $\delta^{15}N$ of biota from the Okavango delta (this study) and lakes of the Yukon Territory (Kidd 1996).

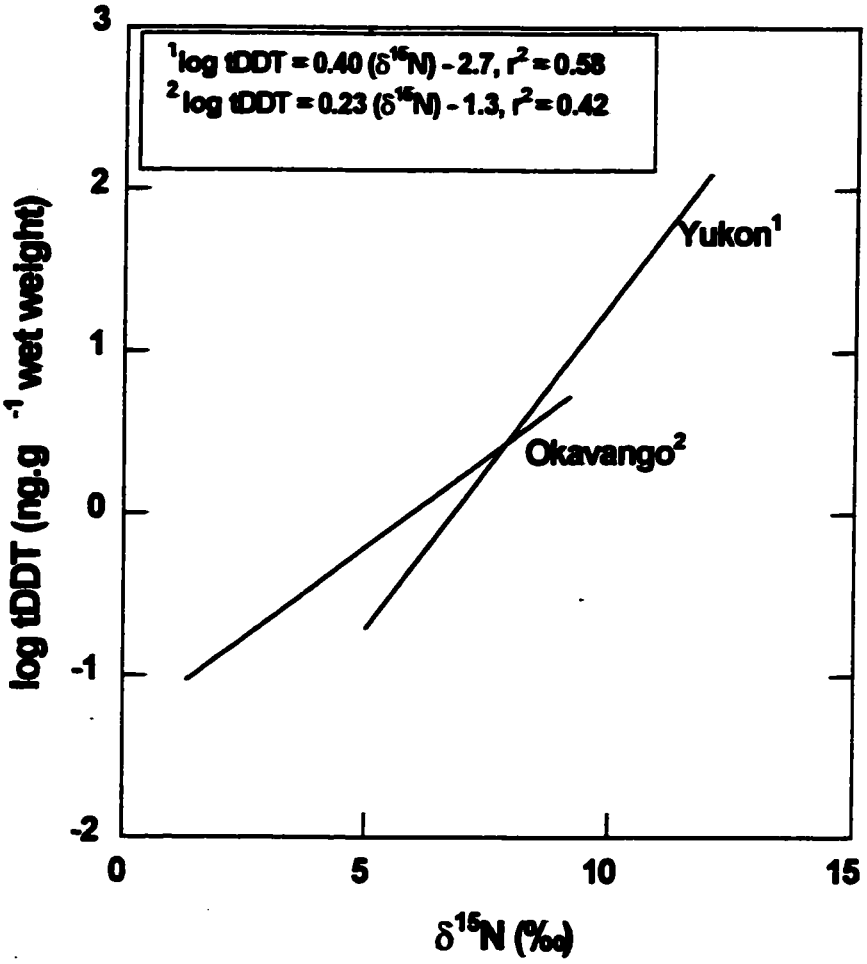
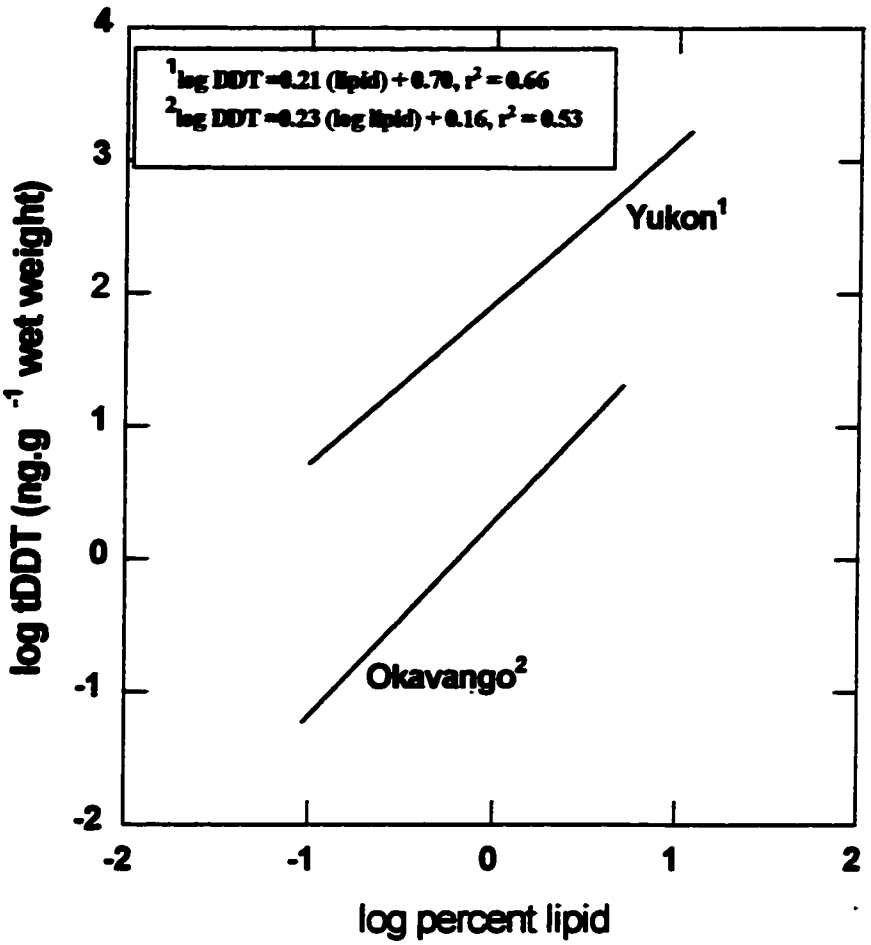


Figure 2.11: A comparison of the relationship between log tDDT concentrations ($ng.g^{-1}$ wet weight) and log percent lipid for biota of lagoons of the Okavango delta (this study) and lakes of the Yukon Territory (Kidd 1996)



Chapter 3

General Conclusions and Implications for Future Research

Ecotoxicology has been an area of high importance in developed countries since the awareness of the public to the hazards of pollution of the environment for biological life in general, hence the ban on DDT in developed nations in the early 1970's. Extensive research has demonstrated how DDT accumulated from lower levels in water (Larson *et al.* 1992) and plankton along the food chain to predatory fish and carnivorous birds by a factor of a 1000 or more (Evans *et al.* 1991, UNEP 1991, WWF 1998). As a result of adverse environmental effects of DDT, especially on non-target species, its accumulation and concentration in food chains, and its range of deleterious effects on reproduction in wildlife, severe restrictions and bans in many developed countries were implemented in the early 1970's (Wassermann *et al.* 1982, Environment Canada 1997). By 1995, at least 49 countries eliminated all uses of DDT because of its persistence, bioaccumulation, hazards to wildlife and other chronic effects (Fasola *et al.* 1997, Jensen 1997, WWF 1998). In addition, DDT was severely restricted or, remained unregistered in 29 other countries (UNEP 1996).

Most importantly, the uptake of DDTs through diet poses a threat to human health and represents a major exposure route, especially in homes where it is being sprayed in the interior of homes for the control of malaria-causing mosquitoes. DDT use for malaria control also has a potential to contribute to increased environmental contamination (WWF 1998). Samples of breast milk from Nicaraguan women have shown DDT levels at astounding amounts of 45 times WHO's tolerance limits (Farah 1994). Other levels of DDT detected in breast milk fat were recently found in China (4.4 ppm DDE and 1.8ppm DDT) and India (4.8ppm DDE and 4.4 ppm DDT) (Farah 1994). In northern South Africa, mothers were

exposed to 24.8 ppm of DDT residues in breast milk fat as a result of house spraying with DDT (Bouwman *et al* 1990a 1990b). According to WWF (1998), by three months of age, some infants' DDT levels reach those of their mothers.

While acknowledging the negative effects of DDT on wildlife and humans, the importance of DDT in improving public health over the years can not be overemphasized. In the field of public health, and especially in malaria control, DDT has saved many lives. Currently, around 2.5 billion people around the world are at risk of contracting malaria (WWF 1998). In developing countries malaria has continued to be one of the leading causes of illness and death, contributing up to 3 million deaths and up to 500 million clinical cases every year (WWF 1998).

Although the use of DDT for the control of tsetse flies has been stopped over ten years ago in Botswana (Davies 1980), its use for the control of malaria has only been stopped in 1999 (Ministry of Health 1999). This study measured DDT concentrations in biota from different locations of the Okavango delta where it has been used for both malaria and sleeping sickness control. Food sources for the species studied, and how they relate to DDT transfer have been investigated using the $\delta^{13}C$, which indicates the carbon source of an organism. Through this technique, food web relationships in the Okavango delta have been determined along with DDT biomagnification. $\delta^{15}N$ explained trophic position of the individual fish and other organisms studied, and provided a measure of biomagnification potential of DDT in the delta and, $\delta^{13}C$, or carbon source, differentiated between food sources that could potentially transfer DDT concentrations to top predators.

Based on these results it was concluded that trophic level (as determined by $\delta^{15}N$) was a significant predictor of DDT concentrations in the food web of the Okavango delta.

The slope of biomagnification (DDT vs $\delta^{15}N$) also revealed that DDT accumulated in the same manner in the Okavango delta food web as it does in temperate and sub-arctic food webs. Fish that consumed other fish had higher DDT concentrations than herbivores. Not only can the use of stable isotopes be used to show the significant value in modelling DDT biomagnification as it has been shown in this study, but can also be used to give a more comprehensive understanding of food web dynamics in the wider objectives of the proper management of the Okavango delta.

DDT levels in biota from the Okavango delta were low, and the metabolite DDE was the most predominant. The low levels can be explained by several factors and in particular the amount of DDT sprayed for both malaria and tsetse control. Compared to many countries, Botswana has used relatively lower amounts of DDT. The physical characteristics of the delta such as its sandy soils and sediments with low organic carbon and, the semi-arid temperatures, are some of the factors which may have played a major role in the low levels of DDT detected in the delta. These conclusions are strengthened by the findings reached by other researchers such as Liechtenstein and Shulz (1959) and Edwards (1973a, 1973b), who found that, the amounts of DDTs recovered from sandy soils, with little organic carbon, were lower than those recovered from soils with higher organic carbon a few months after application. They also found that temperature was an important factor in the persistence of DDT as it facilitates volatilisation. Losses due to volatilization can be as high as 50% in only 5 months due to the high erradiance of sunlight in levels in fish obtained from desert soils such as the Okavango delta.

Malaria areas, which have also been previously sprayed with DDT for tsetse control had significantly higher levels of DDT concentrations than tsetse control areas. These levels

were however driven by biological factors rather than history of DDT application. Besides trophic level, lipid was a significant predictor of DDT concentrations in fish from the delta. Body size, as determined by length and weight of fish, was also significantly related to DDT concentration.

Although it may seem unlikely that DDT levels in fish from the delta would cause any detrimental health impacts on humans, caution has to be exercised in interpreting these data. Humans may be exposed to DDT levels from the air and other food commodities. In the case of Okavango residents and others throughout the country where DDT has been sprayed in residential homes for the control of malaria causing mosquitoes, there already exists the risk of further exposure to DDT.

To further address the fate of DDT in the Okavango delta, and to assess possible environmental and human health risks due to DDT used for malaria control, future studies should be directed to other compartments of the environment and in particular, the levels in the air and breast milk. Studies from neighbouring countries such as Zimbabwe, have shown that DDT levels are higher in the atmosphere due to the warm temperatures (Larson *et al.* 1995). In other African countries, research has also shown that DDT levels are high in breast milk. Area-specific consumption rates of fish will also be needed to assess whether DDT in fish, combined with all other exposure routes, may have detrimental human health impacts.

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APPENDIX 1

DETAILED DESCRIPTION OF FIELD AND LABORATORY METHODS

Sampling

Sampling of biota was conducted in July, August and December 1999. The inaccessibility of the delta by both road and boat and time limitations prevented the collection of one species of fish from all the sampled locations around the delta to allow for the adequate assessment of site to site differences in DDT concentrations.

Temperature Profile and Water Chemistry

Temperature was measured with a thermometer and conductivity measured using YSI meter (Model58) starting at the surface of the water to a depth of 1-2 meters. Water samples were collected at each lagoon (with the exception of Samuchima) in pre-cleaned 2-4 L Nalgene™ bottles from a depth of 1 meter for particulate organic matter (POC), dissolved organic carbon (DOC), dissolved phosphorus (P), total phosphorus (TP), calcium (Ca) iron (Fe), potassium (K), magnesium (Mg), and sodium (Na). All samples were stored immediately in ice-filled coolers.

Water was filtered through ashed 47 mm Whatman™ GF/F filters for DOC and stored immediately in a freezer in petri dishes at the Department of Water Affairs laboratories in Botswana. Filtered water was transferred into clean, pre-labelled 125 ml bottles for dissolved P analysis, and whole water for total P, trace metals, major ions, and DOC were transferred into separate pre-rinsed 125 ml bottles. Samples were kept at 4°C until shipment to Canada, University of Ottawa. Environment Canada, Atlantic Region, completed all water chemistry analysis procedures.

Organochlorine Analyses:

The dorsal muscle of the large fish (including the skin), whole small fish and plant material were homogenized with anhydrous sodium sulfate (precleaned at 600°C for 16 hrs). In order to reduce evaporative losses the mortar and pestle were chilled in the

freezer and placed on dry ice during the homogenization process. Blanks were run with every 10th sample to ensure cleanliness of solvents and glassware. Internal standards of Polychlorinated biphenyl 30 (PCB 30) and Octachloronapthalene (OCN) were then added to samples, which were then Soxhlet extracted overnight with 135-mls of Dichloromethane (DCM). After extraction, the extract volumes were reduced under vacuum at 20°C to a volume of approximately 2 ml. Gel permeation chromatography (GPC) was used to separate the lipid component from the organochlorines mixture in the extraction solution. A solution of 55:45 (v: v) hexane /dichloromethane was used as the elution solvent.

A gel permeation chromatography column (30x3cm o.d) with medium porosity glass frit and 4mm teflon stopcock was prepared adding 50 g (dry weight) of biobeads S-X3 (200-400 mesh) pre-swelled in the elution solvent. Once settled in the column, beads were kept immersed in the elution solvent.

The Elution volumes, which were required to collect the lipid and organic contaminants fractions from the chromatography column, were calibrated using vegetable oil and PCB standard mixture (CCIW, Ontario) For Lipid calibration, 0.3g of Mazola oil was added to a tarred, graduated cylinder and made up to a 4ml volume with hexane. This solution was applied to the surface of the beads in the column together with approximately 3ml hexane rinses of the graduated cylinder used to remove residual oil. Seven separate fractions of the eluant were collected and their respective volumes recorded for a total of 220 ml. Each fraction was then evaporated under vacuum at 20°C to approximately 3ml and transferred into a labeled, pre-weighed aluminum boat. The

remaining solvent was allowed to evaporate in a fume-hood. Samples were then dried at 60°C for two hours and allowed to cool down in a desiccator and weighed.

Elution volumes required for organochlorines were determined in a similar manner by spiking a known amount of PCB and OCN standards to 3ml of hexane. The solution was then added to the surface of the beads then collecting 250 ml of eluent in seven individual fractions. Each fraction was then evaporated under a vacuum at 20°C to approximately 2ml and transferred to a pre-calibrated centrifuge tube. Centrifuge tubes were placed at 30°C on an evaporating unit (Pierce Reati-Therm Heating Module, Model 18780) and eluent volumes reduced to a final volume of 1ml under a steady stream of nitrogen gas. The 1ml solution was placed into labeled gas chromatography vials for subsequent gas chromatography analysis. The elution volume of organochlorines from the chromatography column was determined by the appearance of the contaminants in the one or more of the resulting chromatograms from the seven fractions collected from the column. After calibration, the initial volume of 40 ml contained neither lipid, PCBs nor organochlorine compounds and was regarded as waste fraction. The next 85-ml contained 98 % of the lipids, and the final 120-ml contained the PCBs and organochlorine standards.

Extractable lipids from fish, zooplankton and plant material were determined with 1/10th of the extract. The remaining extract was then fractionated on florisil (8g; 1.2% deactivated with HPLC-grade water) topped with 1g anhydrous sodium sulfate. The first fraction was eluted with hexane, the second with hexane-DCM (85:15) and the third with straight DCM (Kidd *et al.* 1998). Florisil eluates were analyzed by Capillary Gas

Chromatography (GC) with ^{63}Ni electron capture detection on 30 m x 0.25 mm DB-5 column using a HP 6890 (Hewlett Packard©).

APPENDIX 2

$\delta^{15}N$, $\delta^{13}C$, STANDARD LENGTH (mm) WEIGHT (g), PERCENT LIPID AND CONCENTRATIONS OF DDT AND ITS METABOLITES FISH, PLANT MATERIAL, ZOOPLANKTON (ng.g^{-1} wet weight), AND WATER (ng. L^{-1}) FROM THE OKAVANGO DELTA AND GABORONE DAM

Location	Species	Sample #	$\delta^{15}N$	$\delta^{13}C$	Length (mm)	Weight (g)	% lipid	p,p'-DDE	p,p'-DDD	o,p'-DDT	p,p'-DDT	ΣDDT	tDDT	% RECOVERY	
														PCB30	ONC
	Fish														
Xakanaka	Brownspot Largemouth (*Serranochromis thumbergi*)	XGA4	7.34	-21.6	190	108.8	0.4	0.16			0.04	0.04	0.20	87	79
Ikoga	Brownspot Largemouth (*Serranochromis thumbergi*)	IK4	6.92	-27.1	360	706.6	1.1	0.48			0.54	0.54	1.02	89	85
Samuchima	Brownspot Largemouth (*Serranochromis thumbergi*)	SA4	8.35	-23	330	500	0.6	2.32	1.15	0.03	0.76	0.8	4.26	40	50
Samuchima	Brownspot Largemouth (*Serranochromis thumbergi*)	SA7	7.84	-23.1	245	420	0.4	2.09	0.67	0.06	1.04	1.10	3.86	102	76
Samuchima	Thinface Largemouth (*Serranochromis angusticeps*)	SA5	8.17	-24.2	290	500	0.6	2.23	0.39	0.23	1.12	1.35	3.97	86	67
Xakanaka	Thinface Largemouth (*Serranochromis angusticeps*)	EX1	5.04	-23.9	125	138.5	0.2	0.12	0.02				0.14	88	80

Guma	Nembwe (*Serranochromis robustus*)	GU2	9	-23.5	290	500	1.4	1.45	0.26		0.1	0.1	1.81	95	60
Guma	Nembwe (*Serranochromis robustus*	GU3	7.67	-26.3	230	306.5	0.6	0.45	0.05		0.11	0.11	0.61	96	64
Guma	Nembwe *Serranochromis robustus*	GU5	7.94	-25.1	220	333	0.7	0.77	0.37		0.06	0.06	1.2	77	61
Xakanaka	Redbreast Tilapia (*Tilapia rendalli*)	EX5	1.83	-19.8	157	126.9	0.1	0.05			0.03	0.03	0.08	81	83
Ikoga	Threespot Tilapia (*Oreochromis andersonii*)	IK2	6.16	-23.7	255	484	0.4	0.58			0.37	0.37	0.95	93	87
Ikoga	Redbreast Tilapia (*Tilapia rendalli*)	IK3	4.32	-23.5	220	369.7	0.4	0.05			0.06	0.06	0.11	85	78
Ikoga	Threespot tilapia (*Oreochromis andersonii*)	IK10	7.55	-26.4	220	364.3	2.4	3.99	0.31	0.03	2.975	3.01	7.31	67	83
Samuchima	Redbreast Tilapia (*Tilapia rendalli*)	SA1	4.04	-20.3	280	400	1.6	2.68		0.07	1.51	1.58	4.27	80	85
Samuchima	Redbreast Tilapia (*Tilapia rendalli*)	SA3	4.85	-20.2	280	600	0.5	2.36	1.29	0.02	0.43	0.45	4.11	82	69
Guma	Redbreast Tilapia (*Tilapia rendalli*)	GU4	4.29	-24.7	200	255	0.9	1.75					1.75	105	81
Xakanaka	Redbreast tilapia (*Tilapia rendalli*)	EX3	1.3	-18.8	165	163.6	0.4	0.05					0.05	80	30
Xakanaka	Redbreast tilapia (*Tilapia rendalli*)	EX4	2.06	-20.5	159	138.5	0.2	0.16	0.07		0.01	0.01	0.24	74	69
Xakanaka	Redbreast Tilapia (*Tilapia rendalli*)	EX7	1.88	-21.7	175	219.1	0.2	0.01	0.01				0.02	89	66
Xakanaka	Sharptooth Catfish (*Clarias gariepinus*)	EX2	8.46	-21.2	535	1000	0.3	1.34					1.34	111	101
Xakanaka	Sharptooth Catfish	EX6	6.85	-20	365	626.1	3.9	2.33	2.19		0.12	0.12	4.64	77	70

	(*Clarias gariepinus*)														
Ikoga	Sharptooth Catfish (*Clarias gariepinus*)	IK6	8.97	-23.9	550	2000	10.5	7.98	0.95	0.93	5.78	6.71	15.64	85	81
Ikoga	Sharptooth Catfish (*Clarias gariepinus*)	IK7	8.07	-22.8	440	1452	0.6	1.4			0.99	0.99	2.39	84	86
Ikoga	Sharptooth Catfish (*Clarias gariepinus*)	IK11	8.18	-25	470	1500	0.2	2.52			1.00	1.00	3.54	117	85
Xakanaka	Bulldog (*Marcusenius macrolepidotus*)	EX9	5.36	-20.8	240	219.5	10	3.75	0.98	0.45	0.99	1.44	6.17	83	63
Xakanaka	Spotted squeakier (*Synodontis nigromaculatus*)	EX11	7.94	-22.8	205	193.8	5	5.49	0.93	0.67	2.17	2.84	9.26	65	76
Xakanaka	Finetooth squeakier (*Synodontis vanderwaal*)	EX10	7.8	-21	210	191.6	3.2	4.75	0.46		1.60	1.60	6.81	48	60
Ikoga	Threespot Tilapia (*Oreochromis andersonii*)	IK1	6.78	-27.2	240	328.6	2.4	1.97		0.37	1.05	1.42	3.39	82	83
Ikoga	Threespot Tilapia (*Oreochromis andersonii*)	IK8	6.2	-26.3	200	215.2	1	0.06			0.01	0.01	0.07	60	66
Ikoga	Threespot Tilapia (*Oreochromis andersonii*)	IK9	7.8	-27.1	230	395	2.3	1.20			0.98	0.98	2.18	82	97
Xakanaka	Tigerfish (*Hydrocynus vittatus*)	XGA3	8.19	-22.8	395	680.2	2.5	2.42	0.34	0.13	0.58	0.71	3.47	110	92
Samuchima	Tigerfish (Hydrocynus vittatus)	SA2	8.16	-24.2	290	200	1.9	2.57	0.99		3.92	3.92	7.48	93	78
Samuchima	Tigerfish (*Hydrocynus vittatus*)	SA6	9.29	-24.7	500	2400	11.6	9.92	2.13	0.93	5.78	6.71	18.76	98	74

Xakanaka	Tigerfish (*Hydrocynus vittatus*)	EX8	7.57	-22.8	280	458.2	0.3	0.23	0.04				0.27	99	52
Ikoga	Western bottlenose (*Mormyrus lacerda*)	IK5	5.31	-23.8	270	549.8	5.7	3.90	0.53	0.93	2.97	3.90	8.33	73	65
Guma	Striped Robber (*Brycinus lateralis*)	GU 1	6.44	-25.8	80	10.3	13.9	4.45	1.04		1.64	1.64	7.13	103	70
Guma	Striped Robber (*Brycinus lateralis*)	GU 6	6.27	-21.8	85	14.2	10.4	0.32	0.07		0.08	0.08	0.47	102	74
Gaborone	Carp (Caprinus carpio)	GAB4	13.43	-19.8	520	2500	2.1	7.44	0.06				7.50	91	72
Gaborone	Sharptooth Catfish (*Clarias gariepinus*)	GAB1	14.27	-19.3	420	1000	2.5	37.43				2.02	37.43	83	59
Gaborone	Sharptooth Catfish (*Clarias gariepinus*)	GAB2	13.9	-19.3	350	600	0.9	13.75	0.17	0.03	0.03	0.06	13.98	90	67
Gaborone	Sharptooth Catfish (Clarias gariepinus)	GAB3	13.62	-21.8	410	846	2.7	22.99	0.39		0.07	0.07	23.45	99	80
Gaborone	Threespot Tilapia (*Oreochromis andersonii*)	GAB5	10.35	-17.6	210	232.7	0.6	2.34	0.26	0.04	0.17	0.21	2.81	102	86
Gaborone	Threespot Tilapia (*Oreochromis andersonii*)	GAB6	10.12	-19.3	240	358.6	0.1	0.17	0.02				0.19	88	72
Gaborone	Threespot Tilapia (*Oreochromis andersonii*)	GAB7	9.83	-19	230	316.1	0.4	1.32	0.24		0.09	0.09	1.66	88	80
Zooplankton															
Xakanaka		Xzoo	3.213	-22.3			0.4	0.03	0.06		0.09	0.09	0.18	99	52
Gaborone		GABZOO	4.303	-29.5				0.255	0.066		0.049		0.369	94	71

Plant material															
Xakanaka	Tswii (rooted plant)	tswii	1.045	-24.3			0.04	0.08		0.01		0.01	0.09	131	91
Ikoga	Water lily (aquatic leaves)	Ikoly	1.694	-23.1			0.1	0.06			0.11	0.11	0.17	150	84
Suspended particulates															
Xakanaka	Particulate matter	XGsup	1.88	-25.5				0.01			0.02	0.02	0.03	91	72
Ikoga	Particulate matter	Iksup	1.506	-25.5				0.08		0.05	0.06	0.11	0.19	83	59
Guma	Particulate matter	Gusup	0.096	-25				0.04		0.01	0.01	0.02	0.06	90	67
Water															
Xak	water	XGw						0.009					0.009	79	68
Xak	water	Xkw						0.04			0.08		0.12	100	83
Iko	water	IKW1							0.001	0.002	0.019	0.019	0.022	107	78
Iko	water	IKW2							0.001	0.003	0.02	0.02	0.004	106	76
Blanks															
	Blank1	fish												86	69
	Blank2	fish												90	75
	Blank3	fish												99	85
	Blank4	water												102	98
	Blank5	water												106	79

APPENDIX 3

Prey items on stomachs of fish from the Okavango delta*

		Prey Items													
Species (Family)	**Common Name**	**Fish #**	**% lipid**	**δ15N**	**δ13C**	**Fish**	**Periphyton**	**Detritus**	**Aquatic seeds**	**Mollusks**	**Aquatic insects**	**Terrestrial insects**	**Plant**	**unidentified**
Serranochromis thumbergi (Cichlidae)	Brownspot Largemouth	TSEGA4	0.4	7.34	-21.57	X					X			
Serranochromis thumbergi (Cichlidae)	Brownspot Largemouth	MAIK4	1.1	6.92	-27.12			X			X			
Serranochromis thumbergi	Brownspot Largemouth	MASA4	0.6	8.35	-22.98	X				X	X			
Serranochromis thumbergi	Brownspot Largemouth	MASA7	0.4	7.84	-23.08			X			X			
Serranochromis angusticeps (Cichlidae)	Thinface Largemouth	MASA5	0.6	8.17	-24.24	X		X						
Serranochromis angusticeps (Cichlidae)	Thinface largemouth	TSEX1	0.2	5.04	-23.90	Empty Stomach								
Serranochromis robustus jallae (Cichlidae)	Nembwe	MAGU2	1.4	9.00	-23.54	X					X			
Serranochromis robustus jallae (Cichlidae)	Nembwe	MAGU3	0.6	7.67	-26.26	X								
Serranochromis robustus jallae (Cichlidae)	Nembwe	MAGU5	0.7	7.94	-25.09	X					X			
Tilapia rendalli (Cichlidae)	Redbreast Tilapia	TSEX5	0.1	1.83	-19.84								X	
Tilapia rendalli (Cichlidae)	Redbreast Tilapia	MAIK3	0.4	4.32	-23.49		X	X						

Tilapia rendalli (Cichlidae)	Redbreast Tilapia	MASA1	1.6	4.04	-20.30								X	X
Tilapia Rendalli (Cichlidae)	Redbreast Tilapia	MASA3	0.5	4.85	-20.16								X	
Tilapia Rendalli (Cichlidae)	Redbreast Tilapia	MAGU4	0.9	4.29	-24.71		X	X					X	X
Tilapia Rendalli (Cichlidae)	Redbreast Tilapia	TSEX3	0.4	1.30	-18.84		X	X						
Tilapia rendalli (Cichlidae)	Redbreast Tilapia	TSEX4	0.2	2.06	-20.46		X	X						
Tilapia rendalli (Cichlidae)	Redbreast Tilapia	TSEX 7	0.2	1.88	-21.70		X	X			X			
Clarias gariepinus (Clariidae)	Sharptooth catfish	TSEX2	0.3	8.46	-21.21	Empty stomach								
Clarias gariepinus (Clariidae)	Sharptooth catfish	TSEX6	3.9	6.85	-20.01	X		X			X	X		
Clarias gariepinus (Clariidae)	Sharptooth catfish	MAIk6	10.5	8.97	-23.87	X					X			
Clarias gariepinus (Clariidae)	Sharptooth catfish	MAIK7	0.3	8.07	-22.82	X			X					
Clarias gariepinus (Clariidae)	Sharptooth catfish	MAIK11	0.2	8.18	-24.97	X			X			X		
Marcusenius macrolepidotus (Mormyridae)	Bulldog	TSEX9	10	5.36	-20.80		X	X			X	X		
Synodontis nigromaculatus (Mochokidae)	Spotted squeakier	TSEX11	5.0	7.94	-22.78	X	X	X	X					
Synodontis vanderwaali((Mochokidae)	Finetooth squeakier	TSEX10	3.2	7.80	-21.03	X	X	X		X				
Brycinus lateralis (Characidae)	Striped robber	MAGU1	13.9	6.44	-25.78				X		X	X		
Brycinus lateralis (Characidae)	Striped robber	MAGU6	10.4	6.27	-23.87						X	X		
Oreochromis andersonii (Cichlidae)	Threespot Tilapia	MAIK10	2.4	7.55	-26.36		X	X						

Oreochromis andersonii (Cichlidae)	Threespot Tilapia	MAIK2	0.4	6.16	-23.71		X	X	X					
Oreochromis andersonii (Cichlidae)	Threespot Tilapia	MAIK1	2.4	6.78	-27.20			X			X			X
Oreochromis andersonii (Cichlidae)	Threespot Tilapia	MAIK8	1.0	6.20	-26.29			X					X	
Oreochromis andersonii (Cichlidae)	Threespot Tilapia	MAIK9	2.3	7.80	-27.06			X			X			X
Hydrocynus vittatus (Characidae)	Tigerfish	TSEG3	2.5	8.19	-22.83	X					X			X
Hydrocynus vittatus (Characidae)	Tigerfish	MASA2	1.9	8.16	-24.16	X					X			
Hydrocynus vittatus (Characidae)	Tigerfish	MASA6	11.6	9.29	-24.65	X								
Hydrocynus vittatus (Characidae)	Tigerfish	TSEX8	0.3	7.57	-22.80	X								X
Mormyrus Lacerda (Mormyridae)	Western Bottlenose	MAIK5	5.7	5.31	-23.84	X			X	X	X		X	

*** Prey identification done by Melissa Legrand, honours student, University of Ottawa**

APPENDIX 4

Percent Occurrence of prey items in fish stomachs for selected lagoons of the Okavango delta

Species (Family)	Common Name	n	% lipid	δ15N	δ13C	No. of Empty stomachs	Percent Occurrence of Prey Items[1]							
							Fish	Periphyton	Detritus	Aquatic seeds	Mollusks	Aquatic insects	Terrestrial insects	Plant
Serranochromis (Cichlidae)	Brownspot Largemouth	9	1.1	6.92	-27.12	1	75	_	37.5	_	12.5	75	_	_
Tilapia rendalli (Cichlidae)	Redbreast Tilapia	8	0.4	4.32	-23.49	3	_	50	100			20		50
Clarias gariepinus (Clariidae)	Sharptooth catfish	5	0.3	8.46	-21.21	1	100		-	-	-	50	50	25
Marcusenius macrolepidotus (Mormyridae)	Bulldog	1	10	5.36	-20.80	0		100	100	-		100	100	-
Synodontis nigromaculatus (Mochokidae)	Spotted squeaker	2	5.0	7.94	-22.78	0	100	100	100	50	-	-	-	-
Brycinus lateralis (Characidae)	Striped robber	2	10.4	6.27	-23.87	Too small								
Oreochromis andersonni (Cichlidae)	Threespot Tilapia	5	2.4	7.55	-26.36	0	-	60	100	20	-	-	-	-
Hydrocynus vittatus (Characidae)	Tigerfish	4	0.3	7.57	-22.80	0	100	-	-	50	-	-	-	-
Mormyrus Lacerda (Mormyridae)	Western Bottlenose		5.7	5.31	-23.84	100	-	-	100	100	100	-	-	-

* Prey Identification done by Melisa Legrand, Honors student, university of Ottawa

[1]Percent of fish feeding on each taxon calculated as: # of stomachs with taxon / total # of non-empty stomachs

APPENDIX 5

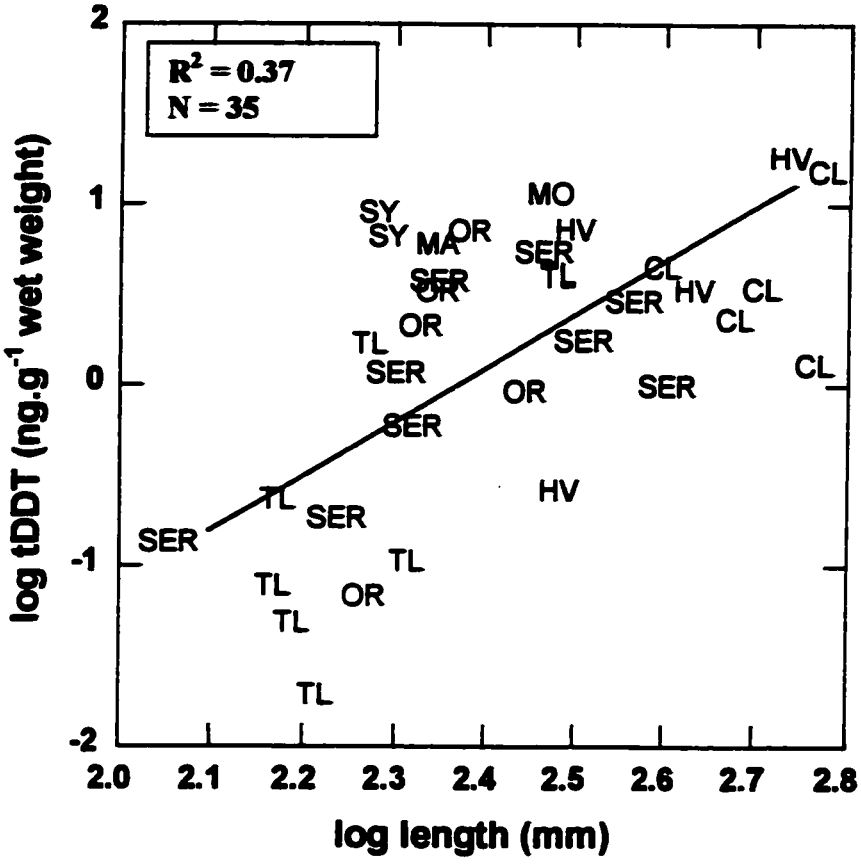
Physical and epilimnic chemical characteristics of Xakanaka Ikoga, and Samuchima, Okavango Delta in Comparison with Gaborone Dam, Botswana and two Canadian lakes, lake Laberge in the Yukon Territory and Lake Ontario. Unless otherwise indicated, the water quality data is from this study and is given as means and ranges in parenthesis.

				Other Locations		
	Xakanaka	Ikoga	Samuchima	Gaborone	Lake Laberge, Canada	Lake Ontario Canada
Conductivity	56.9 (56.6-57.5)	33.6 (32.6-35.1)	27.9 (27.9-2.0)	293(291-295)	113 (111-115)[2]	
TN ($\mu g.L^{-1}$)	150 (140-160)	1000[3]	-	0.20 (0.16-0.23		
TP ($\mu g.L^{-1}$)	7.3 (7.0-8.0)	14 (11-17)	6.5(6.0-7.0)	14	5.1 (3.5-8.1)[2]	7.7
TDP ($\mu g.L^{-1}$)	6.0	9 (6-0.12)	4.0	8.0 (0.007-0.01)	2.5[2]	4.9[1]
Ca ($mg.L^{-1}$)	5.79 (4.43-6.64)	2.85 (1.86-3.74)	1.94(1.33-2.70)	27.8 (27.7-28)		10[1]
Na ($mg.L^{-1}$)	4.09 (3.84-4.29)	1.38 (0.925-1.69)	1.24 (1.03-1.051)	15.50		11.9[1]
DOC $mg.L^{-1}$)	12.8[3]	3.8 (3.2-4.9)			2.61 (1.55-6.2)[2]	7.89[1]
POC ($\mu g.L^{-1}$)	210 (180-240)	426 (398 -471)	0.476 (0.45-0.51)	2.53 (0.98-1.17)		316[1]
PH	7.29 (7.23-7.40)	7.0 (6.99-7.01)	7.0 (6.86-7.10)	8.09		
NO3 (mg.L-1)	0.049[3]				16[2]	
H_20 temp (°C)	18.5	18.75 (18.0-19.5	20.75 (20.0-21.5)	13.7°C		
TDS					62 (54-75)[2]	
TOC ($mg.L^{-1}$)				4.36 (4.1 -4.5)		
K ($mg.L^{-1}$)	2.74(2.61-2.84)	1.83 (0.54-1.11)		7.85 (7.77-7.91)		1.3[1]
Chlorophyll $\alpha(\mu g.L^{-1})$	3.36[3]					1.5

[1]Paterson 1996
[2]Kidd 1996
[3]Cronberg *et al.*1995

APPENDIX 6

Relationship between log tDDT (ng.g^{-1} wet weight) and log length (mm) of fishes of the Okavango delta.



APPENDIX 7

Relationship between log tDDT (ng.g^{-1} wet weight) and log weight (g)of fishes of the Okavango delta.