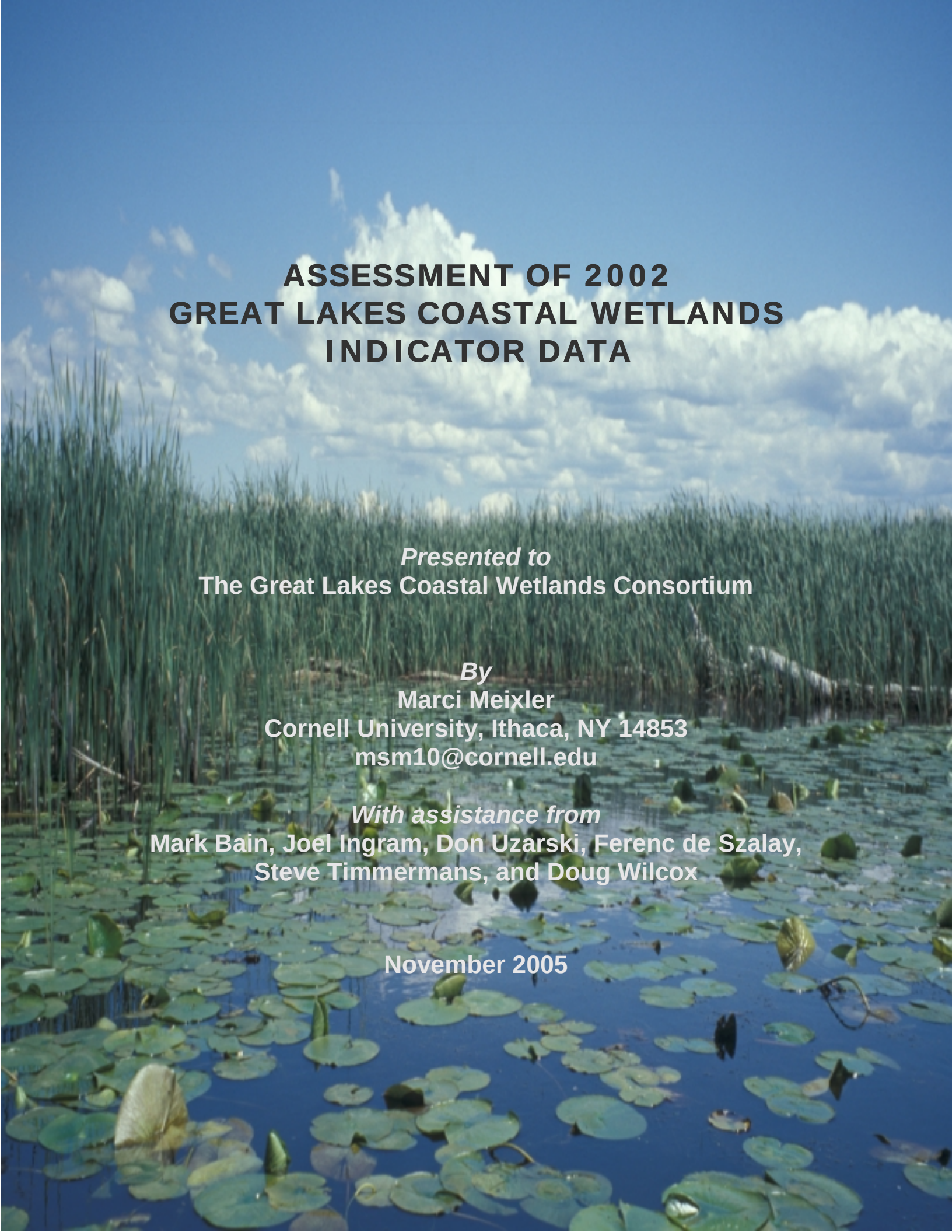

ASSESSMENT OF 2002 GREAT LAKES COASTAL WETLANDS INDICATOR DATA

Presented to
The Great Lakes Coastal Wetlands Consortium

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EXECUTIVE SUMMARY

The overall goal of the Great Lakes Coastal Wetlands Consortium (GLCWC) is to develop a basin-wide biological monitoring program for Great Lakes coastal wetlands that can report on wetland health as it pertains to anthropogenic disturbance. This initiative evolved from State of the Lakes Ecosystem Conferences (SOLEC) and the recognized need for bi-national reporting on Great Lakes ecosystem health.

In an effort to begin evaluation of indicators of wetland degradation, six teams of investigators collected data on fish, macroinvertebrates, birds, amphibians, vegetation, water chemistry, and landscape attributes in 2002. All investigators agreed to collect the same flora, fauna, physical, and landscape level data using standardized protocols.

This report furthers the development of a long-term, bi-national coastal wetland monitoring program by compiling the results of the six teams and evaluating the degree to which each of the indicators can be used to diagnose wetlands status across the basin. Each indicator was evaluated in terms of: cost, measurability, basin-wide applicability of sampling by wetland type, availability of complementary existing research or data, indicator sensitivity to wetland condition changes, and ability to set endpoint or attainment levels. Three combinations of indicators were recommended based on cost, time, and sensitivity optimization.

Based on overall indicator evaluation, we recommend that monitoring programs sample water chemistry, fish, macroinvertebrates, and landscape attributes as these indicators have the highest degree of sensitivity, applicability, measurability and complementary data availability for the lowest cost. If only biological monitoring is to be completed, we recommend fish and macroinvertebrates be considered for inclusion in a bio-monitoring program.

INTRODUCTION

Wetlands are important, highly productive natural systems in the Great Lakes basin as they serve a variety of valuable functions including flood control (Thibodeau and Ostro 1981, Maynard and Wilcox 1997), groundwater filtering and recharge (Maynard and Wilcox 1997), nutrient uptake (Mitsch *et al*. 1979, Whigham *et al*. 1988, Johnston 1991), shoreline stabilization (Wang *et al*. 1997), water quality improvement (Maynard and Wilcox 1997), structural habitat diversity (Jude and Pappas 1992, Brazner and Beals 1997), food chain production (Maynard and Wilcox 1997), and erosion control (Maynard and Wilcox 1997). Further, wetlands exhibit exceptional biodiversity due to their varied structural habitats (Jude and Pappas 1992, Randall *et al*. 1996, Brazner and Beals 1997). Hundreds of species of common and rare macroinvertebrates, fish, amphibians, birds, and mammals inhabit coastal wetlands in the Great Lakes. Additionally, wetlands provide important breeding habitat for macroinvertebrates (Batzer *et al*.1999), fish (Feierabend and Zelazny 1987), amphibians, birds (Wharton *et al*. 1982, Gibbs 1993) and mammals (Gibbs 1993). As sites of natural integrity and ecosystem function, wetlands are inherently valuable components of the region's landscape (Mitsch and Gosselink 2000).

Despite the benefits wetlands provide, Great Lakes coastal wetlands continue to be threatened (Maynard and Wilcox 1997). Currently coastal wetlands in the Great Lakes are estimated to cover an area of approximately 1200 km^2 (Herdendorf *et al*. 1981, Mitsch and Gosselink 1993). This reflects a 60-80% loss in wetland area since European settlement due largely to agricultural practices, land filling, and residential/industrial development (Whillans 1982, Jude and Pappas 1992, Comer *et al*. 1995). Further, no current system is in place to consistently measure or monitor the status of coastal wetlands either in terms of wetland loss or degradation. Concerned scientists, policy makers, managers and other stakeholders established the Great Lakes Coastal Wetland Consortium (GLCWC) to address this need. The goal of the Consortium is to develop and implement a sustainable, long-term, basin-wide monitoring plan for Great Lakes coastal wetlands.

In 2002 the Great Lakes Commission, through the Great Lakes Coastal Wetland Consortium Initiative, funded six teams of investigators to collect data on a suite of indicators in wetlands throughout the Great Lakes. The data collected were then used by select investigators to develop and test multimetric indices of biotic integrity (IBI) that could be employed in a bi-national monitoring program by federal, state, and local agencies to evaluate environmental integrity of Great Lakes Coastal wetlands. This report will further the development of a long-term, bi-national coastal wetland monitoring program by compiling the results of the six teams and making recommendations on the degree to which these techniques can be used to diagnose wetlands status across the basin.

Objectives

The objectives of this report are the following:

- Compile the results from the six collection efforts to determine the effectiveness of each indicator in terms of:
    1. Cost
    2. Measurability
    3. Basin-wide applicability of sampling by wetland type
    4. Availability of complementary existing research or data
    5. Indicator sensitivity to wetland condition changes
    6. Ability to set endpoint or attainment levels
- Define specific standard operating procedures (SOP) for methodologies employed (Appendix B)
- Make recommendations on the degree to which these techniques can be used to diagnose wetland status across the basin

DATA COLLECTION

Site selection

Six project teams sampled a total of 60 wetland sites within the Great Lakes basin (Figure 1; Table 1). Sites were chosen to represent a considerable portion of the Great Lakes coastline, a gradient of human disturbance based on the surrounding land use, and a wide variety of geomorphic wetland types (Minc and Albert 1998, Chow-Fraser and Albert 1998). Wetland types included open lacustrine wetlands, protected lacustrine, barrier beach lagoon, and ridge and swale complexes. Open lacustrine wetlands lack a protected barrier and are largely exposed to wave action and storm surge activity from the lake resulting in decreased organic matter accumulation and vegetation development. Open lacustrine sub-types include open shoreline, characterized by erosion resistant substrate, and open embayment, characterized by gravel, sand, and clay substrate. Protected lacustrine wetlands have some protection by bay or sand-spit formation, resulting in greater organic sediment accumulation and vegetation development than open lacustrine wetalnds. Although these wetlands are protected from wave energy, the wetlands are not hydrologically isolated from the Great Lakes. Sub-types include protected embayments and sand-spit embayments. Barrier beach lagoons form behind sand beach or dune barriers reducing mixing of Great Lakes waters with those of the wetlands. Ridge and swale complexes encompass sand-spit swales, occurring between the recurved fingers of sand-spits and dune and swale complexes, present between relict beach ridges. All wetland types listed above were sampled in the 2002 study. Names, locations, and wetland classifications of the 60 sites sampled are available in Appendix A.

TABLE 1. Project codes and sampling location information for six project teams.

Code	Principle Investigator	Location sampled	Sampling dates	Number of sites
CALE	Timmermans	CA Lake Erie	Apr. - Aug. 2002	11
CALO	Ingram	CA Lake Ontario	Apr. - Aug. 2002	12
USLE	de Szalay	US Lake Erie	June - Aug. 2002	8
USLM	Wilcox	US Lake Michigan	Apr. - Sept. 2002	1
USLO	Bain/Meixler	US Lake Ontario	May. - Aug. 2002	8
USMH	Usarski	US Lakes Michigan and Huron	July - Aug. 2002	20

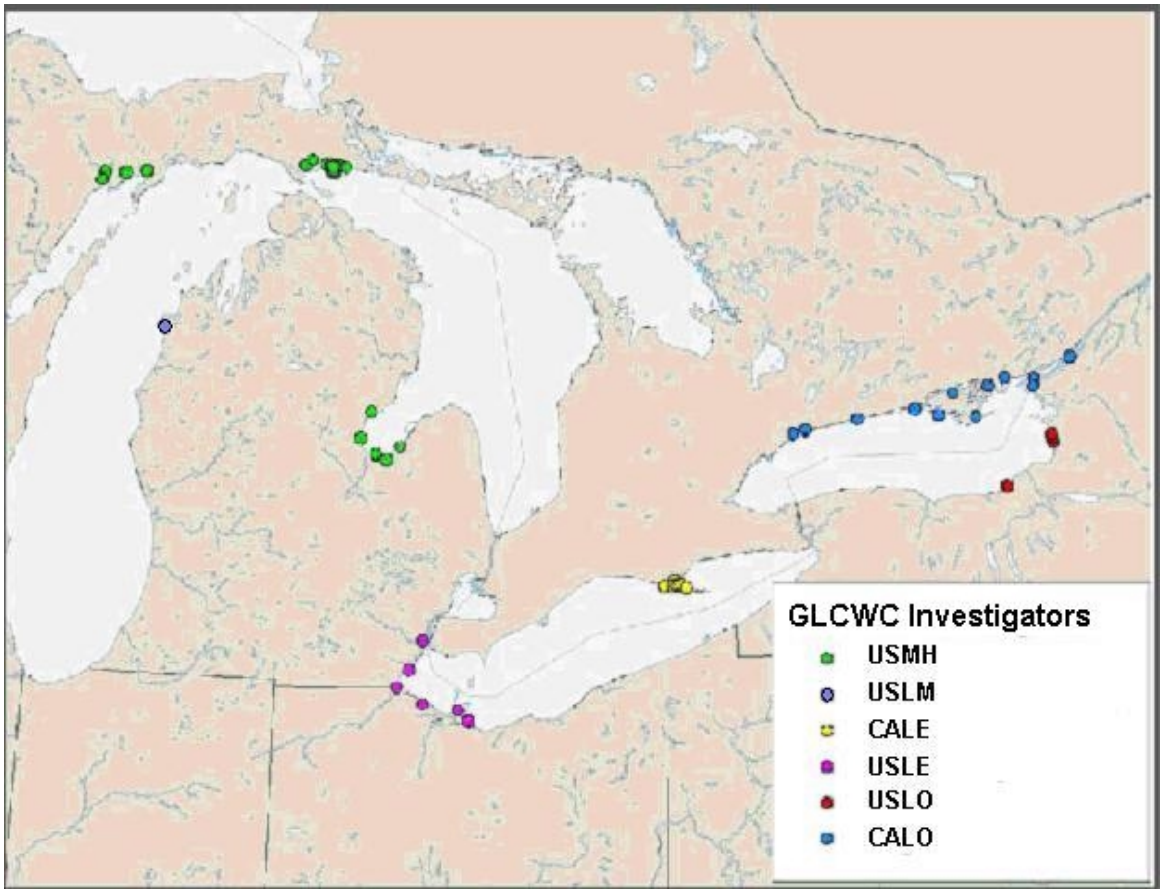

FIGURE 1. Sampling locations for the six project teams.

Indicator sampling

The following indicator metrics (Bertram and Stadler-Salt 2000) were sampled in coastal wetlands between May and September of 2002: fish community health and DELTs (Sanders *et al*. 1999, indicators #4502 and #4503), macroinvertebrate community health (#4501), bird diversity and abundance (#4507), amphibian diversity and abundance (#4504), plant community health (#4513), nitrate and total phosphorus (#4860), contaminated sediments (#119), and landscape attributes (#8132). The indicators sampled by each project team are displayed in Table 2. Project reports (Uzarski *et al*. 2002, Wilcox 2002, Grabas *et al*. 2003, de Szalay *et al*. 2003, Meixler and Bain 2003, Timmermans and Craigie 2003) were used to compile information for indicator evaluation. The use of snapping turtle eggs as an indicator of contaminant exposure

(#4506) was studied by Mayne *et al*. (2004). Their findings will not be included in the indicator evaluation section of this report. Instead, a white paper of their findings is included as Appendix C of this report.

TABLE 2. Indicators sampled by project teams during summer 2002.

	Fish				Macroinvertebrates									
	Fyke nets	Minnow traps	Electro-fishing	Gill nets	Sweep Net	Activity traps	Hester-Dendy	UV Blacklight	Birds	Amphibians	Vegetation	Water chemistry	Sediment	Landscape attributes
CALE	X	X			X				X	X	X	X		X
CALO	X	X			X				X	X	X	X		X
USLE	X	X			X	X		X			X	X		X
USLM	X				X	X		X	X	X	X	X		X
USLO	X	X	X	X	X	X	X				X	X		X
USMH	X	X			X						X	X	X	X

All teams were tasked with following the same set of standardized procedures. Data from teams that did not follow the prescribed procedures were not included in the indicator sensitivity analyses. Procedures are described in detail in Appendix B (Standard Operating Procedures).

Some indicators were sampled using multiple gears or methods. Fish were sampled using fyke nets, gill nets, minnow traps, and electrofishing gear. Macroinvertebrates were sampled with sweep nets, activity traps, Hester-Dendy artificial substrate samplers, and UV blacklight traps. Landscape attributes were assessed both in the field and with Geographic Information System (GIS) methods. We have not undertaken to do a study of appropriate sampling gear for each indicator in this study as only fyke netting and sweep netting were tested through indicator sensitivity analysis. We included all gears in the cost analysis section but only included fyke netting and sweep netting for fish and macroinvertebrate sections respectively in the remainder of the report. Field and GIS methods were lumped under the heading landscape attributes as most teams performed both.

IBI development

The collected data were used by select investigators to develop and test multimetric indices of biotic integrity (IBI) that could be employed in a bi-national monitoring program by federal, state, and local agencies to evaluate environmental integrity of Great Lakes coastal wetlands. Indicators and metrics for wetlands associated with the Great Lakes basin were previously developed for macroinvertebrates (Burton *et al*. 1999, Lougheed and Chow-Fraser 2001), fish (Randall *et al*. 1996, Minns *et al*. 1994) and plant communities (Mack *et al*. 2000). However, much of their work was completed on inland wetlands or coastal wetlands within a limited geographic area and of a specific geomorphic type (Grabas *et al*. 2003). IBIs developed under this effort will strive to have bi-national coverage and applicability in a wide range of geomorphic wetland types. IBIs were created for fish (Uzarski *et al.* submitted), macroinvertebrates (Uzarski *et al.* 2005),

birds (Crewe and Timmermans 2005), amphibians (Crewe and Timmermans 2005), and vegetation (Minc and Albert 2004).

INDICATOR EVALUATION

The six project teams collected data on fish, macroinvertebrates, birds, amphibians, vegetation, water chemistry, sediments, and landscape attributes for evaluation of their use as indicators of wetland degradation. We evaluated these indicators in terms of six factors: cost, measurability, basin-wide applicability and sampling by wetland type, availability of complementary data, indicator sensitivity to wetland condition changes, and ability to set endpoint or attainment levels. Each factor was scored based on a set of appropriate metrics. Scores for each factor were then weighted and combined. The resulting scores can be compared across indicators to evaluate indicator strength and make recommendations regarding optimal surveying protocols. Factors were then re-weighted based on three optimization scenarios: decreased cost, decreased time, and increased indicator sensitivity. The overall indicator scoring and optimization scenarios are robust enough to allow for slight changes in factor values without observing appreciable differences in the results.

Descriptions of the factors, indicator performance evaluations, and metrics used for scoring and weighting are described for each factor in the sections that follow. Overall indicator scoring and optimization scenarios are presented in the "overall indicator scoring and optimization scenarios" section.

Cost

Total labor, effort, and expenses were tracked for each biotic indicator by the six project teams (Table 3). Labor describes the number of personnel required, effort describes the number of person-hours required, and expenses describe the equipment and consumables needed to sample a wetland for each indicator.

Labor

Labor is measured by the number of personnel required to perform a task (e.g., set a net, sample along a transect, visit a birding station, etc.).

Effort

Effort is measured in person-hours which refers to the total number of hours needed to perform a task. When interpreting information from project reports, person-days were assumed to be 8 hours and person-evenings (primarily used to describe bird and amphibian surveys) was assumed to be 4 hours. Time is not included for such activities as identifying monitoring sites, gaining access to sites, training, entering and validating data, analyzing results, etc.

Expenses

Expenses are listed per wetland with approximately 2 plant zones sampled per wetland. All expenses are shown in US dollars; expenses for Canadian projects CALO and CALE were converted to US dollars. Costs for buying large equipment, assumed already owned by agencies, were not included. Likewise, salaries to hire field staff and qualified specialists were not included. Travel and accommodation expenses can be significant depending on site locations however, these varied greatly by project and thus were also not included. Expenses for general field equipment (i.e. equipment used across all indicators) were subdivided among indicators. Expenses shown in Table 3 represent start-up costs, a large portion of which (85%, Grabas *et al*. 2003) are non-consumables (e.g. nets, field guides, etc). Monitoring programs should expect to spend approximately $240 on consumables for each wetland studied per year. The approximate cost of boat

outfitting and maintenance per summer is $368. This includes engine maintenance, tools, necessary emergency equipment, grease, oil, propeller repair, etc.

Fish sampling expenses for fyke netting, minnow trapping, electrofishing, and gill netting include equipment such as: fyke nets, minnow traps, electrofishing boat, gill nets, PVC pipe, ethanol, fish ID books, measuring boards, dip nets, ropes, and buoys. Fish sampling expenses do not include costs for a boat (not rigged for electrofishing; ~$1000), motor (~$1,500), boat trailer (~$900), gas, or boat maintenance despite the fact that generally fyke netting and gill netting use a boat.

Macroinvertebrate sampling expenses for sweep netting, activity trapping, Hester-Dendy sampling, and UV blacklight trapping and associated laboratory identification include equipment such as: sweep nets, activity traps, Hester-Dendy samplers, PVC pipe, sieves, pans, ethanol, macroinvertebrate ID books, counters, tweezers, forceps, vials, ropes, and buoys. Macroinvertebrate expenses do not include the cost of a dissecting microscope (~$1500), bench mat (~$500), or light source (~$200).

Bird and amphibian expense estimates include: binoculars, tape players, field guides, backpack, compass, stopwatch, stakes and flagging. Vegetation sampling expense estimates include: plant bags, plant press and blotters, underwater camera and film, quadrat frame, rake, cooler/fridge, hand lens, and field guides. Vegetation expense estimates do not include: canoe (~$800), PFDs (~$100), and paddles (~$30).

Water chemistry and sediment expense estimates include: standards, mixing bowls, spoons, bottles/flasks, depth and sediment probes, calibration rack, hand pump, filters, graduated cylinders, beakers, pipettes, thermometer, secchi disk, cooler, ice packs, whirlpaks or sample bottles. Water chemistry expenses do not include: laboratory analysis (~$42-200/sample), hydrolab (~$5,300-$12,000), DR890 (~$1,120), or equipment for analysis (e.g., gas chromatograph (~$20,000), mass spectrometer (~$85,000), mercury analyzer (~$25,000), ICP (~$50,000), TOC analyzer (~$20,000),

amonia electrode and ion meter (~$5,000), Sozhlet extraction system (~$10,000), Sample concentrator (~$10,000), and sieves (~$650)).

Landscape attribute expense estimates include: GPS unit, paper, binoculars, batteries, topographical maps, digital planimeter, and air photos. Landscape attribute expenses do not include the cost of hiring a GIS specialist.

General equipment subdivided between all categories includes: waders, depth stick, tape measures, tools, maps/gazetteer, camera/film, clipboards, rite-in-the-rain paper, tape, scissors, pens/markers, flagging, batteries, VHF radio, first aid supplies, dry sacks, cases for equipment, flashlights, GPS unit, and compass.

Scoring and weighting

Median labor, effort, and expense values for each indicator were calculated based on the information submitted by the six project teams (Table 3). We used medians to eliminate biases by projects with substantially low or high labor, effort, and expense requirements. Each metric, labor, effort, and expense, was scored separately based on median values. Only indicator methods used in sensitivity analysis were included in the scoring procedure, therefore minnow traps, electrofishing, gill netting, activity trapping, Hester-Dendy sampling, and UV blacklighting were removed. GIS and field procedures for landscape attribution were combined. Indicator sampling protocols requiring 1 person, 2 people, or more than 2 people were assigned scores of 1, 2/3, and 1/3, respectively, based on the distribution of values. Indicator sampling requiring less than 2 hours, between 2 and 4 hours, or more than 4 hours were assigned scores of 1, 2/3, and 1/3, respectively. Indicator sampling costing less than $50 US, $50-100, or more than $100 per wetland were assigned scores of 1, 2/3, and 1/3, respectively. Thus, when added together, cost scores ranged from 1 to 3 with higher scores being most desirable. Cost is a fairly important factor since agencies are not likely to sample an indicator if the cost is prohibitive. Thus, cost scores greater than 2 were weighted more heavily by multiplying those values by 2 (Table 4).

TABLE 3. Expense ($US), effort (person-hours), and labor (number of personnel) estimates from 2002 sampling of indicators in Great Lakes wetlands. Expense estimates are on a per wetland basis; effort and labor estimates are on a per net/transect/visit basis. Expenses for CALO and CALE were converted to US dollars. Travel time not included.

		Fish				Invertebrates									Landscape attributes	
		Fyke Netting	Minnow Trapping	Electro-fishing	Gill netting	Sweep netting	Activity trapping	Hester Dendy	UV blacklight	Birds	Amphibians	Vegetation	Water Chemistry	Sediments	GIS analysis	Field
CALE	Expenses	$241.32	$13.90			$18.29				$59.06	$12.82	$11.93	$44.82		$6.15	$6.15
	Effort	4	2			0.67*				1.04	1.58	12	-		8.73	0.083
	Labor	2	2			-				-	-	2	-		1	1
CALO	Expenses	$233.31	$20.34			$97.11				$50.54	$27.84	$36.34	$236.01			$107.07
	Effort	3.12	1.54			1.25*				10	15	4	3.16			7.17
	Labor	2	2			3				2	2	2	1			1
USLE	Expenses	-	-			-	-		-			-	-			-
	Effort	6.03	2.97			2.33/13.5	2.16		4.25			8	1.00			1.00
	Labor	3	2			1 field/1 lab	1 field/2 lab		1 field/1 lab			3	1			1
USLM	Expenses	-				-	-		-	-	-	-			-	
	Effort	2.5				1.12*	0.5*		1.65*	1.33	2	8.89			4	
	Labor	2				2	2		2	1	1	2			1	
USLO	Expenses	$504.59	$20.58	$3,170.52	$231.53	$76.75	$44.01	$59.87				$42.50	$273.74		$39.06	$65.56
	Effort	3.5	3.03	0.75	4.03	3.5	1.67	1.67				15.7	2.09		3	0.33
	Labor	2	2	3	2	3 field/1 lab	1 field/1 lab	2 field/1 lab				3	1		1	1
USMH	Expenses	$131.90	$4.55			$24.41						$25.85	-	$55.60		$4.00
	Effort	6	3			11.25						8.35	1.75	4		-
	Labor	-	-			-						-	-	-		-
Median expenses		$237.31	$17.12	$3,170.52	$231.53	$50.58	$44.01	$59.87		$54.80	$20.33	$31.10	$236.01	$55.60	$22.61	$35.86
Median effort		3.75	2.97	0.75	4.03	7.38	1.92	1.67	4.25	1.33	2.00	8.62	1.92	4.00	4.00	0.67
Median labor		2	2	3	2	2.5	2	2	1.5	1.5	1.5	2	1		1	1

*Does not include ID time

TABLE 4. Scoring of indicators for cost. Higher scores indicate lower labor, effort, and expense requirements.

	Fish (Fyke)	Macroinvertebrates (sweep)	Birds	Amphibians	Vegetation	Water chemistry	Sediments	Landscape attributes
Expense score	0.33	0.67	0.67	1.00	1.00	0.33	0.67	0.67
Effort score	0.67	0.33	1.00	0.67	0.33	1.00	0.67	0.33
Labor score	0.67	0.33	0.67	0.67	0.67	1.00	1.00	0.67
Cost score	1.67	1.33	2.33	2.33	2.00	2.33	2.33	1.67
Weighted cost score	1.67	1.33	4.67	4.67	2.00	4.67	4.67	1.67

Measurability

Measurability is evaluated by assessing 1) the level of expertise and training required by staff to implement each of the methodologies and 2) the ease of implementation of each of the methodologies. Detailed descriptions of measurability for each indicator follow.

Fish

Level of expertise and training

The crew leader should possess at least a fish and wildlife technology diploma or a Bachelors degree in fisheries biology with netting and fish identification experience. One of the crew members should also have experience or be able to identify and describe general aquatic plant community types and wetland attributes. All field technicians should be in good physical shape, capable of lifting heavy equipment (i.e., nets full of turtles and fish, outboard motor), be familiar with small outboard motor and boat operation, know how to properly check, process, repair, monitor and store fishing gear, be able to identify fish (adult and young-of-the-year) to species, and have experience with the proper handling and measurement of fish. Turtles are commonly trapped in fyke nets so at least one crew member should be familiar with the proper techniques for handling turtles to ensure that neither the handler nor the turtle suffer injury.

A one-day field trip prior to the beginning of the field season is useful to teach field technicians the proper setting and use of fishing gear, where to set nets and traps, and how to process samples, locate and differentiate vegetation zones, and properly identify fish species and DELTs. Training sessions covering these topics will ensure that fish community data in coastal wetlands are collected in a standardized manner.

Ease of implementation

Fyke-nets can be used in almost any wetland with a suitable bottom profile to accommodate the proper setting of nets. In many locations however, shallow soil made it impossible to drive the fyke net stakes in far enough to secure the nets. Instead, areas within the site with deeper deposits of sand were sought. Conversely sites with deep (>1

m) unconsolidated sediment are difficult to wade through. Thus, the setting and collection of nets and traps took longer in these sites than at most other sites due to the difficulty of maneuvering on foot. Dense, submerged aquatic vegetation rendered the outboard motor useless and further exacerbated the maneuverability problem. Both large and small fyke nets were required to capture fish in vegetation communities of varying water depths. Due to above average lake water levels, attempts to set fyke nets in Scirpus stands were often thwarted because these stands generally occurred at water depths too deep to sample.

Macroinvertebrates

Level of expertise and training

In field sampling of coastal wetlands for macroinvertebrates, the lead field technician should have some experience in macroinvertebrate sampling, however, not all technicians require prior specific training. The lead technician should have a minimum of a college degree in environmental studies, with an emphasis on field techniques, including some level of experience with macroinvertebrate collection and sorting. Prior experience with aquatic macroinvertebrates will produce more uniform and standardized field collection techniques that will better capture the taxonomic diversity of the communities present in coastal wetlands.

Prior to the field season, the lead field technician should conduct a one-day field training exercise where the supporting field staff are taught how to collect macroinvertebrates, where to sample, how to differentiate vegetation zones, and properly sort, pick, and store macroinvertebrates according to protocol. This also allows the technicians to familiarize themselves with sampling macroinvertebrates in a wetland setting and standardizes the techniques.

Lab identification and sorting of marcroinvertebrates by taxonomic group requires skilled technicians trained and familiar with aquatic macroinvertebrate identification. Project teams found that staff members with previous training and/or experience were much more efficient at processing macroinvertebrate samples than staff with no previous training.

Previous training consists of either an undergraduate course in aquatic entomology or on-the-job training.

Ease of implementation

Sweep netting can be affected by a variety of biases. Location bias can have a substantial impact on the results since the selection of a place to sweep is somewhat subjective. The operators may be biased either for or against cryptically-patterned organisms, animals that move quickly as opposed to those that are more sessile, or animals about which they have more knowledge or interest. Some project teams encountered more uncertainties in how to process samples because they often collected considerable aquatic vegetation in the sweep nets. This made it difficult to sub-sample by grids in the sorting pan so they often resorted to opportunistically selecting specimens both directly from the vegetation and from the sorting pan.

Lab-sorting samples may eliminate some of the biases in mentioned above. However, testing of lab-sorting procedures against live-sorting in the field revealed that lab-sorted samples took almost three times longer to process and may not provide any more information than live-sorted samples (de Szalay *et al*. 2003)

Although some difficulties were encountered in sites with extensive floating mats of emergent vegetation, samples obtained from a canoe appeared to yield a macroinvertebrate community similar to locations where the sampler was able to sample by foot.

Project team USMH determined that the ranked order of sites produced by their IBI with data at the higher taxonomic resolution was fairly similar to the order produced by the modified IBI using family-level macroinvertebrate data (Uzarski *et al*. 2005). The modified IBI produced scores on average 2% lower than those of the unmodified IBI. Therefore, macroinvertebrates need only to be identified to the family level to achieve a similar level of accuracy required by their modified IBI.

Birds and amphibians

Level of expertise and training

The Marsh Monitoring Program was designed as a volunteer-based program to survey birds and amphibians throughout the Great Lakes basin in marsh wetland habitats so no specific education is required to conduct surveys (Timmermans and Craigie 2003). The program is designed to accommodate the needs of surveyors ranging from amateur to professional. Still, surveyors must have some knowledge of wetland ecosystems and species identification skills. Specifically, for amphibian surveys, participants must be able to correctly identify the calls of the 13 species of frogs and toads found in the Great Lakes basin. For the marsh bird surveys, participants should be able to correctly identify at least 50 species of wetland birds by both sight and sound. Surveyors are required to complete a habitat description at each station. While this does not require a high level of botanical expertise, participants must be able to recognize and make distinctions between the major groups of wetland plants. In general, preference should be given to volunteers or paid staff with demonstrated experience and expertise in these areas. In terms of training, surveyors need to familiarize themselves with the MMP instruction booklet and training tape provided by Bird Studies Canada (BSC). Surveyors can use the training tape to ensure that they are able to identify all calls/songs of amphibians and/or birds before commencing their surveys. The MMP staff and partner support network often conduct training seminars and field demonstrations and are available to provide additional training upon demand.

Ease of implementation

Amphibian and marsh bird communities have intra- and interspecifically dispersed breeding phonologies. Thus, multiple surveys are often required to adequately measure peak occurrences and abundances across species. This requires at least three survey visits to each station for amphibians and two survey visits to each station for birds. Because timing and weather conditions are critical to gathering accurate and representative observation data for amphibians and birds, special attention was devoted toward deciding when to conduct each survey.

For wetland sites dispersed over great distances, it was challenging for a small team of surveyors to adequately survey all wetland sites for the required number of visits during the appropriate survey periods. Personnel coming from long distances clearly do not have the flexibility of local volunteers who have a greater ability to initiate surveys on short-notice as soon as conditions become ideal. The main difficulty encountered in implementing the bird and amphibian surveying program was created by the lack of complete coverage by volunteers.

The most realistic and viable approach for including marsh bird and amphibian monitoring into a Great Lakes coastal monitoring program would be to engage a combination of volunteer citizens, municipal, state, provincial, federal, or non-government agencies, institutions or organizations, and in certain cases, paid personnel most or all of whom are situated in close proximity to their respective coastal wetland sites. It is likely that volunteers could be engaged to monitor many coastal wetland sites in proximity to human habitation. However, wetlands closer to urban areas or major transport corridors have higher ambient noise than more remote wetlands, thus creating sampling difficulties. This may be further exacerbated if the tape player cannot be heard by another person 100 m away as was sometimes the case for some project teams.

Vegetation

Level of expertise and training

Plant sampling in coastal wetlands requires familiarity with a relatively diverse monocot flora of sedges, rushes, spike-rushes, bulrushes, grasses, pond weeds, and many other genera, often in sterile condition. Typically, specimens require further examination with a microscope for final identification. Coastal wetland sampling requires field technicians who have worked extensively with these aquatic plants. The lead field researcher should be very familiar with the above mentioned submerged and emergent coastal wetland plant communities, be able to identify and key plant taxa to the level of species or sub-species, and be familiar with standard identification references. The ability to correctly identify all species encountered is essential. In addition, the researchers should be able to identify wetland soil types, have knowledge of plant specimen collection and preservation

techniques, and be familiar with transect and quadrat sampling procedures. Further, it would be helpful if sampling personnel were able to properly identify localities for transect sampling through the use of air photo interpretation and mapping. An individual with a degree in an environmental/biological field with an emphasis on botany/plant ecology, and/or experience sampling wetland vegetation communities would have a background in such skills.

Ease of implementation

Some teams found the plant sampling quadrat method to be confusing and difficult to employ, likely a result of inexperience with the methodologies. The placement of sampling points at 25 m intervals resulted in the potential for quadrats surrounding one sampling point to fall into more than one vegetation type (e.g., the sampling point fell at the interface of sedge meadow and mud-flat communities).

Sampling submerged zones required the use of a boat or canoe, and hence represented a greater cost. Sampling of the wet meadow and the emergent zones did not require this equipment, except in situations where access to suitable sampling areas in these zones was greatly facilitated by, or only possible with, the use of such items.

Several teams felt that the incorporation of color infrared air photo interpretation into their methods was important for transect location purposes, especially for sites that were large. Air photos provide easy and accurate evaluation of many landscape characteristics. Air photos can also be used to monitor changes in major vegetation types and invasive vegetation types over time using metrics such as percent wetland in dominant emergent vegetation type and percent wetland in invasive vegetation types. However, aerial photography cannot be relied upon to identify most populations of exotic plants (Uzarski *et al*. 2002). Even in areas where exotic plants are abundant, exotics may not necessarily be abundant or dense enough to be consistently identified from aerial photography.

Random surveys for presence/absence of plant species seemed to be valuable for species richness measurements and were relatively easy to perform. If random surveys are deemed

useful in a vegetation IBI, observations should be completed in all zones of the wetland, including those deemed too small to warrant quadrat sampling.

Sampling during a standardized timeframe (ideally mid July to August) will minimize the influence of seasonal variability of the density, apparent dominance, and frequency of individual plant species.

Water chemistry

Level of expertise and training

Trained field personnel are required to complete water collection and analyses properly. Preference should be given to individuals with field experience who are capable of problem solving within a field or laboratory setting and have at least a college degree in an environmental program. Although direct experience with the equipment (i.e., Quanta Hydrolab) is not necessary, individuals should have experience with other field meters to ensure they are familiar with the operation and the necessity for regular calibration. All personnel should be instructed to use the field sheets as a template to ensure all required data are collected following a standardized procedure in the field. To ensure that QC/QA objectives are met, personnel measuring nutrient parameters must be familiar with and have the means to complete standard curves, analyze blanks and duplicates, and be able to determine repeatability of sample results. Samples should either be processed in an appropriate limnological laboratory by professional staff, or adequately trained project personnel must be able to conduct QC/QA activities under improvised laboratory conditions. In keeping with the objective of producing reproducible and reliable data, trained personnel must also have demonstrated that they have good laboratory technique, either through education, experience or adequate in-house training (i.e., they can run a standard curve in duplicate within acceptable limits).

At least two weeks prior to entering the field, a training session should be completed to ensure that all equipment operates properly, personnel are trained in the field procedures for collecting physical and chemical parameters and calibration of field equipment, and potential problems are identified. Problems that may be encountered include expired

calibration standards, malfunctioning equipment requiring servicing, or logistical issues involving access to sites.

Ease of implementation

Several teams observed that turbidity and other water chemistry factors changed within a wetland from day to day. In particular, turbidity in shallow sites with fine inorganic sediments and sparse emergent vegetation increased on windy days due to wave action churning up the sediments. For water chemistry among sites to be directly comparable in future years, sampling should be completed under calm weather conditions, if possible. However, the nature of open bay sites leaves them more exposed to wind and wave action and thus, sampling in windy conditions may provide more representative, though highly variable, data.

Sediments

No information provided.

Landscape attributes

Level of expertise and training

Surveyors should have experience working in Great Lakes coastal wetlands and be able to identify and distinguish between all hydrologic and landscape alterations surrounding wetland sites, particularly the less obvious activities (i.e., tile inlet, presence of filling, sediment input, etc.) and processes (i.e., dredging, filling, sedimentation, point source pollution, etc.). In addition, surveyors should be in good physical shape and be able to traverse difficult wetland terrain. Skills required for land use data collection are basic air photo interpretation ability and planimeter use. Air photo interpretation skills can be limited to an ability to identify broad land use types incorporated in this study. Personnel need not have prior experience with planimeter use and topographic map reading as these skills are easily learned and applied in a very short time. Specific spatial analytical skills may be required for one or more personnel if GIS techniques are employed. In particular, the ability to manipulate geographically referenced data and digitized spatial datasets.

Ease of implementation

Landscape attribute variables were generally easy to collect. However, because the data were collected from one central location in the wetland, it was difficult to observe all landscape attributes from this location. Many of the variables identified could not be readily investigated in the field without more time consuming preliminary field sampling. For example, cryptic features such as tree removal, tile inlets, and point source inlets can be easily overlooked.

On some sites, access is partially limited by private property limiting the ability to view the entire site. Determination of types of road can be time consuming for large sites. For many variables, this problem was solved by utilizing aerial photography or imagery, which allowed for a more accurate evaluation of land use. However, air photo interpretation requires more time to accurately quantify the variables.

Scoring and weighting

We used the information on level of expertise and training required and ease of implementation to derive scores for the measurability of each of the indicators. Each metric received a score of 1, 2, or 3 depending on the level of expertise and training required and the ease of implementation stated by the six project teams. Higher measurability scores describe indicators with fewer technical requirements and greater ease of implementation. Values were then averaged to arrive at an overall measurability score for each indicator. Measurability is a fairly important factor, as agencies are not likely to survey an indicator if a high degree of skill is required or the sampling methodology is too cumbersome. Thus, measurability scores greater than 2 were weighted more heavily by multiplying each of those values by 2 (Table 5).

TABLE 5. Scoring of indicators for measurability. Higher scores indicate greater measurability.

	Fish	Macro-invertebrates	Birds	Amphibians	Vegetation	Water chemistry	Sediments	Landscape attributes
Level of technical expertise	2	1	2	2	1	2	N/A	2
Ease of implementation	3	3	3	3	2	2	N/A	2
Measurability score	2.5	2	2.5	2.5	1.5	2	N/A	2
Weighted measurability score	5	2	5	5	1.5	2	N/A	2

Basin-wide applicability of sampling by wetland type

Each of the Great Lakes has distinctly different physico-chemical conditions (Maynard and Wilcox 1997). Therefore, biotic communities in coastal wetlands will be different in each of the Great Lakes. Further, several types of coastal wetlands are found in the Great Lakes basin, and these differ in their hydrogeomorphic conditions (Keough *et al*. 1999). The varied spatial extent of the six projects allowed examination of the applicability of the various sampling methodologies across the Great Lakes and in a variety of wetland types. Detailed descriptions of basin-wide applicability for each indicator follow.

Fish

Fyke-netting methods are applicable to virtually any wetland throughout the Great Lakes basin with suitable bottom profiles to accommodate the proper setting of nets. Open bay sites are often more exposed to high wind and waves rendering sampling from a small boat at these sites difficult and potentially dangerous. At open bay sites, even a light wind can make net placement and collection markedly more difficult than in protected bays or barrier beaches. No significant differences were found in fish species richness or fish abundance in fyke nets between protected and open lacustrine wetland types in Lake Erie (de Szalay *et al*. 2003). Therefore, it is likely that this methodology can be applied to most coastal wetland sites throughout the Great Lakes basin.

Macroinvertebrates

The analytical methods used for this research have basin wide acceptance for routine monitoring and environmental research. Furthermore, when tested in Lake Erie sites, there were no significant differences in macroinvertebrate abundance or richness between wetland types in sweep net samples (de Szalay *et al*. 2003). Therefore, it is likely that this methodology can be applied to most coastal wetland sites throughout the Great Lakes basin.

Birds and amphibians

The standardized MMP is designed to accommodate surveys to capture breeding phenologies of existing species at each latitudinal region of the Great Lakes basin and has

been doing so at both coastal and inland marsh wetlands since 1995. Data analysis of bird and amphibian data show no differences with respect to location of the wetland or geomorphic type. At a larger basin-wide scale, the MMP can detect adequately small changes in annual indices for most species if routes are monitored at their current level for ten consecutive years. Survey techniques (equipment, personnel required, and sampling methodology) can be applied to any coastal marsh habitat throughout the basin. Thus, the bi-national MMP program has shown promise as a long-term, basin-wide monitoring strategy.

Vegetation

Although the sites in this study occurred in a highly varied landscape, the methodology employed was easily applied to each wetland. When tested for Lake Erie sites, there were no significant differences in plant abundance or richness between wetland types (de Szalay *et al*. 2003). Therefore, it is likely that this methodology, including sampling protocol and sampling equipment, can be applied to most marsh-type coastal wetland sites throughout the Great Lakes basin. However, this approach seemed to be most appropriate for the smaller wetland sites with plant community zones homogeneously located along a topographic gradient. In larger, more complex sites, the data collected from the quadrat sampling did not seem to characterize the vegetation zone adequately. This was particularly evident in many emergent zones and in large wetlands, where many species occurred in a patchy distribution. For example, within all sites, emergent zones were dominated with considerable stands of common cattail (*Typha spp.*), but many sites also contained significant stands of other emergent species (i.e., arrowhead (*Sagittaria*), wild rice (*Zizania*)) that were beyond the cattail stand (in deeper water). When quadrat sampling began in the cattail stand, the entire transect was often located within the confines of the cattail stand. In large sites, vegetation zones often contained patches of different vegetation types within the zone that were dispersed heterogeneously throughout the site at distances that were beyond the length of the sampling transect. Overall, the protocol appears to provide a characterization of the vegetation in close proximity to the transect only and not the entire wetland. Although, this is considered a rapid assessment technique, and not intended to provide a complete wetland characterization, the patchiness of emergent plant

communities at several wetland sites raises the issue of repeatability of wetland plant community metric values.

One of the differences associated with wetland types is variability in zone width. Typically, transects of coastal wetlands are conducted perpendicular to the shoreline to increase the diversity of species encountered in the zones (wet meadow, emergent, and sometimes submergent). In some of the sites, the zones were too narrow to allow for this, and transects were placed in zones parallel to the shoreline. This change in sampling protocol did not appear to alter the results of sampling significantly.

Water chemistry

There were no water chemistry access, sampling or analysis limitations while collecting data from wetlands. As long as proper equipment, personnel and procedures are included in any water chemistry sampling component of a Great Lakes coastal wetland monitoring program, these methods can be applied to any wetland and wetland type throughout the basin. There appeared to be little to no difference among samples from different wetland geomorphic types. This similarity may be related to the selected locations within the wetland since areas immediately adjacent to aquatic vegetation were chosen. Sampling in an open water location of the wetland, or at least 10 meters from the emergent stand, may prove that differences among the wetland types do occur. Special attention to timing of sample collection within certain wetland types (e.g. beach barrier) may be warranted, as drought conditions can drastically change water levels in some shallow wetlands, which can highly concentrate solids and result in substantially high readings for certain parameters.

Although water chemistry data were shown to be reproducible, reliable, and accurate, the approach of collecting information on water chemistry within three meters of the vegetation stand may not necessarily provide results indicative of the level of disturbance in the entire wetland. In wetlands that only contain a fringe of emergent vegetation, the stand of macrophytes provides a protected area where suspended solids can drop out of suspension and nutrients utilized for vegetative growth. Additional studies are required to

determine if the water quality near the emergent vegetation is representative of the water quality in the entire wetland. Sampling in an open water location of the wetland, or at least 10 meters from the emergent stand, may prove that differences among the wetland types do occur (Grabas *et al*. 2003).

Sediments

No information provided.

Landscape attributes

The landscape attribute and land use data collection methodology was easily applied across all study sites and should be applicable for use across the Great Lakes Basin and all wetland geomorphic types. When assessed specifically at Long Point wetland sites in Lake Erie, there was no discernable relationship between overall disturbance ranking and geomorphic wetland type (Timmermans and Craigie 2003). However, intrinsic features of the wetlands such as wetland size and wetland boundary irregularity may influence the repeatability and accuracy of data.

Scoring and weighting

Scoring for basin-wide applicability of sampling by wetland type was based on two metrics: applicability across all Great Lakes basins and applicability across all wetland types. Each metric received a value of 1, 2, or 3 based on each of these metrics. Higher scores indicate greater applicability of indicators. The values were then averaged to arrive at an overall score of applicability for each indicator. Applicability is a somewhat important factor as agencies are not likely to undertake sampling of an indicator not suited for their location or wetland types. However, indicators can be modified to suit specific locations. Thus, applicability scores greater than 2 were weighted more heavily by multiplying each of those values by 1.5 (Table 6).

TABLE 6. Scoring of indicators for basin-wide applicability of sampling by wetland type. Higher scores indicate greater applicability.

	Fish	Macro-invertebrates	Birds	Amphibians	Vegetation	Water chemistry	Sediments	Landscape attributes
Applicability across basin	3	3	3	3	3	3	N/A	3
Applicability across wetland types	2	3	3	3	2	3	N/A	3
Applicability score	2.5	3	3	3	2.5	3	N/A	3
Weighted applicability score	3.75	4.5	4.5	4.5	3.75	4.5	N/A	4.5

Availability of complementary existing research or data

Available complementary data can be used to augment or enhance collected data to provide more information about wetland condition. Possible sources of complementary data were located or collected from information supplied by project teams.

General

Several research projects that collect data on Great Lakes coastal wetlands have published their data in papers and project reports. The Great Lakes Environmental Indicators project has links to a breadth of information on macroinvertebrates, fish, birds, amphibians, vegetation, and water chemistry (http://glei.nrri.umn.edu/). The USGS Great Lakes Science Center has a section of it's website devoted to wetland ecology with links to current and completed research (http://www.glsc.usgs.gov/). The NOAA Great Lakes Environmental Research Laboratory has biological, chemical and hydrological data easily downloadable from http://www.glerl.noaa.gov/. State and provincial environmental agencies are also likely to have information on coastal wetlands. Contact information for each of these agencies can be accessed at the following websites: USEPA (www.epa.gov), Environment Canada (http://www.ec.gc.ca/), Illinois Department of Natural Resources (http://dnr.state.il.us/), Indiana Department of Natural Resources (http://www.in.gov/dnr/), Michigan Department of Environmental Quality (http://www.michigan.gov/deq), Minnesota Department of Natural Resources (http://www.dnr.state.mn.us/index.html), New York Department of Environmental Conservation (http://www.dec.state.ny.us/), Ohio EPA (http://www.epa.state.oh.us/), Pennsylvania Department of Environmental Protection (http://www.dep.state.pa.us/), Wisconsin Department of Natural Resources (http://www.dnr.state.wi.us/), Ontario Ministry of Natural Resources (http://www.mnr.gov.on.ca/MNR/), and Quebec Environment and Sustainable Development (http://www.gouv.qc.ca/Vision/Territoire/Environnement_en.html)

Fish

In 2002, fish were collected by Don Uzarski and others from 61 sites in both the United States and Canada. For some sites in Lakes Michigan and Huron yearly fish data exist back to 1999 and for a few sites back to 1994.

In Canada, an extensive coastal wetland fish sampling program is ongoing in the western Lake Ontario wetland, Cootes Paradise. This project is lead by Tys Theysemeyer at the Royal Botanical Gardens. In addition, the Durham Region Coastal Wetland Monitoring Project, led by the Canadian Wildlife Service and the Central Lake Ontario Conservation Authority, is repeatedly assessing fish assemblages at 15 Lake Ontario coastal wetlands. This string of coastal wetlands starts just east of Toronto, at the Rouge River and extends east 56 kilometers to the Port of Newcastle. Lastly, the Ontario Ministry of Natural Resources' various Fisheries Assessment Units have reports and publications with data from Great Lakes Canadian coastal wetlands involving a variety of sampling methods.

Several other leading coastal wetlands researchers such as Doug Wilcox, Pat Chow-Fraser, Gerry Niemi, Tom Simon, John Brazner, Kurt Kowalski, Dave Johnson, and Mary Moffet may also have complementary fish data. Care should be taken when performing fish data comparisons to ensure that similar gear and assessment methods have been followed.

Macroinvertebrates

Unfortunately, few studies on Great Lakes coastal wetland macroinvertebrates have been completed in the past. However, Don Uzarski and Tom Burton have accumulated and maintain what is widely considered the largest bi-national macroinvertebrate data set collected for Great Lakes Coastal Wetlands. Pat Hudson and Brian Armitage also have macroinvertebrate data from across the Great Lakes basin. In Canada, aquatic macroinvertebrate collections have been conducted in littoral zones of Lake Ontario by Ora Johannsson and Scott Millard at the Canadian Department of Fisheries and Oceans and by Pat Chow-Fraser from McMaster University in Hamilton, Ontario. Complementary macroinvertebrate community data for Canada may also be available through the Ontario Ministry of Environment. Care should be taken when performing macroinvertebrate data

comparisons to ensure that similar gear and assessment methods have been followed in each study.

Birds and amphibians

Bird and amphibian data are available from Marsh Monitoring Program (MMP) surveys conducted in Lake Ontario and St. Lawrence River coastal wetlands since the program's inception in 1995. Canadian Wildlife Service (CWS) -Ontario is also involved in an International Joint Commission Lake Ontario/St. Lawrence water regulation review study for which data on bird communities were collected during the 2002 breeding season in nine of the 12 GLCWC study sites. These data may be of use as a complement to the bird survey data previously collected. Amphibian and marsh bird data from the STAR – grant monitoring project were collected in a similar manner to the MMP and may be available to complement data collected through the GLCWC. In addition, two other volunteer frog survey programs may yield complementary data: The Ecological Monitoring and Assessment Network (EMAN) supports a national FrogWatch program, and CWS supports a backyard and roadside amphibian call surveying program.

Vegetation

Dennis Albert is believed to have the largest available data set on macrophytes in Great Lakes coastal wetlands. The following individuals are also likely to have complementary aquatic plant data sets: Doug Wilcox, Jim Meeker, Jane Bowles, Greg Grabas, Joel Ingram, and Mary Moffet and her research collaborators at the Duluth EPA Lab.

Doug Wilcox and Jim Meeker have extensive vegetation data sets from ridge and swale wetlands at three locations on Lake Michigan and three locations on Lake Superior. They also studied six other shoreline wetland sites in Saginaw Bay, six drowned river mouths in Lake Michigan, and six barrier beach wetlands in Lake Superior.

In Canada, CWS-Ontario is involved in an International Joint Commission Lake Ontario/St. Lawrence water regulation review study in which vegetation community quadrat data on plant communities was collected in 2003 from 16 Lake Ontario and St.

Lawrence River coastal wetlands. Doug Wilcox added an additional 16 sites on the US side of Lake Ontario with the help of Jim Meeker. Eleven of the wetlands are Year one GLCWC study sites. These data may be of use as a complement to the data collected in 2002.

The International Joint Commission also performed a vegetation study in the late 1980s/early 1990s on 17 Lake Ontario wetlands and 18 Lake Superior wetlands as well as repeat study sites on Lake St. Clair, Saginaw Bay of Lake Huron, and the Kakagon Sloughs of Lake Superior.

Additionally, the Long Point Waterfowl and Wetlands Research Fund has extensive information about plant community composition and locations, as well as an increasing inventory of information about invasive non-native wetland macrophyte communities within Long Point coastal wetlands.

Few projects chose to incorporate interpretation of color infrared air photos into their methods evaluations, however many researchers have maintained that this is a valuable tool for evaluating long-term changes in wetlands, especially those that are large. This data may be available in electronic downloadable form from the following GIS data clearinghouses: the National Spatial Data Clearinghouse program (http://nsdi.usgs.gov/) in the United States and the National Air Photo Library (http://airphotos.nrcan.gc.ca/index_e.php) in Canada.

Water chemistry

Research conducted by Dr. Patricia Chow-Fraser's laboratory at McMaster University in Hamilton, Ontario, Canada may provide data that are comparable to the water chemistry data produced by the GLCWC projects. National water quality data are available for sites within the United States from the Environmental Protection Agency's STORET database program (http://www.epa.gov/storet/dbtop.html) and the United States Geological Survey's water resources program (http://waterdata.usgs.gov/nwis/qw). This database can be sorted by hydrologic unit code or viewed graphically in a GIS using latitude and

longitude coordinates to identify previously collected data from Great Lakes coastal wetlands. In addition, the USEPA Great Lakes National Program Office maintains a large database on water quality and sediment chemistry. Likewise, the NOAA Great Lakes Environmental Research Laboratory provides information on the web on water temperature, streamflow, chemicals, toxics, nutrients, turbidity, and sediment transport data (http://www.glerl.noaa.gov/data/). The use of the same reference methods in this project ensured that program data would be complementary and comparable to other locations in the basin. In Canada, water quality data may be available through the Ontario Ministry of the Environment.

Sediments

The USEPA Great Lakes National Program Office maintains a large database on water quality and sediment chemistry. GLNPO data are collected using the same protocols as this project thus their data are complementary and comparable for other locations in the basin.

Landscape attributes

There are many existing spatial databases for areas of the Great Lakes basin that could be useful in helping to quantify land use and disturbances surrounding coastal wetlands. Aerial photographs, satellite imagery and land use/land cover maps are available for certain areas, but scale and currency of these data may vary considerably among wetland sites. This data may be available in electronic downloadable form from the following GIS data clearinghouses: the National Spatial Data Clearinghouse program (http://nsdi.usgs.gov/) in the United States and the National Air Photo Library (http://airphotos.nrcan.gc.ca/index_e.php) in Canada.

Scoring and weighting

We used the information on available complementary data to score indicators on two metrics: locational data availability and the number of data sources. Each indicator was classified as US, CA, or Both for locational availability and given a numerical value for the number of data sources. Indicators with data only in the US or only in Canada were

assigned scores of 3/4. Those with data in both locations were valued at 1.5. Indicators with 0-2, 3-5, and 5+ data sources were assigned scores of 1/2, 1, and 1.5, respectively. These values were then added to arrive at an overall score of complementary data availability for each indicator (Table 7). Higher scores indicate the existence of better sources of data. Complementary data availability is not an important factor as agencies may still survey an indicator even if complementary data are available as data could be out of date or in the wrong location. Thus, scores were not changed in the weighting for this factor.

TABLE 7. Scoring of indicators for availability of complementary data. Higher scores indicate more availability of complementary data.

	Fish	Macro-invertebrates	Birds	Amphibians	Vegetation	Water chemistry	Sediments	Landscape attributes
Number of identified data sources	12	6	3	4	10	6	1	2
Locational data availability	Both	Both	Both	Both	Both	Both	US	Both
Complementary data score	3.00	3.00	2.50	2.50	3.00	3.00	1.25	2.00
Weighted complementary data score	3.00	3.00	2.50	2.50	3.00	3.00	1.25	2.00

Indicator sensitivity to wetland condition changes

Multimetric IBIs were developed by select investigators for fish, macroinvertebrates, birds, amphibians, and vegetation based on the data collected in 2002. IBIs are built by developing metrics (e.g., taxa diversity, abundance of indicator taxa or guilds, health of individual organisms) believed to be correlated with environmental integrity and calculating from the metrics a composite score (e.g., Karr *et al*. 1986, Hilsenhoff 1987, Plafkin *et al*. 1989, Thoma 1999, Karr and Chu 2000). IBI scores at sampled sites can be compared to scores at reference sites of good quality to determine environmental stress or wetland sites can be tracked over time to gauge long-term trends in habitat quality (National Research Council 2000).

To create and validate wetland IBI models, biological metrics are assessed against levels of disturbance impacting the wetland. Disturbance is estimated by determining the levels of abiotic parameters that are influenced by or directly due to human disturbance within the wetland and surrounding watershed. IBI metrics were assessed for their ability to detect site disturbance and thus an indicator's sensitivity to wetland condition. Details on indicator sensitivity follow.

Fish

Analysis of fish community data from large and small fyke net collections in 61 sites across four wetland types in all five Great Lakes revealed that plant zone was the major driving factor in establishing fish community composition (Uzarski *et al*. submitted). Further analyses suggested that plants, fish communities and the associated abiotic factors were related and predictable. Thus, a fish- based IBI for the entire Great Lakes basin appeared to be feasible. Based on this information, IBIs were developed separately for two vegetation zones, *Scirpus* and *Typha*, for the entire Great Lakes Basin.

Attributes that showed the most promise in the *Scirpus* zone are: mean abundance per net-night, total richness, percent non-native richness, percent omnivore abundance, percent piscivore richness, percent insectivore abundance, percent insectivorous Cyprinidae

abundance, percent carnivore (insectivore+piscivore+zooplanktivore) richness, white sucker *(Catostomus commerson*i) mean abundance per net-night, black bullhead *(Ictalurus melas*) mean abundance per net-night, rock bass *(Ambloplites rupestris*) mean abundance per net-night, alewife *(Alosa psuedoharengus*) mean abundance per net-night, smallmouth bass *(Micropterus dolomie*u) mean abundance per net-night, and pugnose shiner *(Notropis anogenus*) mean abundance per net-night. Attributes that showed the most promise in the *Typha* zone are: percent insectivore abundance, insectivorous Cyprinidae richness, percent Centrarchidae abundance, Centrarchidae richness, mean Shannon Diversity Index, mean evenness, longnose gar *(Lepisosteus osseus*) abundance per net-night, largemouth bass *(Micropterus salmoides*) abundance per net-night, rock bass *(Ambloplites rupestris*) abundance per net-night, bluegill *(Lepomis macrochirus*) abundance per net-night, and *Lepomis* abundance per net-night (Uzarski *et al*. submitted).

A disturbance gradient was developed using the chemical/physical variables turbidity, specific conductance, chloride, nitrate, ammonium, soluble reactive phosphorus concentrations, percent saturation of dissolved oxygen, and pH and the land use/cover variables 1 and 20 km percent land use of developed, agriculture, forest, and wetland+meadow.

Community attributes and indicator species were evaluated based on their ability to order sites according to anthropogenic disturbance. After removing sites with insufficient data, metric scores correlated with disturbance rankings at r = 0.891 for *Scirpus* and r = 0.824 for *Typha* indicating that fish community composition shifted with, and even within, plant zone with increasing nutrients and agriculture (Uzarski *et al*. submitted). Therefore, it appears that fish can be used as reliable indicators of wetland condition.

Project teams CALO and USMH also tested the sensitivity of DELTs. Both found that the level of disturbance at wetland sites did not appear to correlate with the incidence of DELT anomalies in captured fish. DELTs are not likely to serve as good indicators of wetland condition when captured with fyke nets because the majority of fish captured were: (1) migratory (in and out of the wetland), (2) short-lived species, or (3) young of the year

(Uzarski *et al.* 2002). These fish are not likely to have been exposed to contaminants long enough to produce visible effects. Therefore, we do not recommend using DELTs as a tool for evaluating wetland condition.

Macroinvertebrates

Sweep net samples from 41 wetland sites spanning all five Great Lakes and multiple wetland types were used in macroinvertebrate indicator analysis. Great Lake, ecoregion, wetland type, and vegetation type all appeared to contribute to the structure of invertebrate communities in coastal wetlands across the Great Lakes basin (Uzarski *et al.* 2005).

The following metrics were deemed important in the identification of degradation in wetlands: Crustacea+Mollusca richness, Odonata richness, genera richness, % Gastropoda, % Odonata, % Sphaeriidae, Ephemeroptera+Trichoptera richness, % Crustacea+Mollusca, family richness, evenness, Shannon diversity index, Simpson index, % Isopoda, and % Amphipoda (Uzarski *et al*. 2005).

Disturbance gradients were used to evaluate the IBI scores and a Pearson correlation coefficient of 0.674 was found between the overall disturbance gradient and IBI scores (Uzarski *et al*. 2005). Both the IBI and the disturbance ranking seemed to account for the anthropogenic disturbance found at sites across the Great Lakes basin. A number of sites did, however, have IBI scores that were either higher or lower than expected from the disturbance ranking. It is unclear from the data collected in 2002 whether these discrepancies were a result of the disturbance ranking not accounting for certain disturbances that were detected by the IBI or whether the IBI was not sufficiently representing the "reference condition" of these sites (Uzarski *et al*. 2005). Overall, however, the invertebrate IBI was useful in measuring wetland condition in multiple wetland types.

Vegetation

The assessment of potential vegetation IBIs utilized vegetative transect data from Great Lakes coastal wetland sites representing four regions: Northern Lakes Huron and

Michigan, Saginaw Bay, Long Point (Lake Erie), and Lake Ontario. Sampling of aquatic macrophytes was conducted within both wet meadow and emergent marsh zones at 32 sites on the U.S. shoreline of Lakes Huron and Michigan, 16 sites on the Canadian shoreline near Long Point on Lake Erie, and 12 Canadian sites on Lake Ontario (Minc and Albert 2004).

Twenty-six potential IBIs were evaluated against three independent measures of anthropogenic stress, including upland land use within a 5-km buffer, specific or localized sources of stress within a 1-km buffer (Michigan sites only), and field-sampled data on water quality and chemistry. Surprisingly few of the potential IBIs held up to testing against these independent measures and none of the potential IBIs functioned well across the entire Great Lakes region (Minc and Albert 2004). However, several of the potential IBIs appear to have higher predictive values for more localized areas.

This lack of strong land use - plant relationships may partially be a product of not identifying appropriate land use factors and/or conducting analyses at an inappropriate spatial scale. Land-use cover values combine a broad range of activities of varying intensity and spatial scale of impact, ranging from intense local point sources to broad regional influences. Frequently, the appropriate spatial scale of analysis is not readily apparent, and may in fact depend on wetland site configuration and degree of exposure to the Great Lakes (Minc and Albert 2004). In contrast, water chemistry and water quality are subject to temporal variations with major fluctuations over both the short- and long-term. Isolated measurements may not reflect the prevailing chemical environment within which aquatic macrophytes grow (Minc and Albert 2004).

To further complicate matters, chemical and plant sampling were not adequately coordinated during this project to provide a strong relationship between these two data sets. Further, small sample sizes spread across a diversity of wetland types severely limited the ability to define sensitive and robust IBIs. Perhaps because of these many complicating factors, plant community metrics showed limited sensitivity to wetland condition.

Birds

Marsh Monitoring Protocol bird survey data from 88 sites across the Great Lakes in a variety of wetland types were analyzed for their ability to classify wetland sites along a gradient of disturbance (Crewe and Timmermans 2005). The following metrics were tested: bird species richness and abundance of aerial foragers, non-aerial foragers, water foragers (excluding marsh nesters), general nesters, non-area sensitive marsh obligate nesters, area-sensitive marsh obligate nesters, and total species richness or abundance. The abundance of black terns, least bitterns and Virginia rails were also tested for their response to disturbance because these species have shown significant basin-wide population declines. Metrics that were correlated with the disturbance gradient at $p \leq 0.20$ during at least three of the four high-water level years or four of the five low-water level years, and that showed a consistent positive or negative response to disturbance over all high or low water level years, were considered suitable for inclusion in marsh bird IBI development.

Overall, marsh bird IBIs were well correlated (r = -0.6582 for 500 m; r = -0.617 for 1 km) with surrounding landscape disturbance at all scales during high water levels (Crewe and Timmermans 2005). However, marsh bird metric response to disturbance was weak or lacking at all scales except the 20 km scale during low water levels, resulting in the development of only one low water marsh bird IBI, which in turn was not significantly correlated with disturbance. Thus, in general, marsh bird metrics were less responsive to the measured disturbance gradient during low average Great Lakes water levels. Overall, some ability exists for marsh bird IBIs to indicate wetland condition during high water level periods.

Amphibians

Like the bird data analysis, amphibian survey data from 87 sites across the Great Lakes in a variety of wetland types were analyzed for their ability to classify wetland sites along a gradient of disturbance (Crewe and Timmermans 2005). Nine amphibian species guilds were identified, and species richness and maximum calling code of each guild were

considered for inclusion in the amphibian IBI. The guilds identified were: species that commonly occur in species-poor habitats, species associated with woodland habitats, species with basin-wide distributions, disturbance tolerant species, disturbance intolerant species, rare species, declining species (those species that showed significant negative declines in the analysis of MMP data), MMP marsh indicator species and total species richness or calling code. Maximum calling codes of American toads, Northern leopard frogs and wood frogs were also tested because of their tolerance (American toads) and intolerance (leopard frogs, wood frogs) to one or more forms of disturbance. Metrics that were correlated with the disturbance gradient at $p \leq 0.20$ during at least three of the four high-water level years or four of the five low-water level years, and that showed a consistent positive or negative response to disturbance over all high or low water level years, were considered suitable for inclusion in marsh amphibian IBI development.

Overall, marsh amphibian IBIs were well correlated (r = -0.5171 for 500 m; r = -0.4677 for 1 km; r = -0.4224 for 20 km) with surrounding landscape disturbance at all scales during high water levels (Crewe and Timmermans 2005). Although low water amphibian IBIs were strongly correlated with disturbance at the 1 km and overall disturbance scales, amphibian IBIs could not be developed at the other scales due to a lack of metric response. Those amphibian IBIs that were developed also tended to have a greater number of outliers when plotted against disturbance than did IBIs developed for high water levels. Thus, in general, marsh amphibian metrics were less responsive to the measured disturbance gradient during low average Great Lakes water levels. Overall, some ability exists for marsh amphibian IBIs to indicate wetland condition during high water level periods.

Water chemistry

Water chemistry data were used by several groups to rank site disturbance. Some water chemistry variables are better predictors of local disturbance than others, as certain variables may be just as prone to sources of variation in the natural environment as they are to unnatural sources of disturbance and degradation (Timmermans and Craigie 2003). The following variables were often used with their interpretations and sources in parenthesis after each: DO (nutrient enrichment; eutrophication), specific conductivity (dissolved ions

in water; a good indicator of urban run-off), salinity (salting of roads with sodium and potassium chloride), turbidity (sediment run-off from agricultural and ditching operations), chlorophyll a (phytoplankton production; nutrient enrichment), TDS, ORP (nutrient enrichment such as sewage, fertilizer, and manure), chloride (salting of roads with sodium and potassium chloride), sulfate (urban and industry contaminants), nitrate-N (nutrient enrichment such as sewage, fertilizer, and manure), ammonia-N (industries, primarily wastewater treatment plants), soluble reactive phosphorus (nutrient enrichment such as sewage, fertilizer, and manure), and alkalinity. Since water chemistry was used as a measure of wetland degradation, no IBIs were created for this metric. However, this metric can be used on its own as a measure of wetland condition. Thus, water chemistry data have some value as indicators of site disturbance.

Sediments

Project team USMH tested the ability of contaminated sediments to indicate wetland condition at 20 sites in Lakes Michigan and Huron. Few of the sediment parameters measured proved valuable in partitioning sites along gradients of anthropogenic disturbance (Uzarski *et al*. 2002). Most potential sediment contaminants were in concentrations below detection limits. The correlations that were found, between sediment organic matter (measured by %TOC and %solids) and IBI scores and between %TOC and PC 2 scores of the chemical/physical PCA, were likely an effect of underlying differences in wetland morphology between sites (Uzarski *et al*. 2002). No significant correlations occurred between sediment contamination and biotic integrity as measured by IBI scores. The inability to predict sediment contamination may reflect the very low concentrations of most contaminants measured. Thus, analysis of sediment contamination does not appear to be of significant value as part of an ongoing monitoring program (Uzarski *et al*. 2002).

Landscape attributes

A critical step in the process of evaluating the efficacy of utilizing community attributes to monitor the health of a particular ecosystem is the creation of an objective procedure to rank a site's condition as a function of stressor influence. Several groups used landscape attributes to rank sites along a gradient of disturbance. Surrounding land use, qualitative

hydrologic/landscape alterations, and quantified immediate disturbances were employed, in concert with water chemistry parameters, to rank wetland sites. Estimates of surrounding immediate disturbances were reflective of the relative disturbance and relative protection that occurs in proximity to the wetland study sites (Timmermans and Craigie 2003). Similarly, upon comparison with *a priori* classifications, the type and number of both hydrologic and landscape alterations and the land use surrounding wetland sites provided some explanation in separating wetland sites in terms of their relative exposure to certain anthropogenic alterations (Timmermans and Craigie 2003). Since landscape attributes were used as a measure of wetland degradation, no IBIs were created for this metric. However, this metric can be used on its own as a measure of wetland condition. Thus, landscape attributes have some value in indicating wetland site disturbance.

Scoring and weighting

Indicator sensitivity was scored on the basis of four metrics: indicator response to degradation, IBI functionality across space/time, demonstrated use throughout the Great Lakes basin, and demonstrated use in a variety of wetland types. Each metric received a score based on information provided by the six project teams. Indicators that responded to degradation (those classified as good) were given a score of 1; those that did not respond well (classified as poor) were given a score of 0. Indicators with poor IBI functionality across either space (e.g. limited use in only selected wetland types) or time (e.g. only useful in high water years) were given a score of 0; all good ratings were given a score of 1. The remaining two metrics were assigned scores of 0 and 0.5 for N and Y, respectively. Values for each metric were summed to arrive at an overall score for indicator sensitivity. Higher scores indicate increased indicator sensitivity. Indicator sensitivity is a critical factor as data are not worth collecting if they do not reveal information about wetland condition. Thus, sensitivity scores greater than 2 were weighted more heavily by multiplying those values by 3 (Table 8).

TABLE 8. Scoring of indicator sensitivity to wetland condition changes. Higher scores indicate greater sensitivity.

	Fish	Macro-invertebrates	Birds	Amphibians	Vegetation	Water Chemistry	Sediments	Landscape attributes
Indicator response to degradation	Good	Good	Good	Good	Poor	Good	Poor	Good
IBI functionality across space/time	Good	Good	Poor	Poor	Poor	Good	Poor	Good
Demonstrated use throughout Great Lakes	Y	Y	Y	Y	Y	Y	N	Y
Demonstrated use in multiple wetland types	Y	Y	Y	Y	Y	Y	Y	Y
Indicator sensitivity score	3	3	2	2	1	3	0.5	3
Weighted indicator sensitivity score	9	9	2	2	1	9	0.5	9

Ability to set endpoint or attainment levels

Metric values obtained within reference sites can be used to establish appropriate attainment levels and identify requirements for setting endpoints within varying basins and/or wetland types. Data from previously studied reference sites were used to establish endpoints or attainment levels for select indicators. However, meaningful endpoints have not yet been identified for most indicators since generally a series of data over several years must be analyzed to determine trends and patterns and as yet long term, comparative datasets do not exist. In time, this data will be forthcoming and attainment levels will be better defined. Indicators for which we have information on endpoints are elucidated further.

Fish

Attainment levels were set for fish metrics in Uzarski *et al*. (submitted). However, data from only one year (2002) were included. A longer, more established data set could be studied in the future to assist in setting more robust attainment levels.

Macroinvertebrates

Attainment levels were set for macroinvertebrate metrics in Uzarski *et al*. (2005) using sampling data from 2002. Categories include: mildly impacted, moderately impacted and moderately degraded. Like fish, a longer, more established data set could be studied in the future to assist in setting more robust attainment levels.

Birds and amphibians

Resampling and power analysis for the small scale marsh bird IBI during high water levels confirmed three wetland condition classifications of good, fair, and poor (Crewe and Timmermans 2005). Similarly, resampling and power analysis of the differences in amphibian IBI means confirmed four wetland condition classification categories for the small spatial scales during both high and low water levels. These categories were very good, good, fair, and poor (Crewe and Timmermans 2005). A statistical standard of 80% power was used in all cases. Sampling data were from the eight year period 1995-2003.

Vegetation

Few plant IBIs proved meaningful in evaluating wetland condition therefore endpoints have not yet been set (Minc and Albert 2004).

Water chemistry

Attainment levels were defined using 2002 water chemistry data for the purpose of site disturbance ranking. For details see Minc and Albert 2004 and Uzarski *et al*. 2005.

Sediments

Consensus-based guidelines have been published for metals and organic chemicals in sediments in the Great Lakes (MacDonald *et al*. 2000). These guidelines contain Threshold Effect Concentrations (levels below which no environmental effects are expected) and Probable Effect Concentrations (levels above which adverse effects are possible). The presence of published sediment quality guidelines serve as attainment levels (Uzarski *et al*. 2002).

Landscape attributes

Attainment levels were defined using 2002 landscape attribute data for the purpose of site disturbance ranking. For details see Minc and Albert 2004, Crewe and Timmermans 2005, and Uzarski *et al*. 2005.

Scoring and weighting

Attainment levels are scored based on two metrics: the existence of endpoints and the length of sampling period from which endpoints were derived. The metrics were scored based on indicator performance. Indicators with existence of endpoint metric values of Y and N were assigned values of 2.5 or 0, respectively. Sampling periods with $\leq$1, 2-4, 5+ years were assigned values of 0.25, 0.375, and 0.5, respectively. Values for each metric were summed to arrive at an overall score for ability to set attainment levels. Higher attainment scores indicate the existence and validity of attainment levels for each indicator.

The ability to set endpoints is an important factor. Thus, attainment scores greater than 2 were weighted more heavily by multiplying those values by 1.5 (Table 9).

TABLE 9. Scoring of ability to set endpoint or attainment levels. Higher scores indicate increased ability to set endpoints.

	Fish	Macro-invertebrates	Birds	Amphibians	Vegetation	Water chemistry	Sediments	Landscape attributes
End points exist	Y	Y	Y	Y	N	Y	Y	Y
Length of sampling period from which end points were derived	1	1	8	8	-	1	1	1
Attainment score	2.75	2.75	3	3	0	2.75	2.75	2.75
Weighted attainment score	4.125	4.125	4.5	4.5	0	4.125	4.125	4.125

OVERALL INDICATOR SCORING AND OPTIMIZATION SCENARIOS

Weighted scores for each factor were combined to arrive at an overall score for each indicator (Table 10). These data should be used to compare the overall value of indicators with each other. Based on our findings, we recommend that monitoring programs attempt to sample all indicators with scores above 20 if funding and time are not limiting. These include water chemistry (score=27.29), fish (score=26.54), macroinvertebrates (score=23.96), landscape attributes (score=23.29), birds (score=23.17) and amphibians (score=23.17).

TABLE 10. Overall scores for each indicator. Higher scores are more desirable.

	Fish	Macro-invertebrates	Birds	Amphibians	Vegetation	Water chemistry	Sediments	Landscape attributes
Weighted cost score	1.67	1.33	4.67	4.67	2.00	4.67	4.67	1.67
Weighted measurability score	5	2	5	5	1.5	2	N/A	2
Weighted applicability score	3.75	4.5	4.5	4.5	3.75	4.5	N/A	4.5
Weighted complementary data score	3.00	3.00	2.50	2.50	3.00	3.00	1.25	2.00
Weighted indicator sensitivity score	9	9	2	2	1	9	0.5	9
Weighted attainment score	4.125	4.125	4.5	4.5	0	4.125	4.125	4.125
Overall score	26.54	23.96	23.17	23.17	11.25	27.29	10.54	23.29

Given the overall scores in Table 10, several possible combinations of indicators can be created. We will highlight three possible combinations here: 1) optimized for cost 2) optimized for time, and 3) optimized for indicator sensitivity.

Suite of indicators optimized for cost

We used our previously developed unweighted scores to isolate the indicators which provided decent indicator sensitivity and measurability for a low cost. First, we removed applicability, complementary data, and attainment scores from the mix. Then we weighted measurability by 2 and indicator sensitivity by 5. We multiplied the expense scores, a component of the cost scores, first by 3 to equalize them with the rest of the factors (as they comprise only 1/3 of the cost scoring), then weighted them by 10. We also included effort and labor metrics, both of which we multiplied by 3 to equalize them with the rest of the factors, and weighted by 3. The results are displayed in Table 11.

With limited funding, we suggest that wetland assessment be performed using amphibian surveys, bird surveys, and landscape attributes. An indicator assessment using just amphibians would cost approximately $20 US/visit. Adding a bird survey would increase the cost to $75 US. Landscape attribute assessments cost approximately $31 US per wetland, an addition which would bring the total cost to $106 US. These values do not take into account the cost to pay staff or travel expenses as these are difficult to measure. However we attempted to incorporate those costs into our calculations by including effort and labor in the weighting.

Optimization for time

We used our previously developed unweighted scores to optimize a set of indicators for time in much the same way as we optimized for cost. We again removed applicability, complementary data, and attainment scores from the mix. Then weighted measurability by 2 and indicator sensitivity by 5. We multiplied effort scores by 3 to equalize them with the rest of the factors then weighted them by 10. We included expense and labor metrics, both of which we multiplied by 3 to equalize them, but we did not add weights to these metrics. The results are displayed in Table 11.

With limited time, a wetland assessment group might consider sampling water chemistry, birds and fish. Water chemistry takes approximately 2.09 person-hours/sample, bird sampling 1.33 person-hours/visit, and fyke netting 3.75 person-hours/fyke net set for a total of approximately 7.17 person-hours per wetland assessment. Of course, one fyke net set, one water chemistry sample, and one bird survey are not enough to accurately assess the condition of a wetland but all three can be accomplished in one day.

Optimization for indicator sensitivity

We used our previously developed unweighted scores to optimize for indicator sensitivity by including all factors except availability of complementary data. We weighted attainment and applicability factors by 1.5, measurability and cost factors by 2, and indicator sensitivity by 5. The results are displayed in Table 11.

Teams hoping to optimize their knowledge of wetland condition should focus on sampling water chemistry, fish (with fyke nets), landscape attributes, and macroinvertebrates (with sweep nets).

TABLE 11. Optimization scenarios for cost, time, and indicator sensitivity. Higher scores indicate better optimization.

	Fish	Macro-invertebrates	Birds	Amphibians	Vegetation	Water chemistry	Sediments	Landscape attributes
Unweighted cost	1.67	1.33	2.33	2.33	2.00	2.33	2.33	1.67
Unweighted labor	0.67	0.33	0.67	0.67	0.67	1.00	1.00	0.67
Unweighted effort	0.67	0.33	1.00	0.67	0.33	1.00	0.67	0.33
Unweighted expenses	0.33	0.67	0.67	1.00	1.00	0.33	0.67	0.67
Unweighted measurability score	2.5	2	2.5	2.5	1.5	2	N/A	2
Unweighted applicability score	2.5	3	3	3	2.5	3	N/A	3
Unweighted complementary data score	3.00	3.00	2.50	2.50	3.00	3.00	1.25	2.00
Unweighted indicator sensitivity score	3	3	2	2	1	3	0.5	3
Unweighted attainment score	2.75	2.75	3	3	0	2.75	2.75	2.75
Optimized for cost	42.00	45.00	50.00	57.00	47.00	47.00	N/A	48.00
Optimized for time	43.00	32.00	49.00	40.00	23.00	53.00	N/A	33.00
Optimized for indicator sensitivity	31.21	30.29	28.67	28.67	15.75	32.29	N/A	30.96

DISCUSSION

Tools developed for basinwide monitoring must provide information about wetland functions in an efficient, comprehensible and geographically extensive manner. (Timmermans and Craigie 2003). Our purpose in this report was to compile information about indicators in a comprehensive way and make recommendations regarding the feasibility of indicators for use in a bi-national, basin-wide monitoring program.

At the individual indicator level, fish scored high for inclusion into a wetland monitoring program. There is good evidence for its use as a reliable indicator of wetland condition (Uzarski *et al*. submitted), it is easy to measure in the field and does not require much technical skill. The effort, labor, and expense requirements are fairly low if a monitoring program already owns the necessary equipment. However, if net purchase is necessary, nets can be considered a capital expense and once bought, continuing expenses should be minimal. We recommend that fish be included as an indicator in a wetland monitoring program.

Likewise, water chemistry has great promise as an indicator of wetland condition. Water chemistry data are fairly sensitive to changes in wetland condition and have been used to rank site disturbance by several project teams. Data are quick and easy to collect and measure in the field with low technical expertise. The protocols are applicable throughout the basin and in all wetland types. Water chemistry data analysis however can be quite costly, especially if laboratory analysis of samples must be performed at an off-site facility or equipment bought to perform the analyses at an in-house facility (which would also include the cost of hiring skilled laboratory staff to perform the analyses). We recommend that water chemistry be included as an indicator in a wetland monitoring program given that laboratory analysis of samples costs are not prohibitive.

Macroinvertebrates, like fish, have a proven record of sensitivity to wetland condition (Uzarski *et al*. 2005). Attainment levels have been described for macroinvertebrate IBI use across the Great Lakes in a variety of wetland types. Protocols are applicable across the

basin and in most wetland types. The field methodology is easy to accomplish by unskilled technicians, however laboratory analysis of samples should be performed by someone with experience in aquatic macroinvertebrate identification. Both field and laboratory procedures can be somewhat time consuming. We recommend that macroinvertebrates be included as an indicator in a wetland monitoring program if time is not limiting.

Landscape attributes proved useful in ranking site disturbance for several groups (Timmermans and Craigie 2003, Grabas *et al*. 2003). Landscape attributes have applicability across the basin, considerable available supporting data, and easy field protocols. Some project teams however voiced concerns about the difficulty in identifying types of disturbance and ranking the relative significance of disturbance to the health of the wetland (Uzarski *et al*. 2002). As this indicator is new and still under development, changes to the protocols are likely to improve the reliability and robustness of the indicator should it be included in a wetland monitoring program. We recommend that landscape attributes be included as an indicator in a wetland monitoring program if new protocols are developed and tested for improved indicator reliability and robustness.

Birds and amphibians proved somewhat useful in elucidating wetland condition. Field procedures are easy to perform and costs are relatively low. However, both bird and amphibian IBIs proved only reliable in high water conditions therefore the applicability of these methods in determination of wetland condition is somewhat variable. We recommend the use of birds and amphibians as indicators only if cost is limiting.

In the overall analysis of indicators, vegetation and sediments did not perform well as indicators of wetland condition. This is largely due to the fact that preliminary sensitivity analysis failed to reveal convincing evidence that data collected on these indicators could detect changes in wetland condition. It is not clear if more work will be done to further understand the factors limiting sensitivity. However, additional data sets with more compatible physical and biotic data may help to understand these metrics further.

Our optimization procedures revealed that several of the most cost efficient indicators (amphibian and bird surveys) are also ones with lower sensitivity to changes in wetland condition. The indicators most sensitive to changes in wetland condition (fish, water chemistry, landscape attributes, and macroinvertebrates) were variable in cost with landscape attributes and macroinvertebrates inexpensive and fish and water chemistry somewhat costly. However, if monitoring programs already own fyke nets or have access to a water chemistry lab, the expense of fish and water chemistry sampling may become quite reasonable. In the overall indicator analysis, fish, water chemistry, landscape attributes, and macroinvertebrate indicators were revealed to have not only the most optimum sensitivity but also easy measurability, large applicability, and defined attainment levels for the lowest cost with the best complementary data.

Throughout this report we took care to suggest that several indicators be employed in a monitoring program. We make this recommendation based on suppositions that groups of indicators respond differently to physical characteristics in a wetland (i.e., a wetland with low plant biodiversity may still support high fish biodiversity; de Szalay *et al.* 2003). A monitoring program that only samples one group of species (e.g. plants) may not provide enough information to accurately assess the wetland condition as a whole. Therefore, we recommend that monitoring programs consist of more than one taxa group to gain a better understanding of the overall habitat quality in the wetland.

Recommendations

Based on overall indicator evaluation, we recommend that monitoring programs sample fish, water chemistry, landscape attributes, and macroinvertebrates as these indicators have the highest degree of sensitivity, applicability, measurability and complementary data availability for the lowest cost. If only biological monitoring is to be completed, we recommend fish and macroinvertebrates be considered for inclusion in a bio-monitoring program. The results of this study can be used to develop a sustainable, long-term, basin-wide monitoring plan for Great Lakes coastal wetlands.

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APPENDICES

Appendix A. Name, location and wetland classification of 60 Great Lakes coastal wetland sites

Project	Name	Lake/Waterway	Country	Wetland classification	N Latitude	W Longitude
CALE	Big Creek	Erie	CA	Protected lacustrine	42.58275	80.457611
CALE	Bluff Marsh	Erie	CA	Protected lacustrine	42.563638	80.52175
CALE	Booth's Harbour	Erie	CA	Open lacustrine/Open shoreline	42.652361	80.40975
CALE	Crown Marsh	Erie	CA	Open lacustrine/Open shoreline	48.58525	80.414694
CALE	Hahn Marsh	Erie	CA	Protected lacustrine	42.57825	80.521638
CALE	Helmer's Pond	Erie	CA	Protected lacustrine/Sand spit embayment	42.564444	80.249388
CALE	Lee Brown Marsh	Erie	CA	Barrier Beach Lagoon	42.575555	80.51
CALE	Little Rice Bay	Erie	CA	Protected lacustrine/Sand spit embayment	42.594388	80.339833
CALE	Long Point Provincial Park	Erie	CA	Open lacustrine	42.585194	80.379583
CALE	Port Rowan	Erie	CA	Open lacustrine/Open shoreline	42.628527	80.441416
CALE	Smith Marsh	Erie	CA	Open lacustrine	42.611694	80.453666
CALO	Bayfield Bay	St. Lawrence River	CA	Protected lacustrine/Protected embayment	36.985416	76.228
CALO	Button Bay	Ontario	CA	Open lacustrine	44.140194	76.383055
CALO	Frenchman's Bay	Ontario	CA	Barrier Beach Lagoon	43.811694	79.094694
CALO	Hay Bay South	Ontario	CA	Open lacustrine	44.156416	76.885361
CALO	Hill Island East	St. Lawrence River	CA	Protected lacustrine/Protected embayment	44.365666	75.954777
CALO	Huyck's Bay	Ontario	CA	Barrier Beach Lagoon	43.936416	77.483583
CALO	Lynde Creek	Ontario	CA	Barrier Beach Lagoon	43.853777	78.9615
CALO	Parrott's Bay	Ontario	CA	Protected lacustrine/Protected embayment	44.220888	76.694555
CALO	Port Britain	Ontario	CA	Barrier Beach Lagoon	43.931194	78.371222
CALO	Presqu'ile Bay	Ontario	CA	Protected lacustrine/Protected embayment	43.998111	77.72475
CALO	Robinson Cove	Ontario	CA	Open lacustrine	44.112861	77.28
CALO	South Bay	Ontario	CA	Open lacustrine	43.920472	77.0475
USLE	Erie State Game Area (Monroe, Monroe Co., MI)	Erie	US	Open lacustrine	41.746378	83.471953
USLE	Lake Erie Metropark (Monroe, Monroe Co., MI)	Erie	US	Open lacustrine	42.07015	83.192228
USLE	Metzger Marsh WA (Oak Harbor, Lucas Co., OH)	Erie	US	Protected lacustrine/Protected embayment	41.638314	83.223631
USLE	Pointe Mouille State Game Area (Detroit, Monroe Co., MI)	Erie	US	Open lacustrine	41.994903	82.226322
USLE	Potters Pond at Cedar Point (Oak Harbor, Lucas Co., OH)	Erie	US	Open lacustrine	41.679275	83.307711
USLE	Sheldon's Marsh State Nature Preserve (Huron, Erie Co., OH)	Erie	US	Protected lacustrine/Protected embayment	41.421556	82.6121
USLE	Willow Point Marsh WA (Fremont, Erie Co., OH)	Erie	US	Protected lacustrine/Protected embayment	41.437061	82.880175
USLE	Young Marsh at Darby Unit of Ottawa NWR (Oak Harbor, Ottawa Co., OH)	Erie	US	Protected lacustrine/Protected embayment	41.538869	83.005342

Appendix A cont. Name, location and wetland classification of 60 Great Lakes coastal wetland sites

Project	Name	Lake/Waterway	Country	Wetland classification	N Latitude	W Longitude
USLM	Arcadia Lake	Michigan	US	Protected lacustrine/Protected embayment	44.4853	86.2294
USLO	Blind Sodus Bay - East	Ontario	US	Protected lacustrine/Protected embayment	43.329194	76.72425
USLO	Blind Sodus Bay - West	Ontario	US	Protected lacustrine/Protected embayment	43.330917	76.72425
USLO	Floodwood Pond	Ontario	US	Protected lacustrine/Protected embayment	43.7231	76.2
USLO	Little Sodus Bay	Ontario	US	Protected lacustrine/Protected embayment	43.3302	76.7083
USLO	North Sandy Pond - North	Ontario	US	Protected lacustrine/Protected embayment	43.680028	76.184167
USLO	North Sandy Pond - Renshaw Bay	Ontario	US	Protected lacustrine/Protected embayment	43.68075	76.198639
USLO	North Sandy Pond - South	Ontario	US	Protected lacustrine/Protected embayment	43.673167	76.177111
USLO	South Colwell - South	Ontario	US	Protected lacustrine/Protected embayment	43.6982	76.1942
USMH	Allen Rd.	Huron	US	Open lacustrine	43.64172713	83.60922405
USMH	Big Fishdam	Michigan	US	Open lacustrine	45.89271	86.58555
USMH	Bradleyville Rd.	Huron	US	Open lacustrine	43.621203	83.634743
USMH	Cedarville	Huron	US	Protected lacustrine/Protected embayment	45.99678	84.36251
USMH	Escanaba	Michigan	US	Open lacustrine	45.8179	87.05235
USMH	Garden Bay	Michigan	US	Open lacustrine	45.99678	86.57316
USMH	Hessel Bay	Huron	US	Protected lacustrine/Protected embayment	46.00548	84.43411
USMH	Hill Island	Huron	US	Protected lacustrine/Protected embayment	45.98199	84.31723
USMH	Jones Rd.	Huron	US	Open lacustrine	43.642355	83.8142727
USMH	Ludington Park	Michigan	US	Protected lacustrine/Protected embayment	45.73874	87.05646
USMH	Mackinaw Bay	Huron	US	Protected lacustrine/Protected embayment	46.00174	84.40915
USMH	Moscoe Channel	Huron	US	Protected lacustrine/Protected embayment	45.99175	84.31438
USMH	Ogontz Bay	Michigan	US	Open lacustrine	45.83229	86.78177
USMH	Pinconning	Huron	US	Open lacustrine	43.85936705	83.91246923
USMH	Rapid River	Michigan	US	Open lacustrine	45.9137	86.96622
USMH	Shepards Bay	Huron	US	Open lacustrine	45.98346	84.36425
USMH	St. Ignace	Michigan	US	Open lacustrine	45.84523	84.73923
USMH	Vanderbilt Park	Huron	US	Open lacustrine	43.60101653	83.6613501
USMH	Wigwam Bay	Huron	US	Open lacustrine	43.96093699	83.85936686
USMH	Wildfowl Bay	Huron	US	Open lacustrine/Protected	43.80198384	83.46281835

Appendix B. Standard Operating Procedures (SOP)

Standard Operating Procedures for Invertebrate Sampling with Sweep Nets

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Purpose:
To collect invertebrates in Great Lakes coastal wetlands

Equipment needed:
In field:

1) D-frame sweep nets
2) Shallow pans
3) Stop watches
4) Clicker counters
5) Forceps
6) Pipettes
7) Eye droppers
8) Squirt bottles
9) Sample bottles
10) Markers
11) Ethanol
12) Waders

In lab:

1) Forceps
2) Shallow pans
3) Eye droppers
4) Pipettes
5) Petri dishes
6) Scintillation vials
7) Ethanol
8) ID books

9) Dissecting microscope
10) Light sources for scope

Preparation prior to fieldwork:
Prior to starting fieldwork, wetland sites should be chosen and access to the sites secured. Sweep netters should be knowledgeable in the methods of sweep netting, and all involved personnel should know and understand the picking procedures.

Nets are available from a variety of stores including Wildco: http://www.wildco.com

Field procedures:
Sampling
For more detailed information on sampling macroinvertebrate communities with sweep nets, see Burton *et al*. 1999. A 500 micron mesh D-frame dip net should be used to "sweep" several different levels of the water column (surface, mid-water, and just above the sediment at the very least). Sweeps should include both the open water and stems of the dominant emergent vegetation. Each plant zone should be sampled, with at least three replicates in each zone to obtain a measure of spatial variance within each zone. July-August sampling in emergent plant communities will likely yield higher biomass and invertebrates in late instar stages. Late instars are easier to identify than early instars.

Burton, T.M., D.G. Uzarski, J.P. Gathman, J.A. Genet, B.E. Keas, and C.A. Stricker. 1999. Development of a preliminary invertebrate index of biotic integrity for Lake Huron coastal wetlands. Wetlands 19: 869-882.

Picking
Samples should be placed in a gridded shallow pan, where specimens should be picked from one grid at a time. Picking should occur for 30 minutes, or until 150 organisms are picked, whichever comes first. In the event that 150 organisms are not picked within the time limit, picking should continue until the next multiple of 50 is reached. Clicker counters can be used to keep track of organisms picked. Pipettes work well to catch moving organisms. Specimens should then be placed in a marked sample bottle filled with 95% ethanol for later identification in the lab.

Picking sweep net samples

Lab procedures:

Identification

Specimens should be enumerated and identified down to the lowest possible taxonomic group. The following sources may be used for identification:

Merritt, R. W. and K.W. Cummins (eds.). 1996. An introduction to the aquatic insects of North America. Kendall/Hunt Publ. Co, Dubuque, Iowa, 862 pp.

Pennak, R. W. 1989. Fresh-water invertebrates of the United States. Third Edition. John Wiley and Sons, Inc. New York, NY, USA.

Thorp, J.L., and A.P. Covich. 1991. Ecology and classification of North American Freshwater Invertebrates. Academic Press, New York.

Associated data sheet name: datasheet.xls – Sweep net worksheet

Standard Operating Procedures for Invertebrate Sampling with Activity Traps

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Purpose:
To collect mobile invertebrates in Great Lakes coastal wetlands

Equipment needed:
In field:

1) Activity traps
2) PVC pipe (4' in height)
3) Buoys (1 per trap)
4) Rope for attaching buoys
5) Funnel
6) Sieves
7) Ethanol
8) Squirt bottles
9) Sample bottles
10) Markers
11) Waders

In lab:

11) Forceps
12) Shallow pans
13) Eye droppers
14) Pipettes
15) Petri dishes
16) Scintillation vials
17) Ethanol
18) ID books
19) Dissecting microscope
20) Light sources for scope

Preparation prior to fieldwork:
Prior to starting fieldwork, wetland sites should be chosen and access to the sites secured. Ready-made activity traps can be bought from Protofab by emailing: dwik_protofab@hotmail.com.

Special care should be taken to ensure that the soda bottle activity traps are built to proper specifications. See USEPAs "Methods for evaluating wetland condition: developing an invertebrate index of biological integrity for wetlands" (available at: http://www.epa.gov/waterscience/criteria/wetlands/9Invertebrate.pdf). Additional information can be found by viewing the patent at the U.S. Patent and Trademark Office website (http://www.uspto.gov/patft/index.html, patent number 5,561,939) and in Murkin *et al*. 1983.

Murkin, H.R., P.G. Abbott, and J.A. Kadlec. 1983. A comparison of activity traps and sweep nets for sampling nektonic invertebrates in wetlands. Freshw. Invert. Biol. 2:99-106.

Field procedures:
Placement
Unbaited traps should be set at the edge of emergent vegetation with at least one in each plant zone. Traps should be placed horizontally about mid-level in the water column. Be sure to purge the air bubbles out of the trap during placement to reduce the buoyancy of the traps and standardize survival of captured invertebrates. The poles to which traps are affixed should be set deep enough in the substrate so as no to be disturbed by waves. Each trap should have a buoy attached so that it can easily be avoided by others and found by you in the future. Traps may be placed in tandem with fyke nets or minnow traps.

Trap placement

Collection
Collection should occur approximately 24 hours after placement. Prior to removal, plug the end of the activity trap with a cork to keep the sample from draining out as it is lifted from the water. After removal, the contents should be poured through a sieve with 200 micron size mesh and then flushed with ethanol. A 200 micron size mesh will ensure that

the smaller invertebrates, such as microcrustaceans, are captured without clogging by fine silts. The contents should then be stored in 95% ethanol in a marked bottle until the sample can be identified in the lab.

Sample collection

Lab procedures:

Identification

Specimens should be enumerated and identified down to the lowest possible taxonomic group. The following sources may be used for identification:

Merritt, R. W. and K.W. Cummins (eds.). 1996. An introduction to the aquatic insects of North America. Kendall/Hunt Publ. Co, Dubuque, Iowa, 862 pp.

Pennak, R. W. 1989. Fresh-water invertebrates of the United States. Third Edition. John Wiley and Sons, Inc. New York, NY, USA.

Thorp, J.L., and A.P. Covich. 1991. Ecology and classification of North American Freshwater Invertebrates. Academic Press, New York.

Associated data sheet name: datasheet.xls – Activity trap worksheet

Standard Operating Procedures for Invertebrate Sampling with Hester-Dendy Traps

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Purpose:
To collect colonizing invertebrates in Great Lakes coastal wetlands

Equipment needed:
In field:

1) Paving tiles
2) Masonite sheeting
3) Bolts and screws
4) Toothbrushes
5) Mesh netting for bags
6) Wrenches for loosening and tightening bolts
12) Buoys (1 per trap)
13) Rope for attaching buoys
14) Funnel
7) Squirt bottles
8) Sample bottles
9) Ethanol
10) Markers
11) Waders

In lab:

21) Forceps
22) Shallow pans
23) Eye droppers
24) Pipettes
25) Petri dishes
26) Scintillation vials
27) Ethanol
28) ID books
29) Dissecting microscope
30) Light sources for scope

Preparation prior to fieldwork:
Prior to starting fieldwork, wetland sites should be chosen and access to the sites secured. Fieldworkers should know proper placement and retrieval methods before going into the field. Hester-Dendy multi-plate samplers can be bought from Acorn Naturalists among other places:

Acorn Naturalists:
http://www.acornnaturalists.com/store/product1.asp?SID=2&Product_ID=508.

If you choose to construct the samplers yourself, it is important that they be built to proper specifications. Several sources of information exist for this purpose:

Hester, F. E. and J. S. Dendy. 1962. A multiple-plate sampler for aquatic macroinvertebrates. Trans. Am. Fish. Soc 91: 420-421.

Britton, L.J. and Greeson, P.E., eds. 1989. Methods for collection and analysis of aquatic biological and microbiological samples: U.S. Geological Survey Techniques of Water-Resources Investigations, book 5, chap. A4, p. 156-158.

New Hampshire Department of Environmental Services. 2002. Biomonitoring program protocols. World Wide Web electronic publication. Retrieved December 4, 2002 from http://www.des.state.nh.us/wmb/biomonitoring/protocols.pdf.

Masonite can be used for the plates but bear in mind that this material swells in water. Therefore, either use a different material or increase the spacing between the plates.

Field procedures:
Placement
Hester-Dendy samplers (three sets of plates attached to each paving stone) should be set at the edge of the emergent vegetation with the paving stone sitting on the bottom and a buoy marking the location. One sampler should be placed in each plant zone. The paving stones are heavy enough to resist most wave action. Each sampler should be left at a site for 1 month so they have an opportunity to act as artificial substrate and collect colonizing invertebrates.

Collection
Mesh bags should be placed over each set of plates during retrieval so that no specimens escape while bringing the sampler to the surface.

Sample collection

All surfaces of each plate should be gently scrubbed with a toothbrush. The contents should then be stored in ethanol in a marked sample bottle until the sample can be identified in the lab.

Hester-Dendy plate scrubbing

Lab procedures:

Identification

Specimens should be enumerated and identified down to the lowest possible taxonomic group. The following sources may be used for identification:

Merritt, R. W. and K.W. Cummins (eds.). 1996. An introduction to the aquatic insects of North America. Kendall/Hunt Publ. Co, Dubuque, Iowa, 862 pp.

Pennak, R. W. 1989. Fresh-water invertebrates of the United States. Third Edition. John Wiley and Sons, Inc. New York, NY, USA.

Thorp, J.L., and A.P. Covich. 1991. Ecology and classification of North American Freshwater Invertebrates. Academic Press, New York.

Associated data sheet name: datasheet.xls – Hester-Dendy worksheet

Standard Operating Procedures for Invertebrate Sampling using Ultraviolet Light Traps

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Purpose:
To collect caddisflies (Insecta: Tricoptera) in Great Lakes coastal wetlands

Equipment needed:
In field:

1) BioQuip 4-watt battery powered UV light
2) 5-gallon bucket or pan
3) Liquid detergent
4) 85% ethanol

In lab:

31) Forceps
32) Shallow pans
33) Eye droppers
34) Pipettes
35) Petri dishes
36) Scintillation vials
37) Ethanol
38) ID books
39) Dissecting microscope
40) Light sources for scope

Preparation prior to fieldwork:
Prior to starting fieldwork, wetland sites should be chosen and access to the sites secured. It is important to make sure that the blacklight is in proper working order and that the set-up will not fail overnight. More information on light trapping for Trichoptera in coastal wetlands can be found in:

Armitage, B.J., P.L. Hudson, and D.A. Wilcox. 2000. Caddisflies (Insecta: Trichoptera) of fringing wetlands of the Laurentian Great Lakes. Verh. International Verein. Limnol. 27:3420-3424.

Field procedures:

Placement

All traps should be set within the emergent vegetation of the wetland for one night. Four traps may deployed within each wetland. Traps should be set at least 20 m from each other to keep collections independent. Traps are comprised of a 5-gallon bucket staked into the marsh sediment with a BioQuip 4-watt battery powered UV light suspended within (the setup can also be created with a flashlight and F6T5-BLB blacklight tube). The bucket should be filled with approximately 6 cm of water to which several drops of liquid detergent are added (85% ethanol can also be used). When switched on at dusk, insects flying over the bucket (and thus only species associated with the habitat being sampled and not drawn in from a long distance or nearby aquatic habitats) will be attracted to the light. When insects fly into the bucket they land on the soapy water, fall through the surface film and are drowned.

Collection

Specimens should be collected the following morning by sieving the bucket contents and preserving insects in 95% ethanol.

Lab procedures:

Identification

Specimens should be enumerated and identified down to the lowest possible taxonomic group. Trichopteran keys in the following sources may be helpful:

Blickle, R.L. 1979. Hydroptilidae (Trichoptera of America North of Mexico. New Hampsh. Agric. Exp. Sta. Bull. 509: 1–97.

Merritt, R. W. and K.W. Cummins (eds.). 1996. An introduction to the aquatic insects of North America. Kendall/Hunt Publ. Co, Dubuque, Iowa, 862 pp.

Ross, H.H. 1944. The caddisflies or Trichoptera of Illinois. Bulletin of the Illinois Natural History Survey 23(1) 1-326.

Lab identification

Associated data sheet name: datasheet.xls – UV worksheet

Standard Operating Procedures for Fish Sampling with Fyke Nets

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Purpose:
To assess fish populations in Great Lakes coastal wetlands. This method is designed to collect some small but mostly large fish.

Equipment needed:

1) Fyke nets
2) PVC poles or crowbar (4' in height; 4 for each net)
3) Buckets for holding fish
4) Cable ties
5) Buoys (1 per net)
6) Rope for attaching buoys
7) Measuring boards
8) Dip nets
9) Fish ID books
10) Waders

Preparation prior to fieldwork:
Prior to starting fieldwork, wetland sites should be chosen and access to the sites secured. All participants should be trained in the set up and placement of the nets. They should be to sight identify fish to species and be educated in the proper protocol for observing DELTs (deformities, eroded fins, lesions and tumors). The DELT protocol is explained in the following publication:

Sanders, R. E., R. J. Miltner, C. O. Yoder, and E. T. Rankin. 1999. The use of external deformities, erosion, lesions, and tumors (DELT anomalies) in fish assemblages for characterizing aquatic resources: a case study of seven Ohio streams. Pages 225-246 in T. P. Simon (editor). Assessing the sustainability and biological integrity of water resources using fish communities. CRC Press, New York, NY.

Net specifications are as follows:

Large fyke nets - 36" x 36", 2 square throat frames, 5 hoop frames (steel), 1/4" nylon mesh, 24lb., 50 ft. lead and 12 ft. detachable wings (36" high), funnels on the 1st and 3rd hoop, 36" between square frames and after last hoop, 30" between hoops, dyed green.

Small fyke nets - 36" x 18" (height), same specifications as above except lead and wings are 18" high, 24" between square frames and after last hoop, and 18" between hoops.

Field procedures:

Placement

Two net sizes should be used: large (~1.0 m x 1.5 m opening) and small (~0.5 m x 1.0 m opening). Both nets should have similar size mesh and should be placed for no less than 24 hours and no more than 48 hours before checking. Large nets should be placed in water no less than 0.75 m deep, and small nets should be set in water from 0.25 m to 0.75 m in depth. Each sampling site should include each net size, and should sample each of the wetland's plant communities. Nets should be set facing and perpendicular to the shore, or facing and perpendicular to the emergent vegetation (where applicable), with leaders extending into the shore or vegetation. Preferably the leaders should reach into the vegetation, assuming it is not too dense. Wings should be set at a 45 degree angle to the net opening. Funnels should be under water once set. A buoy should be attached to make the net more visible to other boaters.

Net placement

Collection

Care should be taken when collecting fish from net to start at open end and simultaneously hold net up while shaking fish down. Successive hoops should then be lifted while still keeping the opening of the net out of the water. This will move fish down to the cod end of net and keep fish from escaping. Captured fish should be identified to species, enumerated, measured in mm, checked for DELTs (deformities, eroded fins, lesions, and tumors) and released.

Measuring fish from fyke nets

A good source for identification of fish in Canada is:
Scott, W.B., and E.J. Crossman. 1990. Freshwater fishes of Canada. Department of Ichthyology and Herpetology, Royal Ontario Museum, Toronto. 966pp.

Associated data sheet name: datasheet.xls – fyke net worksheet

Standard Operating Procedures for Fish Sampling with Minnow Traps

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Purpose:
To collect fish too small to be caught in fyke nets within Great Lakes coastal wetlands

Equipment needed:

1) Minnow traps
2) Buckets for holding fish
3) Cable ties
4) Bricks for weighting traps
5) Buoys (1 per trap)
6) Rope for attaching buoys
7) Measuring boards
8) Dip nets
9) Fish ID books
10) Waders

Preparation prior to fieldwork:
Prior to starting fieldwork, wetland sites should be chosen and access to the sites secured. All involved participants should be trained in the set up of the traps and trap placement. They should be to sight identify fish to species and be educated in the proper protocol for observing DELTs (deformities, eroded fins, lesions and tumors). The DELT protocol is explained in the following publication:

Sanders, R. E., R. J. Miltner, C. O. Yoder, and E. T. Rankin. 1999. The use of external deformities, erosion, lesions, and tumors (DELT anomalies) in fish assemblages for characterizing aquatic resources: a case study of seven Ohio streams. Pages 225-246 in T.

P. Simon (editor). Assessing the sustainability and biological integrity of water resources using fish communities. CRC Press, New York, NY.

Field procedures:

Placement

A typical commercial minnow trap size is approximately 0.4 m long and 0.2 m in diameter with ~7.5 mm mesh. Traps can be placed in conjunction with fyke nets, in water that is too shallow for fyke nets. They should be placed on or near the bottom, within or near the emergent vegetation. Several can be placed within each site and in each plant community. Bricks should be used to weight the trap and buoys used to mark the location. Traps should be placed for at least 24 hours, and no more than 48 hours.

Trap placement

Collection

Captured fish should be identified to species, enumerated, measured in mm, checked for DELTs (deformities, eroded fins, lesions, and tumors) and released.

Measuring fish from minnow traps

A good source for identification of fish in Canada is:
Scott, W.B., and E.J. Crossman. 1990. Freshwater fishes of Canada. Department of Ichthyology and Herpetology, Royal Ontario Museum, Toronto. 966pp.

Associated data sheet name: datasheet.xls – Minnow trap worksheet

Standard Operating Procedures for Fish Sampling with Electrofishing Gear

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Purpose:
To assess fish of all sizes in Great Lakes coastal wetlands. This methods requires the user to have access to a boat and electrofishing equipment.

Equipment needed:

1) Boat outfitted for electrofishing (~16', flat bottom)
2) 5,000 Watt generator
3) Transformer unit capable of generating 120 pulses/sec DC at 354 Volts
4) Insulated rubber gloves
5) Nets with long handled poles
6) Fuses for transformer
7) Life preservers
8) Buckets for holding fish
9) Measuring boards
10) Dip nets
11) Fish ID books
12) Buoy to mark central location

Preparation prior to fieldwork:
Prior to starting fieldwork, wetland sites should be chosen and access to the sites secured. All staff should be familiar with the care, maintenance, use, and safety requirements of the equipment they are using. They should know the proper techniques for retrieving fish. They should also be to sight identify fish to species and be educated in the proper protocol for observing DELTs (deformities, eroded fins, lesions and tumors). The DELT protocol is explained in the following publication:

Sanders, R. E., R. J. Miltner, C. O. Yoder, and E. T. Rankin. 1999. The use of external deformities, erosion, lesions, and tumors (DELT anomalies) in fish assemblages for characterizing aquatic resources: a case study of seven Ohio streams. Pages 225-246 in T. P. Simon (editor). Assessing the sustainability and biological integrity of water resources using fish communities. CRC Press, New York, NY.

Field procedures:

Collection

Drop a buoy in the center of the site to be sampled. Sampling should occur in water less than 4 m in depth, however, several depths in each area should be fished. Electrofish in the vicinity of the buoy for 15 minutes (clock time) in roughly a circular pattern. Try to collect all fish that are shocked; this may require asking the driver to slow down at times. The driver should have a small hand-held net to collect any fish s/he can reach that the netters missed. Empty nets in live well/buckets frequently to maximize the fishes' chance of survival

The transformer should be set to 120 pulses/sec (DC) with a voltage of 354 (DC). If more than one plant community is sampled per wetland, attempt to sample each for 15 minutes separately.

Captured fish should be identified to species, enumerated, measured in mm, checked for DELTs (deformities, eroded fins, lesions, and tumors) and released.

A good source for identification of fish in Canada is:
Scott, W.B., and E.J. Crossman. 1990. Freshwater fishes of Canada. Department of Ichthyology and Herpetology, Royal Ontario Museum, Toronto. 966pp.

Associated data sheet name: datasheet.xls – electrofishing worksheet

Standard Operating Procedures for Fish Sampling with Gill Nets

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Purpose:
To assess fish populations near Great Lakes coastal wetlands. This method is designed for water 1.5 m or greater in depth so is not for use within emergent vegetation. A variety of mesh sizes can be used to catch a range of fish sizes.

Equipment needed:

1) 100 m gill nets – 1", 2" and 4" mesh
2) Buoys
3) Rope for anchoring buoys
4) Buckets for holding fish
5) Measuring boards
6) Dip nets
7) Fish ID books
8) Life jackets
9) Waders

Preparation prior to fieldwork:
Prior to starting fieldwork, wetland sites should be chosen and access to the sites secured. All field staff should be knowledgeable in the setting and placement of gill nets as well as the retrieval of the nets before fieldwork begins. They should be to sight identify fish to species and be educated in the proper protocol for observing DELTs (deformities, eroded fins, lesions and tumors). The DELT protocol is explained in the following publication:

Sanders, R. E., R. J. Miltner, C. O. Yoder, and E. T. Rankin. 1999. The use of external deformities, erosion, lesions, and tumors (DELT anomalies) in fish assemblages for characterizing aquatic resources: a case study of seven Ohio streams. Pages 225-246 in T. P. Simon (editor). Assessing the sustainability and biological integrity of water resources using fish communities. CRC Press, New York, NY.

Field procedures:
Placement

Several lengths and mesh sizes of net should be used. Net lengths between 9.14 m and 32 m are adequate. Mesh sizes should be between 1 and 4 inches. Net heights of 1.83 m are typical. Nets should be set in from 1.5 –4.5 m of water for approximately 2 hours. Nets can be set in shallow areas but do not need to be set within wetlands or emergent vegetation.

Collection
Nets can be lifted from the water and fish removed after two hours. Captured fish should be identified to species, enumerated, measured in mm, checked for DELTs (deformities, eroded fins, lesions, and tumors) and released.

A good source for identification of fish in Canada is:
Scott, W.B., and E.J. Crossman. 1990. Freshwater fishes of Canada. Department of Ichthyology and Herpetology, Royal Ontario Museum, Toronto. 966pp.

Associated data sheet name: datasheet.xls – gill net worksheet

Standard Operating Procedures for Bird Sampling

Purpose:
To assess bird populations in Great Lakes coastal wetlands

Equipment needed:

1) Binoculars
2) Stop watch
3) Measuring tape
4) Field guides
5) Tape player
6) Bird call tapes
7) GPS unit
8) Flashlight
9) Meter and volt-ohm
10) Electric conduit
11) Waders

Preparation prior to fieldwork:
Prior to starting fieldwork, wetland sites should be chosen and access to the sites secured. Individuals associated with bird monitoring should be familiar with the point count method used in the Marsh Monitoring Program Survey Protocol, and should be able to identify at least 50 common bird species by sight and sound with an emphasis on marsh birds. Individuals should also know how to set up monitoring stations and if more than one station is set up at one site, should know proper spacing of the stations. Training kits and instructions explaining all of this and more information can be obtained from the Marsh Monitoring Program (http://www.bsc-eoc.org/mmpmain.html).

Field procedures:
Station setup
Before survey routes are established, a detailed topographical map should be obtained for each wetland to aid in sample station placement and thus ensure that sampling is completed in areas where species abundance and diversity are greatest. If possible, routes

should be established in marsh habitat (i.e., dominated by non-woody emergent plants) and each survey station established within areas where greater than 50% of the wetland is dominated by marsh habitat characteristics. It may be necessary to establish multiple routes to ensure that all available habitats are surveyed. Each point count method survey should be conducted from a fixed point within each marsh habitat. Focal points in each sample station may be marked with a metal stake and flagging for easy future relocation. The sample area is a semi-circle with a 100 m radius that extends from this point. This is known as the sample station. Sample stations should be at least 250 m apart in order to ensure that organisms are not counted twice.

Sampling

Surveys are performed from a few hours before dusk until sunset on evenings with good visibility, warm temperatures (>16° C), no precipitation, and little or no wind (3 of less on the Beaufort scale). A point count is conducted using a tape of bird calls to elicit calls from normally secretive species. During the beginning of each ten-minute survey period, a five-minute broadcast tape was broadcast at each station with a tape recorder held at chest height and aimed so that it broadcast toward the 100 m radius semi-circular survey area. Five minutes of calling is followed by five additional minutes of observation. Typical calls used are: virginia rail *(Rallus limicola* Vieillot), sora *(Porzana carolina* L.), least bittern *(Ixobrychus exilis* Gmelin), pied-billed grebe *(Podilymbus podiceps* L.), and a combination of common moorhen *(Gallinula chloropus* L.) / american coot *(Fulica americana* Gmelin). The tape featured 30 seconds of calls followed by 30 seconds of silence for each species. The 10 minute timer begins at the sound of the first call on the tape and all marsh birds detected both visually and aurally within the survey station area should be recorded. The five minute call period is followed by a five-minute count period. All marsh birds should be recorded until the timer signals the end of the survey.

Each bird observed should be assigned to one of three categories:

Mapped Observation: all birds observed or heard actually residing within the boundaries of the 100 m radius semi-circle. These birds make actual, physical contact with the sample area.

Aerial Foragers: all birds observed actively foraging in the air within the sample area, no higher than 100 m, and not otherwise using the sample area.

Outside/Flythrus: all additional species of marsh birds observed during the ten-minute point count outside the sample area or flying through the sample area without landing.

Additional classification of birds should follow the procedure identified in the Marsh Monitoring Protocol (MMP).

Associated data sheet name: datasheet.xls – Bird worksheet

Standard Operating Procedures for Amphibian Sampling

Purpose:
To assess amphibian populations in Great Lakes coastal wetlands

Equipment needed:
1) Tape player
2) Flashlight
3) Batteries
4) Compass
5) Stopwatch
6) Field guides
7) Binoculars
8) Tape measure
9) Waders

Preparation prior to fieldwork:
Prior to starting fieldwork, wetland sites should be chosen and access to the sites secured. Individuals associated with amphibian monitoring should be familiar with the point count method used in the Marsh Monitoring Program Survey Protocol. Individuals should also know how to set up monitoring stations and if more than one station is set up at one site, should know proper spacing of the stations. Training kits and instructions explaining all of this and more information can be obtained from the Marsh Monitoring Program (http://www.bsc-eoc.org/mmpmain.html).

Field procedures:
Station setup
Before survey routes are established, a detailed topographical map should be obtained for each wetland to aid in sample station placement and thus ensure that sampling is completed in areas where species abundance and diversity are greatest. If possible, routes should be established in marsh habitat (i.e., dominated by non-woody emergent plants) and each survey station established within areas where greater than 50% of the wetland is dominated by marsh habitat characteristics. It may be necessary to establish multiple routes to ensure that all available habitats are surveyed. The focal point of the station, from which the point count method occurs, is a semicircular area with a 100 m radius.

Focal points in each sample station may be marked with a metal stake and flagging for easy future relocation. Between one and eight sample stations can be used within a site, but they should be separated by at least 500 m, to ensure that new organisms are counted.

Sampling

Sampling should occur in spring between the beginning of April and the middle of June, with at least 15 days passing between samples, to maximize surveying during breeding periods. The survey should occur shortly after sunset, and finish before midnight. Because peak amphibian calling periods are strongly associated with temperature and precipitation rather than date, visits should be scheduled to occur on evenings with little wind, moist conditions, and a minimum night air temperature of 5-17 °C. A one minute quiet period should be held prior to starting each survey. During 3-minute survey periods, all species heard within a 100 m radius semi-circle in front of the observer should be recorded on a map, and assigned a Call Level Code to each species detected to estimate the number of calling amphibians in each wetland.

Call Level Code 1: Calling individuals can be counted and calls were not simultaneous. In this instance, exact counts could be made of the number of calling individuals and surveyors recorded both code and their count.

Call Level Code 2: Calls of individuals could be distinguished but some calling was simultaneous. Under these conditions, an exact count was not possible but surveyors were able to make reliable estimates of the number of individuals calling. Surveyors were asked to record both the code and their count estimate.

Call Level Code 3: A full calling chorus with calls continuous and overlapping. Reliable counts and estimates were unrealistic at this level of calling intensity and no counts were requested.

Additional classification of calls should be categorized according the Marsh Monitoring Protocol. A lengthened sampling season from April to early July will enable nearly all of the species of frogs and toads potentially present to be detected coincident with their breeding season

Associated data sheet name: datasheet.xls – Amphibian worksheet

Standard Operating Procedures for Vegetation Sampling

Purpose:
To assess emergent and submerged vegetation in Great Lakes coastal wetlands

Equipment needed:

1) Specimen bag
2) Compass
3) Hand lens
4) Backpack
5) Plant press
6) Quadrat frame
7) Rake
8) Field guides
9) GPS unit
10) Waders

Preparation prior to fieldwork:
Prior to starting fieldwork, wetland sites should be chosen and access to the sites secured. Color infrared aerial photographs at the 1:10,000-scale may aid in identifying locations for placing plant community sampling transects within each wetland site. All helpers should know and understand the methods of the sampling procedure prior to starting fieldwork. Information on emergent plant sampling can be found in the following publications:

Albert, D. A., and L. D. Minc. 2001. Abiotic and Floristic Characterization of Laurentian Great Lakes Coastal Wetlands. Verh. Internat. Verein. Limnol. 27(6):3413-3419.

Minc, L. D. 1997. Great Lakes Coastal Wetlands: An Overview of Abiotic Factors Affecting their Distribution, Form, and Species Composition. A Report in 3 Parts. Michigan Natural Features Inventory. 307 pp.

Minc, L. D. and D. A. Albert. 1998. Great Lakes Coastal Wetlands: Abiotic and Floristic Characterization. Michigan Natural Features Inventory. 36 pp.

Quadrats can be constructed from ½" metal electrical conduit joined at the corners with ½" metal pull-through elbows. Cylindrical pieces of polyethylene foam may be added to two opposite sides of the square quadrat frame to provide flotation for quadrats in the submerged zone.

Field procedures:

Emergent Sampling

Sampling should be conducted on foot along transects perpendicular to the hydrologic gradient. Five randomly located 0.5 m square quadrats should be sampled in each vegetation zone along each transect. The starting point of the transect should be randomly located beginning within 25 m of the upland edge of the wet meadow zone. Subsequent sampling points should be approximately 25 m apart. The location of each sampling quadrat around a sampling point may be selected using randomly selected compass bearings and distances from 1 to 9 m.

Within each quadrat, the total vegetation cover and grouped estimates of emergent, submerged, and floating-leafed plant cover should be recorded as percentages. Cover estimates for each species should also be recorded. The total coverage within a quadrat could exceed 100% because more than one individual may occupy the same area in the two dimensional sampling plane created by the quadrat. For example, when the quadrat is placed in an area with dense floating-leafed plants, the coverage of a floating leafed plant species in the quadrat could be 75%. In addition, submerged macrophytes may be present under the floating leaves at a density of 50%. The resulting total coverage would then be 125%. A fifteen minute or longer random observation may also be completed within each plant zone for the evaluation of the overall species diversity. Plants should be identified to species level, except for sterile plants (mainly grasses and sedges) that require flowering parts for identification.

Sampling in each quadrat may be combined with measurements of water depth, Ecological Land Classification community code (herbaceous, shrub swamp, tree swamp, emergent marsh, submergent marsh), substrate, organic depth, and water clarity (using secchi disk). This method is most appropriate for sampling in the wet meadow and emergent/submergent zones. If a wide submergent zone without emergent vegetation is present, five additional sampling points may be included.

Evaluation of invasive exotic plants requires 1) identification and aerial photo quantification of dense monoculture stands; and 2) ground verification. Thus, it was important to determine presence/absence of lower densities of invasive plants in the 5 random quadrats along transects while in the field. Presence of invasive species and diversity of different invasive species present should be measured along transects.

Emergent plant sampling

Submergent sampling

When present, the submergent zone may be sampled from a canoe within each quadrat while performing emergent plant sampling. If sampling submergent vegetation only, sampling may occur using the rake toss method from a canoe. Samples should be identified to species, and percent cover should be estimated for each plot/quadrat.

Submergent plant sampling

Associated data sheet name: datasheet.xls – Vegetation worksheet

Standard Operating Procedures for Water Quality Analysis

Purpose:
To assess water quality in Great Lakes coastal wetlands

Equipment needed:
In field:

1) Meters – Quanta Hydrolab, turbidimeter, SCT, DO meter, etc.
2) Filtering unit and filters
3) Sample bottles or whirlpaks
4) Cooler
5) Ice packs
6) Thermometers
7) Markers
8) Gloves
9) Waders

In lab:

1) Graduated cylinders
2) Volumetric flasks
3) Beakers
4) Pipette
5) Pipette tips
6) Syringes
7) Syringe filters
8) GFC filters
9) Sample tubes
10) Test tube rack
11) Kimwipes

12) Standards*

*Depends on the type of sampling (i.e. sediment, water, etc.), and tools (i.e. no need for turbidity std. if Quanta Hydrolab is used).

Preparation prior to fieldwork:

Prior to starting fieldwork, wetland sites should be chosen and access to the sites secured. Field staff should be instructed in water quality analysis procedures and maintenance and calibration of equipment prior to beginning fieldwork. A good reference for water quality analytical procedures is the following:

APHA. 1998. Standard Methods for the Evaluation of Water and Wastewater. 20th edition. American Public Health Association. Washington, DC.

Water chemistry data for given sites may also be available from EPA's computerized environmental data system called STORET: http://www.epa.gov/storet/

Field procedures:

Collection/sampling

Water quality parameters should be measured for each vegetation zone. Samples should all be collected within a 24 to 48 hour period to minimize temporal variation in results. In general, water samples should be collected adjacent to the emergent vegetation zone. Care should be taken to avoid disturbing the sediment while samples are being collected. Thus, water quality assessment should be performed prior to biotic sampling and if possible from a canoe or boat.

Measuring water temperature

Turbidity, pH, water temperature, air temperature, dissolved oxygen, chlorophyll a, redox potential, conductivity, and total dissolved solids may all be sampled in the field with electronic devices such as the Quanta Hydrolab or specific meters (Hack 2100P turbidimeter for turbidity, Hanna H1991300 for pH and conductivity, YSI 55 for dissolved oxygen and temperature, etc). Less sophisticated methods (i.e. thermometer) may also be used.

Taking measurements with a YSI meter

Three or more water samples should be taken from each plant zone for parameters not easily measured in the field. These are: alkalinity, chloride and sulfate, nitrogen, and phosphorus. The type of nitrogen or phosphorus sampled (total nitrogen/phosphorus, ammonia (NH4), nitrate (NO3), soluble reactive phosphorus (SRP), etc.) may vary according to applicability. Water samples should be collected approximately 20 cm below the surface using sterilized 500 ml sample bottles or whirlpaks, each rinsed three times with sample water prior to collection of sample. Each sample should then be filtered through a membrane into another acid-washed, rinsed or sterilized bottle and placed on ice until brought to a lab and frozen.

Lab procedures:
Qualified personnel or outside laboratory should be used to test water samples for all parameters not measured in the field.

Standardized methods (USEPA 1979, APHA 1998) may be followed to analyze water samples for: total suspended solids, soluble reactive phosphorus, total phosphorus, nitrate, nitrite, ammonia, total Kjeldahl nitrogen, chloride, sulfate, soluble reactive silica, and specific conductance.

For SRP, NH3 and NO3, sample blanks (<0.01 mg/L for nitrogen compounds, and <0.02 mg/L for SRP) should be run with each batch of samples. For QA/QC, initial, matrix

spikes and duplicate matrix spikes should be measured and recorded for every tenth sample, using known parameter spike concentrations of 0.10 mg/L. Percent measurement recovery should be recorded by comparing matrix spikes with initial readings, and a standard curve produced.

USEPA. 1979. Methods for analysis of water and wastes. EPA 600/4-79-020, US Environmental Protection Agency. Washington, D.C.

Associated data sheet name: datasheet.xls – Water Quality worksheet

Standard Operating Procedures for Contaminated Sediment Sampling

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Purpose:

To assess sediment contamination in Great Lakes coastal wetlands

Equipment needed:
In field:

1.) Piston core device
2.) Large SS mixing bowls
3.) SS spoons
4.) Sample bottles plastic (cs of 90)
5.) Sample bottles glass (cs of 12)

In Laboratory:

1.) Gas chromatograph
2.) Gas chromatograph/mass spectrometer
3.) Mercury analyzer
4.) ICP
5.) TOC analyzer
6.) Ammonia electrode and ion meter
7.) Soxhlet extraction system
8.) Sample concentrator
9.) USGS sieves (10)

Preparation prior to fieldwork:
Prior to starting fieldwork, wetland sites should be chosen and access to the sites secured. Field staff should be instructed in sediment collection procedures and use of equipment prior to beginning fieldwork.

Field procedures:
Unconsolidated sediment samples should be collected for each vegetation zone with a 5 cm core and transferred to a 4 L glass jar. Up to five samples from each vegetative zone per

wetland should be sampled. Samples should be kept separate from each other until transported back to the laboratory.

Collection of sediment core samples

Lab procedures:
A small sample size of sediment cores may be cost prohibitive to set up and analyze individually. An alternative would be to have samples analyzed commercially.

Discrete samples should be composited in the laboratory before analysis. The intent is to analyze individual samples to determine spatial distribution of contaminants if composite samples exceed Threshold Effect Levels (MacDonald et al 2000).

Standardized methods (USEPA 1996) should be used to analyze samples for arsenic, cadmium, lead, chromium, mercury, copper, polychlorinated biphenyls, DDT homologs, PAH compounds, total organic carbon, grain size distribution, and ammonia.

MacDonald, D. D., C. G. Ingersoll, and T. A. Berger. 2000. Development and Evaluation of Consensus-Based Sediment Quality Guidelines for Freshwater Ecosystems. Archives of Environmental Contamination and Toxicology 39:20-31.

USEPA. 1996. Test methods for evaluating solid waste, physical/chemical methods (SW-846). 3rd edition. United States Environmental Protection Agency, Office of Solid Waste and Emergency Response, Washington, DC.

Associated data sheet name: datasheet.xls – Contaminated sediments worksheet

Standard Operating Procedures for Landscape Attribution

Purpose:
To identify natural and anthropogenic influences in Great Lakes coastal wetlands using both field and GIS methods

Equipment needed:
In field:

5) Topographic maps
6) Maps
7) GPS
8) Aerial photographs
9) Binoculars
10) Rite-in-the-rain paper

In GIS lab:

1) GIS data
2) GIS software

Preparation prior to fieldwork:
Prior to starting fieldwork, wetland sites should be chosen and access to the sites secured.

Field procedures:
Field surveying
Sites should be surveyed for qualitative (presence/absence) hydrologic and landscape alteration aspects of the wetland. Numerical data should be collected on presence of structures in or near the wetland. Descriptive data should include habitat types, landuse, construction, and various adjacent human activities. See datasheet for more comprehensive overview. Categories of interest follow:

HYDROLOGIC ALTERATION
dewatering in or near wetland
point source inlet

installed outlet, weir
ditch inlet
tile inlet
unnatural connection to other waters
presence of barriers (dams, waterfalls)

LANDSCAPE ALTERATION: *Vegetation removal/disturbances*
tree removal
tree plantations
mowing or grazing
shrub removal
Coarse woody debris removal
Removal or emergent vegetation

LANDSCAPE ALTERATION: *Substrate/soil disturbances*
presence of livestock hooves
presence of vehicle use
presence of grading/bulldozing
presence of filling
presence of dredging
sediment input (from inflow or erosional)
areas of land in high public use

NUMERICAL PARAMETERS:
proximity to navigable channels
proximity to recreational boating activity
Proximity to roadways that receive regular (daily) traffic
# of dwellings
# of industries
of 'other' buildings
of boat docks
# of paved parking lots
# of dirt parking lots
# of boat launches
% hardened shoreline
% eroding shoreline
% shoreline containing a visible dirt road
% shoreline containing a visible paved road

DESCRIPTIVE PARAMETERS:

- habitat types adjacent to wetland
- land-use classes adjacent to wetland
- Construction sites or obvious sedimentation
- Highway, rail, levees, berms, boardwalks or other such structures built in or around wetland including whether or not the structure appears to restrict hydrological connection
- Categorical degree and type of direct human activity

Mapping

GIS and aerial photography can be used to gain detailed quantitative data about each wetland site. Information may thus be collected on: percent of each adjacent landuse,

percent impervious surface, wetland area, number of adjacent dwellings, number of connecting drainage ditches, watershed area above wetland, length of streams in watershed, high/low elevation in watershed, ecoregion, and chemical pollution. Buffers of 500 m, 1 km and 20 km may be drawn around each wetland site to gain insight into distance of disturbance surrounding each site.

Black and white aerial photography is available for the United States from: http://terraserver.homeadvisor.msn.com/

Associated data sheet name: datasheet.xls – Site Attribute worksheet

Appendix C. The use of snapping turtle eggs as an indicator of contaminant exposure in coastal wetlands of the Great Lakes – St. Lawrence basin

THE USE OF SNAPPING TURTLE EGGS AS AN INDICATOR OF CONTAMINANT EXPOSURE IN COASTAL WETLANDS OF THE GREAT LAKES – ST. LAWRENCE BASIN

Presented to

The Great Lakes Coastal Wetland Consortium

22 July 2004

By

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Executive Summary

- Eggs of the common snapping turtle are excellent indicators of wetland health and contaminant bioavailability. Snapping turtle eggs provide excellent temporal and spatial trends information concerning organochlorine pesticides (e.g., DDT), polychlorinated biphenyls (PCBs), dioxins and furans. In addition, the eggs of this species are capable of providing information for such trends concerning newly emerging chemicals of concern (e.g., polybrominated diphenyl ethers (PBDEs).

- The snapping turtle has been ranked seventh out of 25 vertebrate species used as indicators of persistent organic pollutants (Golden and Rattner 2003).

- The health of snapping turtles has been adversely affected by contaminant exposure in the Great Lakes Basin (Bishop et al., 1991; Bishop et al., 1998).

- Snapping turtles inhabit many types of wetlands when suitable habitat is available, and have small home ranges with limited movement. These characteristics make this species a good reflector of local (point) sources of contaminants, as well as different chemical mixtures (e.g., Aroclors) of contaminants, in a wide variety of wetland types. They are also excellent indicators of the bioaccumulation of chemicals through the food chain.

- In a monitoring program, the annual collection of data is preferred to less frequent sampling in terms of providing the most robust data/information in the fewest number of years (Hebert and Weseloh, 2003).

- Multiple state and provincial agencies, volunteers, and paid staff with one coordinating agency, will have to be involved to adequately cover multiple wetlands/sites across a wide geographical area such as the Great Lakes Basin or even both Canadian and American sides of one of the Great Lakes. The extent of each agency's participation in this monitoring plan will have to be discussed individually. Snapping turtles lay their eggs during the same two week period

(usually the second and third weeks) of June regardless of their location in the Great Lakes Basin. They have not been found on the northern shores of Lake Superior.

- **A pilot project for the first three years of the program** is proposed for Lake Ontario or Lake Erie, with each of the three appropriate wetland types (lacustrine, riverine, barrier-protected) represented relative to their known or anticipated contaminant levels (high, medium and low; alternatively use a high-low contaminant classification); a total of 9 sites will be needed for this pilot study. Lake Michigan may be considered as an alternative for this pilot project but will obviously negate the bi-national aspect of the pilot monitoring program. Protected lacustrine, drowned river-mouth riverine, and barrier beach lagoon wetlands should contain high densities of snapping turtles, but, open lacustrine, connecting channel, delta riverine wetlands, and barrier-protected swale complexes, will have much lower densities of snapping turtles making sampling difficult. In order to achieve a good representation of lake-wide contaminant patterns, coastal wetlands should be located throughout the lake's shoreline although some clustering is likely to occur.

- **Subsequent to the pilot project**, an assessment of the data should be completed to determine if the type of wetland affects the contaminant concentrations found in snapping turtle eggs. If no effect of wetland type is found on these concentrations, then this factor should be removed from the experimental design. In order to determine an overall assessment of contaminant trends on a lake-wide basis, four locations (two Canadian, two American) within each of the three contaminant concentration categories (high, medium, low) should be selected on each of lakes Michigan, Huron, Erie and Ontario. This experimental design will provide a total of 48 sites and data for a bi-national assessment of the contaminants trends in coastal wetlands across all the Great lakes except Lake Superior. The number of sites may be reduced by only using sites that are of high or low levels of contamination; the total number of sites would be 32 using this design.

Alternatively, if wetland type does affect the contaminants levels in snapping turtle eggs, then a different experimental design will have to be employed to determine trends in contaminant levels using snapping turtle eggs. Wetland type (3) within each of the contaminant categories (high, medium, low) on each side (Canadian, American) of each of the Great Lakes (4) will result in the monitoring of 72 sites, or 48 sites if the contaminant categories are restricted to high, low classification.

Whether or not the experimental design accounts for wetland type, selected wetlands should be located throughout the basin of each lake, with the realization that some clustering will occur depending on the location of most wetlands.

- **Estimated Project Budget**: Based on 2004 project costs for snapping turtle work by the Canadian Wildlife Service, we estimate that the pilot study will cost approximately $171,025 CDN per year or a total of $513,075 CDN for three years for work completed on Lake Ontario. Sampling and analysis of eggs and data from each field collection site will cost approximately $12,925 CDN per year, but the costs for a full-time person, statistical analysis, and report writing (total $75,000 CDN) must still be accounted for. Following the pilot study, the cost for a basin-wide (four lakes) monitoring plan using snapping turtles is estimated to cost between $0.494 M CDN (32 sites) and $0.701 M CDN (48 sites) regardless of wetland type, or between $0.701 M CDN (48 sites) and $1.012 M CDN (72 sites) when accounting for wetland type. For these basin-wide program budgets, the $75 K CDN for the full-time person (coordinate program, complete statistical analysis and report writing) and eight additional agency co-ordinators ($60.0 K CDN) are included. These budgets may be pro-rated according to the number of sites in each state/provincial jurisdiction.

- A monitoring program for contaminants in snapping turtle eggs must involve the coordination of people, agencies, and groups to insure comparability and robustness of data, and that all protocols are followed in an appropriate manner. Consequently, the program must follow the approved protocol outlined in the

Quality Assurance Project Plan (QAPP) (see Appendix) to ensure the development and implementation of an integrated, bi-national monitoring program. The groups involved in the monitoring activities will coordinate their efforts through the use of this protocol in sampling procedures, sample and data analysis, and reporting methods, to insure a basin-wide, bi-lateral consistency in data collection and methodologies, thereby enhancing the comparability and value of the data in identifying spatial and temporal trends in contaminant levels.

1.0 Introduction

1.1 *Preamble*.

In preparing the documents involving the snapping turtle as a model for monitoring contaminants as requested by the Great Lakes Wetlands Consortium, the Monitoring Plan and the White Paper (Literature Review) have been combined in this report. Section 2.0 of this report outlines the monitoring plan for using snapping turtle eggs to determine trends in chemical concentrations found in coastal wetlands of the Great Lakes Basin, and hence the integrity of these coastal wetlands. Following the monitoring plan in Section 2.0, the White Paper addresses the six criteria previously established by the Consortium, relating to the utility, cost and validity of using snapping turtle eggs for measuring contaminant concentrations. In addition, we have appended the approved Quality Assurance Project Plan (QAPP) document (Project # WETLANDS2-EPA-05, Revision #3) required by the United States Environmental Protection Agency. All criteria for this project as stipulated by the Great Lakes Wetlands Consortium in the Request for Proposal are outlined below in the Introduction.

1.2 *Introduction*

This white paper describes the utility of the common snapping turtle (*Chelydra serpentina serpentina*) as an indicator of persistent organic contaminants in Great Lakes coastal wetlands. It originates from a need to consistently measure and monitor the status of wetland systems in terms of their degradation due to anthropogenic, persistent, organic chemicals. Our major goal is to present a framework for a sustainable, long-term, basin-

wide wetland contaminants monitoring plan. While monitoring chemical parameters in water and sediment generally reflect the degree of pollution, the measurement of contaminant concentrations in tissues of snapping turtles will provide a gauge of toxicant bioavailability in wetland environments. Thus, this white paper also validates the common snapping turtle as an indicator of chemical exposure, particularly local but non-specific sources of contaminants. The snapping turtle provides many advantages for monitoring contaminant levels in wetlands, including its wide geographic distribution, abundance in a variety of wetland systems, longevity, sedentary nature, its potential for bio-accumulating organic contaminants through its diet, and the ability of adult turtles to store high concentrations of polychlorinated biphenyls (PCBs) in their adipose tissue without apparent adverse effects (Meyers-Schöne and Walton, 1994). Moreover, egg samples for analysis of contaminant concentrations may be taken in sufficient quantities without seriously impacting adult populations (Cunnington and Brooks 1996).

The White Paper addresses six criteria that originate from the Request for Proposals (RFP) disseminated by the Great Lakes Commission on behalf of the Great Lakes Coastal Wetlands Consortium. These criteria fall under the "Scope of Work" in the RFP as one of the goals "to test the feasibility of applying indicators in a monitoring plan." The following are a list of questions posed by the Consortium that serve as the basis for the information discussed in this white paper:

- What is the cost of implementing a program using snapping turtle eggs to measure organochlorine contamination and pesticides, as well as the cost and availability of analytical methods to measure other chemicals of concern?
- Are contaminants measurable in snapping turtle eggs? What is the design and methodology best suited to obtain geographic and temporal contaminant trends in coastal wetlands, and how will wetland sites be chosen for the monitoring plan?
- How applicable and reliable is the snapping turtle in terms of measuring/monitoring contaminants in various wetland types across the upper and lower Great Lakes basin?
- What complementary existing research and data are available that is relevant to using the common snapping turtle to monitor contaminant levels?

- Are snapping turtles sensitive in terms of detecting changes in contaminant concentrations of wetlands over time and space?
- How useful is the snapping turtle for a monitoring plan in terms of being able to set endpoint(s) or attainment levels relative to contaminant levels in wetlands of the Great Lakes basin?

2.0 A Proposed Bi-National Monitoring Plan Utilizing the Snapping Turtle as a Sentinel Species for Contaminant Concentrations in Coastal Wetlands of the Great Lakes Basin

This section of the report will outline the proposed plan for monitoring the quality of Great Lakes coastal wetlands in terms of their degradation due to persistent organic contaminants utilizing snapping turtle eggs. The rationale for this plan, and the validation of using the snapping turtle as a basin-wide and within-lake indicator of contaminant bioavailability, are provided in subsequent sections of the White Paper.

In the RFP, the major objective of the snapping turtle monitoring program was to determine spatial and temporal trends in contaminant concentrations in the three types of coastal wetlands (lacustrine, riverine, and barrier-protected system) regardless of the location(s) of contaminant sources. However, the location of a wetland relative to the contaminant source, will determine the levels of contamination within that wetland as well. Consequently, we recommend that several wetlands of each type, at varying distances from contaminant sources, be selected on each lake (with the exception of Lake Superior). Such an approach will provide a better understanding of contaminant trends in different types of coastal wetlands at the larger scale of the individual lake and the basin as a whole. However, there are many coastal wetlands along the shoreline of each lake and the sampling of two or three wetlands of each type would not be properly representative of the coastal wetlands of that lake.

In selecting the wetlands for use in this snapping turtle monitoring plan, three other considerations must also be taken into account: (1) suitable habitat for adult snapping turtles to inhabit and to lay eggs must be present at the wetland; (2) egg laying by snapping turtles generally occurs during the same 14 d period in the middle of June, regardless of their location within the Great Lakes Basin; (3) snapping turtles are not found along the

northern shores of Lake Superior in Canada, nor are they likely to be found since existing wetlands do not have appropriate habitat and the Lake Superior environment is too cold.

In addition, the monitoring program must follow the approved protocol outlined in the Quality Assurance Project Plan (QAPP) (see Appendix). Development and implementation of an integrated, bi-national monitoring program requires that all participants have the most current version of the approved QAPP (Appendix). The groups involved in the monitoring activities will coordinate their efforts through the use of this protocol in sampling procedures, sample and data analysis, and reporting methods, to insure a basin-wide, bi-lateral consistency in data collection and methodologies, thereby enhancing the comparability and value of the data in identifying spatial and temporal trends in contaminant levels.

We recommend the following monitoring plan using snapping turtle eggs to achieve the objective of the Great Lakes Wetlands Consortium:

- Coastal Wetland Selection:
    - **A pilot project for the first three years of the program**: On Lake Ontario or Lake Erie, each of the three appropriate wetland types will be represented (if possible) relative to their contaminant levels (high, medium and low to serve as the reference site); a total of 9 sites will be needed for this pilot study. Lake Michigan may be considered as an alternative for this pilot project.
        - We recommend sampling protected lacustrine, drowned river-mouth riverine, and barrier beach lagoon wetlands as such habitats are likely to contain high densities of snapping turtles. However, open lacustrine, connecting channel, and delta riverine wetlands will have much lower densities of snapping turtles making sampling difficult; sampling of wetlands and creeks near to these large wetlands may be an alternative. Similarly, barrier-protected swale complexes may also prove difficult as habitat will likely be unsuitable for snapping turtles.
        - In order to achieve a good representation of the lake basin, wetlands should be located throughout the lake basin as much as possible. For example, most coastal wetlands in Lake Ontario are located in the

eastern basin, so many of the sampling points will be located here. However, it is important that other wetlands of all types be chosen from the other areas throughout Lake Ontario in order to gain an understanding of lake-wide trends in contaminants in coastal wetlands.

- For many wetlands, contaminants levels are unlikely to be known but contaminant concentrations for water and sediment samples are available for many sites through universities and/or government agencies. In addition, selecting sites according to the distance from known contaminant sources (e.g., industry, sewage treatment plants, agricultural inputs; urban vs. rural areas) will aid in determining approximate contaminant levels in a wetland.
- Alternatively, only a reference site and a highly-contaminated site within each wetland type may be selected for the pilot work.

- **Subsequent to pilot project**: Following the pilot project, an assessment of the data should be completed to determine if the type of wetland affects the contaminant concentrations found in snapping turtle eggs. If no effect of wetland type is found on these concentrations, then this factor should be removed from the experimental design. In order to determine an overall assessment of contaminant trends on a lake-wide basis, four locations (two Canadian, two American) within each of the three contaminant concentration categories (high, medium, low as a reference site) should be selected on each of lakes Michigan, Huron, Erie and Ontario. This experimental design will provide a total of 48 sites and data for a bi-national assessment of the contaminants trends in coastal wetlands. The number of sites may be reduced by only using sites that are of high and low levels of contamination; the total number of sites would be 32 using this design.

  Alternatively, if wetland type does affect the contaminants levels in snapping turtle eggs, then a different experimental design will have to

be employed to determine trends in contaminant levels using snapping turtle eggs. Wetland type (3) within each of the contaminant categories (high, medium, low) on each side (Canadian, American) of each of the Great Lakes (4) will result in the monitoring of 72 sites, or 48 sites if the contaminant categories are restricted to high, low classification.

Whether or not the experimental design accounts for wetland type, selected coastal wetlands should be located throughout the shoreline of each lake in order to characterize lake-wide contaminant patterns, with the realization that some clustering will occur depending on the location of most coastal wetlands.

- Site Selection: Suitable coastal wetland sites with historical contaminants data for snapping turtle eggs should be included when possible. In addition, all sites should have known high density populations of snapping turtles to insure collection of eggs in a timely manner within the 14 day period. Speaking with local residents, fishers, and fish biologists at universities and state/provincial agencies, is helpful in determining the existence and density of snapping turtles in nearby water bodies.

  Herdendorf (2004) provides an excellent classification of the significant coastal wetlands of the Great Lakes; this classification system differs from the one used by the Great Lakes Wetlands Consortium. Below, is a list of possible Canadian wetland sites known to have snapping turtles.

  a. St Clair River: St. Clair National Wildlife Area (barrier-protected diked wetland), Walpole Island (riverine delta). Contaminant levels are relatively low compared to other Canadian sites.
  b. Detroit River: Turkey Creek – a riverine wetland with high contaminant levels; Canard River Marshes – estuarine/diked wetland, but historically difficult to locate snapping turtle eggs.

c. Lake Erie: Wheatley Provincial Park (barrier-protected but the barrier is washed out quite regularly resulting in a lacustrine wetland each summer), Rondeau Provincial Park, Long Point National Wildlife Area (lacustrine wetland). These sites are moderately to highly contaminated.
d. Niagara River: Lyons Creek – a riverine or diked wetland; water is pumped into the Creek from the Welland Canal. Snapping turtle eggs from this area indicate a point source of PCB contaminants.
e. Lake Ontario: Cootes Paradise – riverine wetland and one of the most contaminated sites. Oshawa Second Marsh (lacustrine), the Bay of Quinte (lacustrine), Lynde Creek although the current existence of snapping turtles in this area is questionable.
f. St. Lawrence River: Upper Canada Bird Sanctuary near Ingleside ON – barrier-protected diked wetland (north side of UCBS) and open lacustrine wetland (west side of UCBS). Contaminant levels were relatively low at this site in 2003.

- Frequency of Collection: Egg samples should be collected yearly for the three year pilot study, and then yearly or once every two years from each site following the pilot study. Preferably, all sites should be collected from within the same year. An assessment as to the frequency necessary to determine trends should be conducted after the first three collections.

- Site monitoring: Each year, each collection site will be monitored to determine when the snapping turtles commence nesting (usually for 10 – 14 days during the middle of June, depending on the location within the Basin). Eggs must be collected as soon as possible after laying since 99% of nests are predated by raccoons or other mammalian predators within 12 hours of laying; furthermore, embryonic development is minimal at this time.

- Sample Size: At each site, five clutches of eggs should be collected for contaminant analysis. Five eggs taken from throughout each clutch should be collected. In order

to minimize sample loss during shipping, the eggs from each clutch may be broken open and the contents put into hexane-rinsed jars. Clutches should be kept separately. The jars (or shipping container) need to be labeled with site location, date of collection, contact information for the collector. The samples from each site need to be shipped immediately after egg collection is complete, to the coordinating agency. The coordinating agency will log the locations and numbers of samples per location, and then forward all of the egg samples to the contract lab for specific contaminant analysis.

- Multiple agencies will have to participate in order to successfully conduct this monitoring program. Discussions with each individual agency will have to be conducted to determine the extent of their participation. Possible agencies include: universities and natural history groups; state and provincial groups (e.g., New York Department of Environmental Conservation (NYDEC), Michigan Department of Natural Resources (DNR), Ohio DNR, Wisconsin DNR, Minnesota DNR, Ontario Ministry of Natural Resources); and federal agencies (e.g., Canadian Wildlife Service, U.S. Fish and Wildlife Service).

- Estimated Project Budget: Based on 2004 project costs for snapping turtle work by the Canadian Wildlife Service, we estimate that the pilot study will cost approximately $171,025 CDN per year or a total of $513,075 CDN for three years for work to be completed on Lake Ontario. The details are provided in the table immediately below and are best estimates only; please note that some costs may have been overlooked. Egg collections and chemical analyses for each site is likely to cost approximately $12,925 CDN per year, but the costs for a full-time person who will act as the main project coordinator and complete the statistical analysis and report writing (total $75,000 CDN), must still be accounted for. Following the pilot study, the cost for a basin-wide (four lakes) monitoring plan using snapping turtles is estimated to cost between $0.494 M CDN (32 sites) and $0.701 M CDN (48 sites) regardless of wetland type, or between $0.701 M CDN (48 sites) and $1.012 M CDN (72 sites) when accounting for wetland type; the $75K CDN for the

full-time person, statistical analysis and report writing, as well as the $60 K CDN for hiring eight agency co-ordinators, are included in all of these budgets.

Pilot study: 9 sites on Lake Ontario (all costs are listed in Canadian dollars)

(Lake Erie would require additional contractors, field costs for field collections & as contact for main coordinator)

		per Site		**Per Year**
Contaminants	5 pools of 5 eggs each/site		45 pools/year	
egg preparation ($25/egg)	25 eggs/site	$625		$5,625
OC pesticides ($350/sample)	5 samples/site	$1,750	45 samples*350	$15,750
dioxins ($1200/sample)	1 sample/site	$1,200	9 pools *1200	$10,800
BDEs ($350/sample)	5 samples/site	$1,750	45 samples*350	$15,750
Total mercury ($30/sample)	5 samples/site	$150		$1,350
Total: contaminant analyses		**$5,475**		**$49,275**
Field collection costs				
per diem per person ($150/d * 4 d at each site); 2 people (for safety reasons)		$1,200		$10,800
Food per day ($75/d * 4 d/site) per person; 2 people/site		$600		$5,400
hotels (4 nights/site*$100/d*2 people)		$800		$7,200
Total: field collection costs		**$2,600**		**$23,400**
Travel, vehicle costs				
van rental (14 d * $100/d)		$1,400		$5,600
insurance & gasoline (best estimate only)		$1,000		$4,000
Total: travel, vehicle costs		**$2,400**		**$9,600**
Staffing costs				
1 full-time (overall project co-ordination, statistical analysis, report writing)				$67,500
1 full-time person as agency co-ordinator				$7,500
1 contractor (agency co-ordinator; $150/d*50d)		$1,700		$7,500
Total: staffing costs		**$1,700**		**$82,500**
Miscellaneous costs				
courier costs (btwn sites, lab prep, central lab, reports)		$500		$4,000
Field equipment (containers, vermiculite, water)		$250		$2,250
Total: miscellaneous costs		**$750**		**$6,250**
Grand total costs		**$12,925/site**		**$171,025/year**

- Analytical costs:
  - PCBs, organochlorine (OC) pesticides, polybrominated diphenyl ethers (PBDEs): Currently (2004), the Great Lakes Institute of Environmental Research at the University of Windsor, a contract lab used by the CWS, charges $350 CDN per sample for PCB and organochlorine pesticides and an additional $350 CDN per sample for PBDEs. One sample per clutch is usually analyzed for these contaminants.
  - Non-ortho PCBs, dioxins, and furans: AXYS Analytical, another contract laboratory used by the CWS, currently charges $1200 CDN per sample for non-ortho PCBs, dioxins. One pooled sample per site (sub-samples from all clutches from one site pooled into one sample) is usually analyzed for dioxins, furans and non-ortho PCBs. The Great Lakes Institute of Environmental Research at the University of Windsor does not conduct this type of chemical analysis (Dr. K. Drouillard, University of Windsor, pers. comm.).
  - Total mercury (Hg) (approximately $30 CDN /sample) is also measured but not the biologically important form of methyl-mercury (approximately $100 CDN /sample); one sample per clutch is usually selected for analysis from those sites in which total mercury is a suspected problem. Philip Analytical Services (Halifax, NS) is a contract laboratory that will analyze Hg in wildlife tissues.

- Statistical analysis and reporting of results will be completed after each collection, although the time required for laboratory chemical analysis may not make annual reporting feasible.

- Endpoint for Chemical Monitoring: Monitoring of chemical concentrations using snapping turtle eggs may be ceased when concentrations of toxic chemicals are similar among inland reference site(s) and the various coastal wetland sites located within the Great Lakes Basin. This endpoint definition is used by the CWS in its herring gull chemical monitoring program which has been run since 1974.

The following sections of this report provide the rationale for this monitoring program and the scientific background for using snapping turtle eggs as a means to monitor chemical concentrations in coastal wetlands in the Great Lakes Basin.

3.0 Program budget and analytical costs

3.1 *Program Budget*

Based on 2004 project costs for snapping turtle work by the Canadian Wildlife Service, we estimate that the pilot study will cost $171,025 CDN per year or a total of $513,025 CDN for three years. Budget details for the project have been provided in the preceding section, and are based on a best estimate only; some costs may have been overlooked although this was not intentional. Each field collection site is likely to cost approximately $12,925 CDN per year, but the costs for a full-time person, statistical analysis, and report writing (total $75,000 CDN) must be taken into account. Following the pilot study, the cost for a basin-wide (four lakes) monitoring plan using snapping turtles is estimated to cost between $0.494 M CDN (32 sites) and $0.701 M CDN (48 sites) regardless of wetland type, or between $0.701 M CDN (48 sites) and $1.012 M CDN when accounting for wetland type; the $75 K CDN for the full-time person, statistical analysis and report writing, as well as the $60 K CDN for the eight agency co-ordinators, are included in all of these budgets. These budget figures include the estimated costs for hiring of staff (full-time person, one contractor per agency), purchasing of field equipment and materials, travel (hotel, food, gas, vehicles, insurance), courier shipping of egg samples and other materials, statistical analysis of data, and the presentation and reporting of results. Depending on the timeliness of the chemical analysis, statistical analysis and reporting of results should be completed after each collection.

3.2 *Analytical Costs*

The analytical cost associated with quantitative analysis of organochlorine (OC) pesticides and polychlorinated biphenyl compounds (PCBs) is currently $350 CDN per sample as charged by the contract laboratory at the Great Lakes Institute of Environmental Research at the University of Windsor; a sample may consist of individual or pooled eggs.

The organochlorine pesticides and compounds that are typically measured by the CWS in snapping turtle eggs include: *p,p'*-dichlorodiphenyltrichloroethane (*p,p*'-DDT); 1,1-dichloro-2,2-bis (p-chlorophenyl)ethylene (*p,p*'-DDE); *p,p*'-DDD, *alpha*-, *beta*,- and *gamma*-hexachlorocyclohexane (HCH); hexachlorobenzene (HCB); octachlorostyrene (OCS); mirex; dieldrin; photomirex; *cis*- and *oxy*- chlordane; *cis*- and *trans*- nonachlor, and heptachlor epoxide (HC Epox). The PCB congeners that are currently measured in routine OC analyses by the CWS include the following 59 congeners: #16/32; 17;18; 22; 28; 31; 33/20; 42; 44; 47; 49; 52; 56/60; 64; 66; 70/76/ 74; 85; 87; 92; 95; 97; 99; 101/90; 105; 110; 118; 128; 130; 137; 138; 141; 146; 149; 151; 153; 156; 157; 158; 170/190; 171; 172; 174; 176; 177; 178; 179; 180; 183; 187; 194; 195; 196/203; 201; 200; 202; 206; 207, and 208. In addition, the Aroclor 1260 and Aroclor 1254:1260 (1:1) equivalents are provided with most analytical reports and provide important information that allows for historical comparisons when fewer congeners were measured.

Quantitative analysis of dibenzodioxins (PCDDs), dibenzofurans (PCDFs), and non-ortho PCBs (congeners # 37, 77, 81, 126, 189) is currently $1200 CDN per sample as charged by the contract laboratory of AXYS Analytical (Vancouver, British Columbia). The Great Lakes Institute of Environmental Research at the University of Windsor does not complete this type of chemical analysis (Dr. Ken Drouillard, University of Windsor, personal communication 2004). The following PCDD congeners are provided with most analytical reports: 2,3,7,8-TCDD; 1,2,3,7,8-PeCDD; 1,2,3,4,7,8-HxCDD; 1,2,3,6,7,8-HxCDD; 1,2,3,7,8,9-HxCDD; 1,2,3,4,6,7,8-HpCDD, and OCDD. The PCDF congeners most often reported are: 2,3,7,8-TCDF; 2,3,7,8-TCDF(C); 1,2,3,7,8-PeCDF; 2,3,4,7,8-PeCDF; 1,2,3,4,7,8-HxCDF; 1,2,3,6,7,8-HxCDF; 1,2,3,7,8,9-HxCDF; 2,3,4,6,7,8-HxCDF; 1,2,3,4,6,7,8-HpCDF; 1,2,3,4,7,8,9-HpCDF, and OCDF. In addition, the following non-ortho PCB congerners are provided: PCB 37 (3,4,4' TriCB); PCB 77 (3,3',4,4' TetraCB); PCB 126 (3,3',4,4',5 PentaCB); PCB 169 (3,3',4,4',5,5' HexaCB), and PCB 189 (2,3,3',4,4',5,5' HeptaCB).

Of recent concern are brominated flame retardants (BFRs), especially polybrominated diphenyl ethers (PBDEs). At this time, nine brominated diphenyl ether congeners are measured: BDE-28; -47; -49; -99; -100; -138; 153; -154, and –183. The cost associated with analysis of PBDEs is estimated at approximately $350 CDN (Great Lakes

Institute of Environmental Research) or $1200 CDN (AXYS Analytical) per individual or pooled sample, depending on the type of analytical method employed.

Total mercury is also routinely measured in snapping turtle eggs collected from sites where mercury concentrations are expected to be of concern. Methyl mercury is the biologically active form of mercury, but it is only measured when health effects from mercury exposure are suspected. Methyl mercury (approximately $100-150 CDN /sample) is much more expensive to analyze than total mercury ($30 CDN /sample).

4.0 Are Contaminants Measurable in Snapping Turtle Eggs?

The monitoring program should follow the approved protocol outlined in the Quality Assurance Project Plan (QAPP) (see Appendix). The methodology is divided into six specific components that will facilitate data collection and analysis: 1) wetland site selection; 2) sample collection and handling; 3) sampling frequency; 4) tissue storage, shipment, and preparation; 5) sample analysis and quality control; and 6) reporting and sharing of data.

Development and implementation of an integrated, bi-national monitoring program requires that participating researchers have the most current version of the approved QAPP (Appendix). It is important that the groups involved in monitoring activities should coordinate their efforts through the use of this protocol in sampling procedures, sample and data analysis, and reporting methods. This approach ensures a basin-wide (bi-lateral) consistency in data collection and methodologies among participating agencies in Canada and the United States, thereby enhancing the comparability and value of the data in identifying spatial and temporal trends in contaminant levels.

4.1 *Wetland Site Selection*

A selection of different types of lacustrine, riverine, and barrier-protected system wetlands with suitable habitat for snapping turtles may be included in this contaminants monitoring program to characterize contaminant levels in coastal wetland systems. Lacustrine system wetlands are controlled by waters of the Great Lakes and are strongly

affected by lake-level fluctuations, near-shore currents, seiches, and ice scour. Riverine system wetlands occur in rivers and creeks that flow into or between the Great Lakes. Riverine wetlands within the Great Lakes also include those wetlands found along large connecting channels between the Great Lakes with different dynamics than smaller tributary rivers and streams. Barrier-protected system wetlands have originated from either coastal or fluvial processes. Under the influence of coastal processes, the wetlands have become separated from the Great Lakes by a barrier beach or other barrier feature.

Great Lakes coastal wetlands within these hydrologically based systems are further classified based on their geomorphic features and shoreline processes. For a complete summary of Great Lakes coastal wetlands classifications, refer to the classifications summary document on the Great Lakes Wetland Consortium web page (http://www.glc.org/wetlands/pdf/wetlands-class-scheme.pdf).

Snapping turtles throughout the Great Lakes region have a nesting season which generally overlaps during the middle two weeks of June; however, the laying of clutches may begin prior to this in the southern part of the Great Lakes range, or slightly later in the north (Ernst et al., 1994). Since persistent organic pollutants such as DDE have decreased during the first 10 days of incubation in loggerhead sea turtles (*Caretta caretta*) (Clark and Krynitsky, 1985), eggs for contaminant analysis should be collected during this two week laying period to ensure freshness and minimal embryonic development. The utilization of fresh or recently laid eggs (< 48 hours) removes the uncertainty of changes in contaminant concentrations by Phase I and Phase II metabolic enzymes (Bishop et al., 1995a).

Site monitoring should be conducted daily until all egg collections have been completed for the site because predation is extremely high (> 98%) within hours of the turtle eggs being laid. Nesting activity is greatest in the morning between 0500 and 0900, with less activity between 1700 and 2100. Mornings prove to be the most efficient and practical time to collect eggs, unless predation by raccoons is a serious concern. Nesting sites involve a variety of substrate types including sand, loam, clay, or vegetable debris. However, we have also found turtle eggs buried in hard, pebbly areas such as along roadsides and ditch culverts. Sites tend to have substantial exposure to sunlight and generally have sparse vegetation at the time of oviposition. Natural sites, roadsides, railways, or dams are typically used. Shortly after laying, the nest is identifiable by two

distinct mounds of excavated earth upon which the female may urinate giving the excavated material a granular appearance. The nest has been described as bowl shaped, with a narrow opening descending at an angle to a large chamber 7 to 18 cm below the surface (Ernst et al., 1994).

4.2 *Sample Collection and Handling*

Although Bishop et al. (1995) reported a non-significant intra-clutch variation in contaminant levels among freshly laid eggs, the first five eggs contained the highest mean concentration of all chemicals on a wet-weight basis and the highest mean lipid values relative to the last five eggs collected. To mitigate the potential for intra-clutch contaminant variation, the CWS collects a composite subsample of five eggs from throughout each clutch. The first eggs oviposited are considered to be the last eggs found at the bottom of the nest cavity, and the last eggs laid are likely the first eggs encountered when the nest is excavated from the soil surface. A composite sample is obtained in the field by the following method: eggs are removed from the nest and placed in a plastic Tupperware® container filled with moistened vermiculite to prevent desiccation and breakage; the eggs are placed in order from first egg laid to the last; each clutch is divided into five groups of approximately equal size, and from within each group, an egg is selected haphazardly (de Solla and Fernie, in press). The total number of eggs, the wetland site, latitude and longitude of the collection site, the collection date, as well as the collector's name and contact information, should be recorded on the top and sides of each shipping container for each clutch. The eggs not intended for contaminant analysis are immediately returned and reburied in the nest.

Since organochlorine concentrations among clutches can be highly variable within a snapping turtle population (Bishop et al., 1991; Struger et al., 1993, Bonin et al., 1995), it is preferable to use 5 to 10 clutches of eggs per site for biomonitoring in order to obtain robust statistical comparisons among sites and among years within a site (Portelli and Bishop, 2000). Bishop et al. (1994) report that the coefficients of variation ranged from 38.6% to 55.9% among 15 clutches. This level of variation is comparable to studies using

Great Lakes herring gulls, Atlantic puffins (*Fratercula arctica*), Leach's petrels (*Oceanodroma leucorhoa*) and spottail shiners (*Notropis hudsonius*) (Bishop et al., 1994).

4.3 *Sampling Frequency*

Data collected as part of long-term contaminant monitoring programs undoubtedly advance our understanding of the sources and fate of contaminants, as well as provide spatial and temporal trend information for assessing improvement in controlling contaminant outputs (Pekarik and Weseloh, 1998; Braune et al., 2001). However, the frequency of field sampling can affect the ability to detect temporal changes in persistent contaminant levels. Hebert and Weseloh (2003) examined the effect of different sampling frequencies (i.e., every year; every second year; every third year; every fourth year; and every fifth year) on the ability to identify significant temporal declines in persistent organic contaminant levels in the Great Lakes. The data used by these authors were taken from the analysis of 13 herring gull eggs collected annually from each of five Great Lakes colonies between 1980 and 2001. This study confirms that programs of shorter duration that sampled at widely spaced intervals produced data with a limited capability of detecting significant temporal changes in contaminant levels in the environment. This was attributed to the decreased statistical power associated with analyses of few data points. Sampling at every two, three or four year intervals was able to detect changes in contaminant levels, however, identifying a significant change in levels is delayed by years relative to results from annual monitoring efforts. Hebert and Weseloh (2003) indicate that frequent temporal trend data regarding the bioavailabilty of environmental contaminants is most important when timely information is needed; for example, in assessing the effectiveness of Remedial Action Plans. In addition, the design of a monitoring program must strike a balance among costs, logistics, quality of data, and program objectives.

4.4 *Measurement and Data Acquisition*

For information on Sample Handling, Analytical Methods, Quality Control Requirements and Data Management, please refer to the approved Quality Assurance Project Plan (Revision 3- QAPP Wetlands2-EPA-05) in the Appendix of this report.

5.0 Applicability and Reliability of Snapping Turtles to Measure Contaminants

Knowledge of the life history and patterns of movement of the snapping turtle is essential to understanding the potential for their exposure to environmental contaminants. Ernst et al. (1994) provides a comprehensive overview of the snapping turtle and its ecology.

According to Lower and Kendall (1990), the utility of a given species for biomonitoring is based upon its geographic distribution, home range, presence in a particular habitat, and availability and specificity of biological endpoints. Golden and Rattner (2003) rank the suitability of vertebrate species as sentinels of contaminant exposure based upon their geographic occurrence, exposure potential, ease of collection, and quantity of existing exposure and effects data. Here, we address each of these criteria in order to assess the utility of the snapping turtle as an indicator of contaminant exposure.

Snapping turtle populations are more sensitive to "crashing" (mortality) as a result of lethal sampling of adults than to mortality from sampling eggs (Struger et al., 1993; Bishop et al., 1996; Cunnington and Brooks 1996). Although blood sampling of adults is another viable method for monitoring contaminants in snapping turtles (de Solla et al. 1998), concentrations of PCBs increase with body size in adult males, whereas contaminants in eggs are independent of body size of the laying females (Bishop et al.,1994). Due to the relatively low lipid levels in blood plasma, concentrations of contaminants in blood plasma are much lower than those found in eggs. Furthermore, trapping adult snapping turtles is labor- and time-intensive relative to egg collection. Consequently, the collection of eggs is the most ecologically sound and practical approach to monitoring contaminant exposure. Given our focus on the utility of turtle eggs as an indicator of contaminant exposure, we also include information on the transfer of contaminants from female turtles to their eggs in section 4.6.

5.1 *Geographic Distribution*

The range of the common snapping turtle encompasses the St. Lawrence River and the shores of the Great Lakes, excluding most of the northern shore of Lake Superior in Ontario, Canada (Ernst et al., 1994). The range includes that area west of Thunder Bay, Ontario, on the northern shore of Lake Superior and south of the St. Marys River, a 112 km connecting channel between Lakes Superior and Huron. However, according to a report that identified species of reptiles native to 17 Ontario Areas of Concern (AOCs), no snapping turtles had been sighted in the above mentioned areas up until the time of the report's publication in 1996 (Shirose and Bishop, 1996). The most important factors affecting the northern distribution of snapping turtles are the lower temperatures during egg incubation, a shorter growing season, and differences in habitat quality (Bobyn and Brooks, 1993).

Snapping turtles are inhabitants of wetlands, and they may be found in a wide variety of habitats where there is abundant aquatic vegetation in slow moving, permanent water bodies such as swamps, marshes, ponds, lakes, streams and rivers. Sites with soft muddy bottoms are preferred. Snapping turtles are sedentary, full-time residents of wetlands, and over-wintering turtles hibernate beneath a covering of mud, logs or plant debris on pond bottoms, under riverbanks or in muskrat burrows. Depending on latitude, snapping turtles may enter hibernation as early as September and emerge in March or April when water temperatures are between 5 ^{o}C to 7.5 ^{o}C (Ernst et al., 1994).

5.2 *Home Range*

The small home range and short migration distances reported in the literature indicate that turtles nesting in wetlands live and feed within these systems, and so an adult snapping turtle and her eggs, reflect contaminant concentrations within that wetland system. Several studies (Table 1) indicate that the home range of snapping turtles is small. Ernst et al. (1968) estimated home range size of snapping turtles in Pennsylvania to be 1.84 ha (n = 9 live captured turtles). Using radio-telemetry, Murphy and Sharber (1973, cited in Obbard and Brooks 1981) estimated the mean home range to be 0.65 ha in a Tennessee

river. In small areas, home range size can be severely constrained; the mean home range size in a 0.8 ha pond was only 0.02 ha (Froese, 1974). While the home range of the common snapping turtle is thought to be determined by variation in food resources, body size, density and shelter (Galbraith et al., 1988; Brown, 1992), these parameters did not influence the home range of snapping turtles in a protected embayment in Hamilton Harbour, Lake Ontario (Pettit et al., 1995). These investigators determined that the mean home range did not vary significantly between 1990 (8.6 ha for females and 2.2 ha for males) and 1991 (9.7 ha for females and 3.4 ha for males) (Pettit et al., 1995). In a separate study, the mean home range of radio-tracked adults did not differ by habitat or wetland size, being similar among Lake Sasajewun (a 43.5 ha lake), Cootes Paradise (a 370 ha wetland exiting into Hamilton Harbour), and Lynde Creek Marsh (a 40 ha cattail marsh opening into Lake Ontario) (Brown et al., 1994).

In Algonquin Provincial Park, Lake Sasajewun is a small lake interconnected by the North Madawaska River to three other smaller lakes. In the late 1970s, nine radio-tracked snapping turtles remained within Lake Sasajewun, and the mean home range size was only 3.44 ha (min-max = 0.95 to 8.38; Obbard and Brooks 1981). A female caught in the North Madawaska River, immediately downstream of Lake Sasajewun, had a home range of 1.3 ha and was never observed to enter the lake (Obbard and Brooks 1981). In two separate studies in Algonguin Provincial Park, the mean home range size of snapping turtles was 8.14 ha in 1987-1990 (Brown, 1992) and 8.64 ha in 1991 (Brown et al., 1994). Movement of males, however, has occurred on rare occasions in this area. For instance, four male turtles made unusual but brief forays outside of their home ranges, traveling up to 1500 m away in May and early June but never after the nesting season (Obbard and Brooks, 1981). The maximum nesting migration distance for female snapping turtles in Cootes Paradise, Lake Ontario was 2020 m (Pettit et al., 1995), but this study indicated that no turtles moved from this site into the adjacent Hamilton Harbour. This is comparable to migration distances in South Dakota (Hammer, 1969) and in southeastern Michigan (Congdon et al., 1987.) These findings indicate that snapping turtles have a high site affinity.

5.3 *Exposure Potential*

Exposure potential is a measure of the likelihood of an individual's exposure to a contaminant by the oral, dermal, or inhalation route. Specific elements that affect exposure may include dietary and habitat preference, longevity, feeding habits, foraging strategy and use of agricultural, industrial, or urbanized areas with anthropogenic contaminant input. The extent of exposure of the snapping turtle to persistent, organic pollutants is related to the chemical availability, and the species' propensity to bio-accumulate these compounds. The principal route of exposure for the snapping turtle is from bioaccumulation through the diet. The snapping turtle is an omnivorous opportunist, basically consuming whatever is available. Food items include vegetation, insects, shellfish, earthworms, leaches, fish, amphibians, small turtles, snakes, birds, and small mammals (Ernst et al., 1994). The contents of 470 stomachs from snapping turtles studied in Michigan were composed of 36.5% plant matter and 54.1% animals by volume. Fish are known to constitute approximately one third of the turtle's diet (Alexander, 1943). Because of the snapping turtle's predatory nature, feeding on large fish, small ducklings and cygnets, as well as carrion (Ernst et al., 1994), it is further subject to food chain biomagnification, and thus is exposed to the greatest concentrations of persistent organic contaminants.

Most organic pollutants are highly lipophilic and thus can be retained by fatty tissues for long periods while the organism is continually exposed. Thus, the longevity of a species can also affect its accumulation of organic pollutants. In one Ontario population, adult females were thought to have an average life span of 40 years based on annual rings on carapace scutes (Galbraith and Brooks, 1989). However, this is probably an underestimate, as Brooks et al. (1997) have since attempted to validate annuli counts, and they were shown to greatly underestimate snapping turtle age.

5.4 *Ease of Collection*

Turtles are common in their range except where populations have been over harvested for human consumption. Factors that determine the ease of sample collection of an indicator species include social structure, abundance, accessibility of sampling unit,

ease of capture, and management status in the proposed study sites (Golden and Rattner, 2003).

Snapping turtles can be very abundant in areas with high primary productivity, and populations vary greatly in density and biomass density. In a pond with high nutrient levels and primary productivity in Hamilton, Ontario, the density was 66 turtles/ha (biomass, 340kg/ha) (Galbraith et al., 1988). This is similar to a study by Major (1975), which reported 60.5 turtles/ha in western West Virginia. In a more northern oligotrophic pond in Algonquin Provincial Park, Ontario the density was 2.4 turtles/ha (biomass, 16 kg/ha) (Galbraith et al., 1988), similar to a Wisconsin lake with a density of 1.9 turtles/ha (Petokas, 1981). The density appears to be negatively correlated with latitude and surface area of suitable habitat (Galbraith et al., 1988). The primary productivity of a habitat appears to be the most important parameter influencing the density of snapping turtle populations. High-density snapping turtle populations appear to be concentrated in marshes and other highly eutrophic bodies of water, whereas low-density populations occur in lakes and other mesotrophic or oligotrophic systems (Galbraith et al., 1988). Other factors such as predation by other turtles, trapping by humans, and predation by *Mustelids* during hibernation may affect population density (Ernst et al., 1994). Snapping turtles are quite tolerant of habitat disturbance, and thus can be found in highly modified wetlands within an urban landscape, even in areas that otherwise have low species diversity due to anthropogenic impacts. A summary of 17 reports on density or biomass of snapping turtle populations from the U.S. and Canada was published by Galbraith et al. (1988).

Collection of snapping turtle eggs for contaminant analysis is made easy because the nests are usually accessible and clutches contain sufficient eggs for analysis. Nesting sites can often be found much earlier than the beginning of oviposition, as the presence of egg shells from the previous year may be apparent. Only one clutch per female is laid in a given year and the number of eggs per clutch varies widely (12 to 72 in our own study). While egg collection may be hampered in locations with limited suitable nesting areas, eggs from as many as 10 clutches have been collected in a single morning in areas with high density populations (Shane de Solla, personal communication, 2003).

5.5 *Quantity of Existing Exposure and Effects Data*

This information will be covered in the section entitled "Availability of Complementary Existing Research Data" (Section 5.0).

5.6 *Maternal Transfer of Contaminants to Eggs*

There are two major periods during which egg development occurs in the one-year snapping turtle reproductive cycle. Between mid-summer and late fall, egg growth is most rapid and follicular development is dependent upon energy assimilated from recently harvested food sources. Egg development resumes following hibernation and consists of final follicular growth, embryo development, shelling, ovulation and oviposition (Ernst et al., 1994). The use of eggs to assess exposure of wildlife to persistent, organic contaminants illustrates the widely held belief that chemicals in eggs are derived from the adult female, and turtle eggs are useful indicators of localized geographic contamination (Stone et al., 1980; Helwig and Hora, 1983; Olaffson et al., 1983; Hebert et al., 1993; Struger et al., 1993; Bishop et al., 1996).

Research on a variety of vertebrate species indicates that concentrations of organic chemicals in eggs closely reflect the concentration in maternal tissues when the concentrations are expressed on a lipid-weight basis (Mineau, 1982; Pagano et al., 1999; Russell et al., 1999). During ovogenesis, chemical transport from maternal tissues to the eggs follows a set of passive transport processes resulting in a chemical equilibrium among maternal tissues and eggs (Russell et al., 1999). Organic chemicals are rapidly distributed because of their lipophilic nature and result in a homogeneous tissue distribution when concentrations are expressed on a lipid basis. The development of eggs in oviparous species involves the transfer of lipoproteins from maternal tissues to eggs, and there is very negligible biotransformation of organic chemicals in eggs because phase I and phase II enzymes are not yet active (Kleinow et al., 1999).

For the most part, available data are consistent with the model that chemical concentrations in eggs and maternal tissues achieve equilibrium. A wide-ranging collection of maternal transfer data was published by Russell et al. (1999), in which these investigators combined existing data with the results of field studies on Lake Erie to

determine maternal transfer, and in ovo bioaccumulation of 44 hydrophobic organic chemicals in nine species of fish, herring gulls, and the common snapping turtle. When chemical concentrations in the eggs and the females were adjusted for lipid content, the egg/female concentration ratios were normally distributed with a mean of 1.2. The mean egg/female concentration ratio for 24 chemicals in the snapping turtle, however, was 0.4. This suggests that snapping turtle eggs do not hold as much chemical as the equilibrium model predicted. These results, however, may have been due to a small sample size of only three snapping turtles. Nonetheless, when examining such a broad range of species, it is interesting to note that concentrations in eggs and maternal tissues were strikingly similar. The results of this study indicate that at the time of egg deposition, contaminant concentrations in the eggs and maternal tissues of fish, turtles, and birds are close to chemical equilibrium.

In a separate study, Pagano et al. (1999) analyzed tissues of six gravid snapping turtles within and outside of the Great Lakes Basin to determine if eggs can be used as indicators of maternal contaminant burdens. Based on the congener specific (mole percent) data, and average chlorine/biphenyl values (which allows assessment of the level of chlorination among maternal tissues and eggs), the results indicated that the primary source of energy for follicle growth was derived from recent food sources. In addition, a significant and positive correlation was found between concentrations of congener-specific PCBs, DDE, mirex and hexachlorobenzene (HCB) in maternal tissues (adipose tissue and liver) and eggs from highly (Massena, Industrial NY), moderately (Hudson River, Annandale NY), and low-level (Sodus Bay, Rice Creek NY) contaminated sites (r-values > 0.95). This indicated that *in ovo* exposure of developing embryos in various classes of oviparous organisms to persistent hydrophobic organic pollutants is similar to the exposure of the adults who deposit the eggs. Pagano et al. (1999) concluded that their findings support previous research that environmental contaminants are maternally transferred, and that snapping turtle eggs are useful indicators of localized geographic contamination.

5.7 *Limitations*

Because relationships have been found between body mass and PCB and organochlorine pesticide concentrations in the liver (Hebert et al., 1993), blood plasma (de Solla et al 1998), and PCBs in the fat of snapping turtles (Bishop, 1990), it would be advantageous to age females which oviposit the eggs used for contaminant analysis. Unfortunately, no such method exists and calculations of annual growth rate as a tool for ageing is problematical as growth annuli may not be formed each year. For instance, juveniles in Algonquin Provincial Park, Ontario, formed one growth annulus on scutes of the carapace each winter, however, approximately 50% of the adults did not add a growth annulus between captures one year apart (Galbraith and Brooks, 1987, 1989). Furthermore, in a validation study in which two casts were taken of the carapaces of adults and juveniles taken ten years apart, Brooks et al. (1997) determined that the number of annuli did not vary between the two age periods for adults. Consequently, age of females cannot be incorporated into models of contaminant exposure or fate. Body size is, however, correlated with the age of turtles, and thus body size of the laying females can be incorporated into models.

Body mass, clutch size, and clutch mass can vary among females within a population (Congdon et al., 1987). Bishop et al. (1994), however, found no significant correlation between body size (body mass, carapace length and width, and plastron length) and chlorinated hydrocarbon concentrations in eggs from 15 snapping turtle nests. These authors suggest that ecological parameters, individual food preferences, and/or foraging activities are more likely to cause variation in chemical concentrations among clutches of snapping turtle eggs in a population. Nonetheless, a larger sample size from other areas within the Great Lakes is needed to further examine and confirm the relationship between size of adult females and the contaminant concentrations in their eggs.

6.0 Availability of Complementary Research Data

The following section provides a chronological overview of published materials on PCBs, PCDDs, PCDFs and p, p'-DDE concentrations measured in snapping turtle eggs;

supplementary data are provided in Table 2 with field sites used in these studies shown in Fig. 1.

Persistent organic compounds have a great affinity for tissues with high lipid content, with the highest concentrations measured in fat, followed by eggs, testes, liver, kidneys and muscle (Portelli and Bishop, 2000). The review here focuses on the results from contaminants analyzed in snapping turtle eggs. For information on contaminant data in other tissue types, and data on a suite of turtle species occurring in North America, the reader is referred to Hall (1980), Meyers-Schöne and Walton (1994), Bishop and Gendron (1998), and Portelli and Bishop (2000).

Snapping turtles have been used as a sentinel species of persistent organic contaminant exposure in wetland environments since the 1970s (Campbell, 1974; Stone et al., 1980), including by the CWS since 1984. In the early 1990s, work by the CWS focused on contaminant-related effects of contaminants on the snapping turtle (Bishop et al., 1991; 1998; de Solla et al., 1998). These efforts have increased the amount of information available on contaminant levels and their effects on snapping turtles inhabiting wetlands of the Great Lakes Basin. Since the organochlorine insecticide DDT, and especially its primary metabolite p, p'-DDE, continue to be found at high concentrations in wildlife, p,p'-DDE is included in this review. Other organochlorine insecticides such as aldrin, dieldrin, chlordane, endrin, heptachlor, mirex, and toxaphene are detected in egg samples and have been reviewed by Portelli and Bishop, (2000).

Improvements in instrument technology, methods and detection limits, has resulted in a substantial increase in the number and type of PCB congeners measured. Currently, as many as 71 PCB congeners may be included in routine organochlorine analytical reports. This is a significant improvement over early research which quantified PCBs as a mixture of Aroclors 1254:1260 (1:1) using PCB-128 (2,3,4,2',4',5'-hexachlorobiphenyl) as a surrogate. In order to compare older data with more recent analyses, Turle et al. (1991) developed conversion factors by using the analytical results of 41 PCB congeners and Aroclor 1254:1260 measured in herring gull eggs. This approach was used by Struger et al. (1993) to calculate total PCB concentrations in turtle eggs collected from 1981-1984. Using Aroclor equivalents also has the advantage of greater comparability when analyzing data from different sources, however, a possible disadvantage is misrepresenting the actual

PCB levels in animal tissues. Although a combination of Aroclor 1254 and 1260 are the dominant mixtures in the lower Great Lakes, there are important local sources of these and other Aroclors in the Great Lakes, such as 1248 and 1242 (Oliver and Bourbonniere, 1985; Sokol et al., 1994, Martin et al., 2003). Large deviations in exposure from the 1:1 Aroclor 1254:1260 may considerably affect the accuracy of the Aroclor equivalent estimates. For example, 1254:1260 (1:1) over-estimated sum PCBs in herring gulls compared to sum PCBs (71 congeners) in the Great Lakes by 1.7 times (Hebert et al., 2000). To this day, there are slight differences in the number and type of PCB congeners measured; therefore, it is advisable that the reader consult the primary literature for details relevant to contaminant values listed in Table 2.

Less information is available on PCDD and PCDF concentrations in turtle eggs compared to total PCBs. Typically egg samples from one site are not analyzed individually for PCDDs and PCDFs, but rather are pooled into one composite sample for the site for analysis; this is due to the cost of analyzing these chemicals. Most studies indicate that the congener 2,3,7,8-tetrachlorodibenzodioxin (2,3,7,8-TCDD) is the predominant form of PCDDs in turtle eggs (Bishop et al., 1991; Struger et al; 1993; de Solla et al., 2001). However, the predominance of this dioxin congener depends on various factors such as differential metabolism of congeners and point sources of PCBs. With regards to PCDF compounds measured in turtle eggs, 2,3,4,7,8,-pentachlorodibenzofuran and 2,3,7,8-tetrachlorodibenzofuran are generally dominant (Bishop et al., 1991; Struger et al., 1993; de Solla et al., 2001).

As previously stated, monitoring chemical parameters in water and sediment generally reflect the degree of pollution in a particular locality; however, the measurement of contaminant concentrations in tissues of snapping turtles provides a gauge of wildlife exposure and of toxicant bioavailability in wetland environments. Recently, the CWS has been working to determine the value of an effects monitoring program using snapping turtles as indicators of contaminant exposure and effects. Since 2001, the CWS (K. Fernie, project leader) has been systematically measuring contaminant exposure and effects in snapping turtles from wetlands in Canadian Areas of Concern (AOCs) in Lake Erie, Lake Ontario, and the St. Lawrence River.

6.1 *Polychlorinated Biphenyls, Dioxins, Furans, and p,p-DDE*

One of the first accounts of contaminated snapping turtle eggs was reported by the CWS in 1974. Snapping turtle eggs from Rondeau Provincial Park, located on the shores of northern Lake Erie, contained 0.21µg/g p,p'-DDE. Unfortunately, no data are available for total PCBs, dioxins or furans (Campbell, 1974, from Bishop and Gendron, 1998). Outside of Canada, Stone et al. (1980) reported that unlaid eggs from six gravid snapping turtles collected from the highly contaminated Hudson River in New York State contained 28.9 :g/g total PCBs (lipid weight). A second report also comes from the Hudson River, in which Bryan and colleagues (1987) compared the difference in partitioning of organochlorine compounds between the yolk versus the albumen (egg white) and egg shell. The lipid rich yolk had between 6.0 and 9.4 times more total PCB concentration than the albumen or egg shells.

Struger and colleagues (1993) later tested the hypothesis that snapping turtle eggs would be useful indicators of geographic variation in contaminant levels among Great Lakes wetlands. In 1981, eggs were collected from a site with low background contamination located in Algonquin Provincial Park, Ontario, and Big Creek Marsh National Wildlife Area, Ontario (Lake Erie). Since Algonquin Provincial Park has no known local PCB sources, the PCB concentrations in the turtle eggs were assumed to represent background airborne exposure to PCBs. Eggs from eight other wetland locations on Lake Ontario, Lake Erie and along the St. Lawrence River in Canada were collected in 1984. The mean summed concentration of PCB congeners at the reference site was 1.87 µg/g, while the Great Lakes and St. Lawrence River sites ranged from 1.00 to 4.76 µg/g. Eggs from Grindstone Creek in Hamilton Harbour contained the highest mean total PCB and p,p'-DDE concentrations from among the ten sample locations (Struger et al., 1993). When compared to other data, turtle eggs were generally more contaminated than spottail shiners collected at the mouth of the Hamilton Harbour, but less contaminated with chlorinated hydrocarbons than herring gull eggs. Unexpectedly, a combined extract of sub-samples from three clutches collected at the mouth of Grindstone Creek in Hamilton Harbour contained similar or higher concentrations of certain dioxin congeners than eggs of herring gulls. This suggests that there are inter-specific differences in the metabolism of

organic contaminants among species. The results of this study clearly revealed widespread and geographically variable contamination among Great Lakes wetland ecosystems, particularly those associated with areas of high contaminant loads.

Between 1986 and 1989, Bishop et al. (1991) measured concentrations of PCBs, PCDDs, PCDFs, and organochlorine pesticides in eggs collected from the reference site in Algonquin Provincial Park, and four wetlands located on the shores of Lake Ontario and Lake Erie. Eggs collected from each site were also incubated to determine hatching success, and the incidence of deformities in embryos and hatchlings. Significant geographic variation in persistent organic chemical concentrations was similar to the results of Struger et al. (1993). Results of the four-year study showed that eggs from Cootes Paradise and Lynde Creek contained the highest concentrations of summed PCBs and p,p-DDE among the five sites. Eggs from Cranberry Marsh and Big Creek Marsh were comparable and moderately contaminated, while reference eggs from Lake Sasajewun (0.028 μg/g w.w.) were the least contaminated. Lynde Creek also had the highest concentrations and the highest number of dioxin and furan congeners. The developmental study revealed that eggs from Cootes Paradise, Lynde Creek and Cranberry Marsh had higher incidences of poor hatching success and deformities relative to eggs collected from Big Creek Marsh and the reference site in Algonquin Provincial Park in all years of study.

Bonin et al. (1995) compared organochlorine and PCB levels in 39 snapping turtle clutches collected from 10 sites along a highly polluted stretch of the St. Lawrence River in Canada (Cornwall, Ontario through to the east end of Montreal Island) and the much less polluted Ottawa River in Canada. Similar to Bishop et al. (1991) and Struger et al. (1993), sites demonstrated a high inter-site variability in contaminant levels. Eggs collected from Raquette River near Masena NY, were highly contaminated with PCBs reaching concentrations of 10.97 μg/g (w.w.). The total PCB concentrations were much lower at the Ottawa River site where the maximum PCB level did not exceed 0.173 μg/g. Among the organochlorine pesticides, p,p'-DDE was generally detected at the highest level. Ingleside, a site upstream from Cornwall, had the highest p,p'-DDE concentration, while turtle eggs from the Ottawa River site had the lowest.

Bishop et al. (1996) compared the geographic contaminant patterns in eggs collected from five sites in Ontario, Canada. Collections were made by Struger et al.

(1993) in 1981 and 1984, and later collections were made by Bishop and colleagues between 1988 and 1991. The pattern of significant geographic variation in organochlorine concentrations in snapping turtle eggs among locations was consistent from 1988 – 1991, and consistent with patterns reported by Struger et al. (1993). In 1989, when eggs were collected from all six sites, the general contaminant pattern for sum PCBs was as follows: Lynde Creek > Cootes Paradise > Rondeau Park > Cranberry Marsh > Big Creek Marsh > Algonquin Provincial Park. A similar pattern however, was not detected for p,p'-DDE: Cootes Paradise > Lynde Creek > Cranberry Marsh >Big Creek Marsh = Rondeau Park > Algonquin Provincial Park. The PCDD and PCDF concentrations in eggs consistently indicated that Lynde Creek was the most contaminated site, while eggs from Cootes Paradise and Cranberry Marsh were the next most contaminated. Eggs from Algonquin Provincial Park had the lowest PCB concentrations, non-detectable levels of PCDFs and PCDD congeners with the exception of octachlorodibenzo-p-dioxin.

Similar to a previous study by Bishop et al. (1991), snapping turtle eggs were again collected for contaminant analysis and incubation to assess developmental abnormalities (Bishop et al., 1998). Eggs were collected from eight sites in Ontario, Canada and Akwesasne, New York, USA during the reproductive seasons from 1989 to 1991. Several of the sample sites such as Lake Sasajewun, Lynde Creek, Cootes Paradise, Cranberry Marsh, Big Creek Marsh, and Rondeau Provincial Park, were common to the 1991 study. Eggs were also collected from the Trent River, a site which drains into the Bay of Quinte, Ontario. In the St. Lawrence River area, samples were collected from Raquette River, St. Regis River, and the Snye marshlands, all within the boundaries of Akwesasne, New York. Eggs from the reference site at Algonquin Provincial Park, Ontario contained the lowest PCB concentrations. Egg samples collected from shoreline sites of Lake Erie and Lake Ontario, ranged from 0.24 μg/g (w.w.) at Cranberry Marsh, to 3.57 μg/g (w.w.) at Cootes Paradise. Eggs from the Trent River contained 0.83 μg/g (w.w.) and Akwesasne samples were the most contaminated with 3.95 μg/g (w.w.) of PCBs. Rankings for PCDDs and PCDFs were somewhat different with low or non-detectable concentrations in eggs from Algonquin Provincial Park, Big Creek, and Rondeau, and higher concentrations and a greater number of detectable congeners in eggs from Cranberry Marsh, Akwesasne, Trent River, Hamilton Harbour, and Lynde Creek. Variations in egg concentrations of p,p'-DDE

showed similar trends to those of total PCBs, except that Cootes Paradise had the highest levels. The authors report a significant increase in abnormal development with increasing concentrations of 10 PCBs, 7 PCDDs, and 11 PCDFs. Cytochrome P-4501A and 7-ethoxyresorufin O-deethylase (EROD), both indicators of exposure to dioxin-like compounds and Ah-receptor mediated response (Safe, 1994), were also measured in livers of hatchling turtles. The mean EROD activity was 8 times higher and the mean CYP1A was 50 times higher in hatchlings from Lynde Creek compared to Algonquin hatchlings.

In 1998, de Solla et al. (2001) collected eggs from the shorelines of marshes within the Mohawk Nation in Akwesasne, New York. The sites were located two to 13 km downstream from PCB-contaminated landfill sites, and included St. Regis River, Raquette River, Snye Marsh, and Turtle Creek. Total PCB concentrations ranged from 2.37 μg/g at Snye Marsh, to 737.68 μg/g at Turtle Creek. The total PCB concentrations measured in eggs at Turtle Creek are among the highest recorded in any tissue of a free-ranging animal. In a pooled sample of eggs from all four sites, the summed concentrations of non-ortho PCBs were 54.54 ng/g, and the summed dioxin and furan concentrations was 85.8 ng/g. Concentrations of p,p'-DDE followed the same pattern and ranged from 9.80 ng/g to 852 ng/g.

Ashpole et al. (2003) collected turtle eggs in 1999 and 2000 from Algonquin Provincial Park, four sites on the St. Lawrence River, Cootes Paradise in Hamilton Harbour, and Walpole Island in the St. Clair River AOC. Total mean PCB concentrations from Walpole Island were 0.239 μg/g (w.w.). Total mean PCBs were 1.93 μg/g in eggs from Hamilton Harbour, while they ranged from 0.17 μg/g to 60.96 in the St Lawrence River at the Cornwall/ Massena AOC. From lowest to highest, egg p,p'-DDE concentrations were as follows: Cooper Marsh < Algonquin Provincial Park , Walpole Island < Greys Creek = Snye Marsh < Raquette Rive < Cootes Paradise. From the one-pooled sample (five clutches with 5 eggs/clutch or 25 eggs/pool), the concentrations of PCDDs (2.44 ng/kg w.w.) and PCDFs (1.18 ng/kg w.w.) measured in eggs were low compared to the results from Hamilton Harbour (total PCDDs 7.81 ng/kg w.w.; total PCDFs 5.19 ng/kg w.w.) and the Cornwall-Massena AOC area (total PCDDs ranging from 11.64 to 26.9 ng/kg w.w.; total PCDFs ranging from 1.58 to 57.94 ng/kg w.w.).

7.0 The Sensitivity of Snapping Turtles to Changes in Contaminant Levels in Wetlands

The major goal of this White Paper was to validate the use of snapping turtle eggs as indicators of wetland health relative to contamination, as well as geographic and temporal trends in environmental contaminant levels. The collective body of literature on the common snapping turtle reveals that they are excellent indicators of the geographic variation in persistent organic contaminants. In a review by Golden and Rattner (2003), the snapping turtle is ranked seventh out of 25 contaminant indicator species evaluated. With regards to temporal trends in contaminant concentrations in a given wetland, it is important to realize that detection of changes over time is facilitated by frequent data collection. Limited information is available on concentrations of persistent, organic contaminants in snapping turtle eggs collected from sites over a long time span. Here, the spatial analysis of organochlorine and pesticide concentrations in snapping turtle eggs involves multiple sites across the Lake Erie, Lake Ontario, and St. Lawrence River basins (described below), while the temporal analysis has been completed for Algonquin Provincial Park, Cootes Paradise in the Hamilton Harbour AOC, Akwesasne in the St. Lawrence River AOC, and Walpole Island in the St. Clair River AOC.

7.1 *Sample Collection*

Currently, data for geographic contaminant patterns in snapping turtle eggs are being gathered since 2001 as part of a Fish and Wildlife Health Effects and Exposure Study (K. Fernie, CWS, unpublished data). Turtle eggs were collected from Canadian AOCs on the lower Great Lakes, as designated by International Joint Commission (IJC). An AOC is defined as a geographic area that has experienced environmental degradation due to an excess of nutrients in the water (eutrophication), bacteria or chemical contaminants in the environment, or loss of fish and wildlife habitat. For temporal trends in contaminants in turtle eggs, eggs were analyzed from the CWS Tissue Bank to supplement existing data from Struger et al. (1993), Bishop et al. (1996), and Fernie (CWS, unpublished data). Turtle eggs were collected with the greatest frequency from Algonquin

Provincial Park and Cootes Paradise in Hamilton Harbour. Other sites were sampled less often, but still provide sufficient data for temporal trend analysis. Data are also presented from the recent analysis of polybrominated diphenyl ether (PBDE) concentrations in turtle eggs, the first time that this chemical has been analyzed in snapping turtle tissues.

The reference sites and AOCs surveyed were divided into geographical regions as follows (Fig. 1):

1. Reference Sites: from 2001 to 2003, a traditional reference site located near Lake Sasajewun in Algonquin Provincial Park, remote from any industrial or agricultural contaminant sources, was used by Canadian Wildlife Service researchers. Tiny Marsh was chosen as a second reference site and is situated south of Georgian Bay near Midland, Ontario.
2. Lake Erie basin: in 2001, three AOCs within the Lake Erie basin were used: the Detroit River AOC (Turkey Creek drains both the city of LaSalle, and an industrial zone in Windsor, Ontario); the St. Clair River AOC (the St. Clair National Wildlife Area (NWA) and Big Point Hunt Club); the Wheatley Harbour AOC (Muddy Creek, located within the Wheatley Harbour AOC, and Wheatley Provincial Park, and Hillman Marsh Conservation Area, both located approximately 2-3 km of the Wheatley Harbour AOC boundaries).
3. Lake Ontario: in 2002, snapping turtle eggs were collected from two Lake Ontario AOCs: the Hamilton Harbour AOC (Grindstone Creek, Cootes Paradise) and the Niagara River AOC (Lyons Creek near Welland, Ontario). In addition, eggs from Wheatley Provincial Park on Lake Erie and Turkey Creek were collected to increase the sample size from the previous year, and to measure dioxin and furan concentrations. In 2003, two sites from the Toronto AOC located on the Humber River were sampled.
4. St. Lawrence River: in 2003, eggs were collected from both the Canadian and Akwesasne/American sides of the St. Lawrence River AOC: one site upstream from Cornwall at the Upper Canada Bird Sanctuary at Ingleside, and east of Cornwall along the Raisin River; sites also included the Snye Marsh in Akwesasne.

Industrial facilities located in Cornwall, Ontario and Massena, New York historically discharged significant quantities of contaminants, including mercury, zinc, polychlorinated biphenyls (PCBs) and lead to the St. Lawrence River.

7.2 *Chemical Analysis*

Both p,p'-DDE and PCB congener concentrations in turtle eggs collected from 1981 to 1991 were measured using analytical procedures outlined by Bishop et al. (1996). The limit of detection for PCBs was 0.005 mg/kg wet weight (w.w.), and 0.0025 mg/kg w.w. for p,p'-DDE. The value of total PCBs reported is the sum concentration of the following congeners which were measured individually in 1989-1990 samples: 28, 31, 42, 44, 47, 49, 52, 60, 64, 66, 70, 87, 97, 99, 101, 105, 110, 118, 128, 129, 137, 138, 141, 146, 151, 153, 158, 170, 171,172, 174, 180, 182, 183,185, 194, 200, 201, 203, 206; in 1991: all congeners measured in 1989-1990 except #47 and PCBs #74, 149; in 1988, only 16 congeners were measured: PCB #28, 31, 52, 99, 101, 105, 110, 118, 138, 153, 174, 180, 170/190, 194, 66/95, 182/187 (IUPAC number; Ballschmiter and Zell, 1980).

Egg samples were analyzed by Dr. Ken Drouillard and Dr. Robert Letcher of the Great Lakes Institute of Environmental Research (GLIER, University of Windsor, Windsor, ON). The egg samples were thawed to room temperature and extracted with dichloromethane (DCM):hexane (1:1 v/v) after the samples were dehydrated with anhydrous Na_2SO_4. The lipids and biogenic material were removed using gel permeation chromatography and cleaned by florisil column chromatography. All of the 2002 samples were analysed using capillary gas chromatography coupled with an electron capture detector (GC/ECD), whereas the samples analyzed in 2003 used a mass selective detector (GC/MSD). Each cleaned sample was injected to determine organochlorine compounds by using twenty-one organochlorine standards. The method quantification limits (10 x the detection limits) ranged between 0.01 to 0.09 ng/g for the eggs samples analysed at GLIER. Non detectable concentrations were treated as 0.05 ng/g. The PCB congeners measured in 2002 were #42, 44, 49, 52, 60, 64, 70, 74, 87, 97, 99, 101, 105, 110, 118, 128, 138, 141, 146, 151, 153, 171,172, 174, 177, 178, 179, 180,183, 194, 195, 200, 201, 203,

206, 31/28, 66/95, 170/190, 182/187. In all samples, Aroclor 1260 was estimated as (PCB 180/10.96)×100, and Aroclor 1254:1260 (1:1) was estimated as (PCB 138 /14.6)×200.

For some contaminant analyses, certain congeners would co-elute, and the individual congeners could not be distinguished from one another, although the quantity of the total co-eluting congeners could be determined. In order to increase the comparability of the data, congeners that sometimes co-eluted were pooled for all analyses. Occasionally, the second co-eluting congener was not reported. Generally, one of the two (or three) co-eluting congeners have much lower concentrations than the other, so the failure to include the less common congener would have a negligible effect on the final concentrations. For example, PCB 132 co-eluted with PCB 153 using GC-MSD in 2003 samples; in 2001 samples they were reported separately, but PCB 132 contributed only on average 0.34% to the sum of PCB 132 and 153. Similarly, PCBs 56 and 60, and PCBs 70 and 76 were pooled. Non-detection limits varied among methods and laboratories, thus we treated non-detection levels as 0.

7.3 *Statistics*

Since the number of congeners varied among years, we report the sum of only those PCB congeners (n = 34) which were common to all analyses for spatial and temporal comparisons. The thirty-four congeners common among all analyses and so used for measuring sum PCBs, included: 42, 44, 49, 52, 56/60, 64, 70/76, 87, 97, 99, 101, 105, 110, 118, 128, 138, 141, 146, 151, 153/132, 158, 170, 171, 172, 174, 180, 182/187, 183, 194, 195, 196/203, 200, 201, and 206. The sum PCBs using these 34 common congeners was only 7.4% lower than the sum of all 71 congeners used in various studies. Since Aroclor equivalents are not dependent upon the number of congeners measured, Aroclor 1254:1260 (1:1) was also used for statistical comparisons.

For the geographic pattern of contaminant concentrations, a non-parametric procedure, Kruskal-Wallis one-way analysis of ranks, was used to compare PCB and p,p-DDE levels among sites. A simple regression was used to analyze the temporal trend in contaminants within each site. A general linear model (GLM) was used to compare mean PBDE levels among sites. Contaminant data were log transformed prior to analysis, unless

otherwise stated; however, graphical data were presented as untransformed values. GLM was also used to determine if the relative contribution of each BDE congener to sum PBDE varied with relative exposure. Tukey HSD tests were used for post-hoc comparisons.

Identifying PCB mixtures based on congener patterns is important as it helps to determine point sources and because there are toxicity differences among Aroclor mixtures. Therefore, the geographic PCB congener patterns characteristic of different mixtures were examined using ANOVA and Principal Components Analysis (PCA). Contaminants were not transformed, and were expressed on a wet weight basis for comparisons. Patterns of PCB congeners in eggs were examined using ANOVA and Principal Components Analysis (PCA) using varimax normalized rotation on untransformed contaminant concentrations. The 15 most prevalent PCB congeners were included, and were expressed as a proportion of the sum PCBs. Fishers LSD test was used for multiple comparisons of the factor scores among sites. Due to the large number of sites, only a select number of sites from 2001 – 2003 were included for illustrative purposes.

7.4.0 Results and Discussion

7.4.1 *Geographic Contaminant Patterns in Areas of Concern*

Contaminant concentrations in snapping turtle eggs varied among the St. Clair River, Detroit River and Lake Erie AOCs. de Solla and Fernie (in press) also differentiated study sites based upon the profile of the PCB congener profiles in eggs. Concentrations of sum PCBs were highest in the snapping turtle eggs from Turkey Creek (0.327-1.902 µg/g), followed by Wheatley Provincial Park (0.249-0.950 µg/g) and Canard River (0.067-0.896 µg/g). When the PCB congener profile among the Lake Erie and St. Clair River sites was examined, results indicated that snapping turtle eggs from Canard River had a similar profile to eggs from both Turkey Creek and the St. Clair River AOC. The PCB congener profile in the turtle eggs from Turkey Creek reflects the historical Aroclor 1260 sources. The largest single source of the majority of organics, including PCBs and organochlorine pesticides in Lake Erie, is thought to originate from contaminant input from the Detroit River (Kelly et al., 1991). However, the main source of PCB contamination occurring in

Wheatley Harbour is thought to be derived from the local discharge of fish offal from Lake Erie fish processing plants (Bedard, 1985, cited in de Solla and Fernie, in press). Although the PCB source at Wheatley Harbour is unknown, the PCB congener profile in these turtle eggs suggests an Aroclor 1260 source. Concentrations of *p,p*'-DDE in turtle eggs were highest in the areas near the Wheatley Harbour AOC (0.017-0.038 µg/g). This is not surprising given the intensive agricultural activity of this area. Turkey Creek had the next highest *p,p*'-DDE concentration (0.011-0.036 µg/g), while eggs from Tiny Marsh, St. Clair NWA AOC and Canard River had similar and low concentrations (0.0047 µg/g to 0.0059 µg/g). The lowest *p,p'*-DDE concentrations were found in the eggs from Algonquin Provincial Park (0.0008-0.0016 µg/g).

Within the Lake Ontario AOCs, turtle eggs collected from Grindstone Creek (0.715-3.275 µg/g) and Cootes Paradise (0.361-2.058 µg/g) in Hamilton Harbour contained the highest sum PCB concentrations followed by Lyons Creek (0.220-2.793 µg/g) in the Niagara River AOC (near Welland ON), then the Humber River (0.278-1.165 µg/g) draining into Lake Ontario at Toronto. Egg p,p'-DDE concentrations were lowest in the industrialized Niagara River-Welland region (0.001-0.018 µg/g) and highest in eggs collected from Grindstone Creek in Hamilton Harbour (0.130-0.182 µg/g). Sources in Hamilton Harbour most likely originated from local historical agricultural use of DDT, and were deposited to the Harbour by fluvial processes via the Grindstone and Spencer Creeks.

Within the St. Lawrence River, turtle eggs from the Snye Marsh contained the highest total PCB concentrations (0.013 to 1.339 µg/g), followed by eggs from Ingleside (0.010 to 0.197 µg/g) and the Raisin River (0.005 to 0.336 µg/g). In contrast, eggs from Ingleside contained the highest p,p'-DDE concentrations (0.0005 to 0.048 µg/g) relative to the Raisin River (0.0005 to 0.022 µg/g) and the Snye Marsh (0.0003 to 0.031 µg/g).

Basin-wide comparisons of contaminant concentrations in turtle eggs revealed significant differences (Kruskal-Wallis test, $P < 0.01$) among the Great Lakes basin AOC sites sampled. Turtle eggs from Hamilton Harbour contained the highest sum PCB congener concentration, and exceeded reference values by approximately 50 times (Fig. 2). The geographic contaminant patterns reported here are similar to trends observed by Struger et al. (1993) and Bishop et al. (1996). Eggs collected from Turkey Creek and Lyons Creek were next highest in total PCB concentration, and were approximately 40

times greater than reference turtle eggs. Significant differences were also found between these sites and the less contaminated sites at Lake St. Clair and the St. Lawrence River sites at Ingleside and the Raisin River. Eggs from the Canard River, Wheatley Harbour, Humber River, and the Snye Marsh were moderate in PCB concentrations and fell between these two groups (Fig. 2). When the Aroclor equivalent (1254:1260) was compared among sites, a similar contaminant pattern emerged (Kruskal-Wallis test, $P < 0.01$), however, eggs from Hamilton Harbour had the highest Aroclor equivalent, and differed significantly from all other sites except Turkey Creek . Significant differences were also found between Turkey Creek and eggs from the reference sites, Lake St. Clair and Ingleside and the Raisin River on the St. Lawrence River (Fig. 3).

Concentrations of p,p'-DDE also differed significantly among AOC and reference sites (Kruskal-Wallis test, $P < 0.01$). Turtle eggs from Hamilton Harbour contained p,p'-DDE concentrations that were approximately 40 times above reference values, and were significantly higher than all other sites monitored within the Great Lakes- St. Lawrence basin. There was a high variation within each site, such that no differences were detected among the remaining AOC sites. Egg p,p-DDE concentrations were comparable among these sites and ranged from 0.003 to 0.058 μg/g wet weight. These results are similar to that reported for p,p'-DDE by Struger et al. (1993) and Bishop et al. (1996).

7.4.2 *Principal Component Analysis*

Four principal components were extracted, accounting for 83.0% of the total variance, and the first component explained 47.8% of the variance. The factor scores for the first component varied among sites (PC1, $F_{[5,50]} = 13.7$, $P < 0.0001$). Snye Marsh had significantly lower scores than any other site, Lyons Creek and UCBS had the next lowest scores, although Algonquin Provincial Park and UCBS were not significantly different from each other. There were no differences among Algonquin Provincial Park, Turkey Creek, and Cootes Paradise. PC1 was positively correlated (> 0.6) with PCBs 180 and 170, which are characteristic of Aroclor 1260, and negatively correlated with PCBs 118 and 105, which are characteristic of Aroclor 1254 (Fig 4a, Frame, 1997). The factor scores for the second component varied among sites (PC2, $F_{[5,50]} = 15.1$, $P < 0.0001$). Lyons and

Algonquin Provincial Park had lower scores than any other site, whereas Cootes Paradise and Turkey Creek had higher scores than any other site except UCBS. Snye Marsh and UCBS had intermediate scores. PC2 was highly positively correlated with PCBs 138 and 128, which are characteristic of Aroclor 1260, and negatively correlated with PCBs 66/95, which are characteristic of Aroclor 1254 (Fig 4a, Frame, 1997). Using the PCA analysis, the different sites were grouped according to the PCB burden in the eggs (Fig. 4b): in general, turtles from Snye Marsh, Lyons Creek and UCBS had the largest relative exposure to Aroclor 1254, whereas Cootes Paradise and Turkey Creek had the largest relative exposure to Aroclor 1260.

The PCA analyses demonstrated that the congener profile in snapping turtles varies geographically, and these differences were associated with different Aroclor sources. It is unlikely that differences in volatilization or trophic transfer could account for differences in congener composition among these sites. Previous studies have demonstrated the difference in Aroclor use throughout the lower Great Lakes region (Oliver and Bourbonniere, 1985; Sokol et al., 1994, Martin et al., 2003). Examining the PCB source is not just pertinent if there is a point source, but is also important in areas in which there are no local sources of PCBs. Algonquin Provincial Park has no known local sources of PCBs (Bishop et al. 1991), and consequently the PCB burdens likely reflect background PCB contamination via airborne deposition. Aroclor 1260 is dominated by hexa and hepta biphenyls, which may explain the similarity of the congener profile in Algonquin Provincial Park to Aroclor 1260.

Biota used for monitoring purposes should be able to discriminate among PCB sources, particularly in situations where there is a prominent point source. Sedentary snapping turtles typically have very small home ranges. Consequently, the maternal burden would reflect the environment throughout their home range. Although Russell et al, (1999) found that the ratio of contaminants between eggs and muscle in snapping turtles deviated from the equilibrium partitioning model, there was good agreement in relative concentrations between maternal and egg burdens (Pagano et al. 1999), and the partitioning of contaminants among tissues was independent of the octanol:water partition coefficients (Russell et al. 1999). Consequently, snapping turtle eggs adequately reflect local contamination. Certainly, the data show the utility of snapping turtle eggs to monitor local

contamination adequately, and can be used to discriminate among different locations within the lower Great Lakes Basin.

7.4.3 *Temporal Variation Within Sites*

Algonquin Provincial Park

Concentrations of PCB 1260 in from turtle eggs Algonquin Provincial Park decreased by 86% ($R^2 = 0.3102$; $P < 0.01$) (Fig. 5) and the Aroclor equivalent (1260:1254) decreased by 65% ($R^2 = 0.3187$; $P < 0.01$) from 1981 to 2003. No temporal change was observed in the total PCB concentrations when the common sum of PCB congeners was examined between the years 1989 and 2003. However, a significant decreasing trend in PCB concentrations was found between 1993 and 2003 ($R^2 = 0.3111$; $P < 0.01$). These results generally correspond to those reported by Bishop et al. (1996), who observed a significant decrease in total PCB concentration in Algonquin Provincial Park eggs examined between 1981 and 1991. Our results also revealed that egg p,p'-DDE concentrations show little change over time, suggesting that p,p'-DDE levels possibly have reached a steady state in wetlands of this region.

Cootes Paradise/ Hamilton Harbour

Turtle eggs from Cootes Paradise collected from 1984 to 2002 revealed a 54% reduction in PCB 1260 concentrations ($R^2 = 0.1194$; $P < 0.01$) (Fig. 6). a decrease of 65% in the Aroclor equivalent (1260:1254) ($R^2 = 0.1818$; $p < 0.01$) and a decrease of 60% in p,p'-DDE concentrations ($R^2 = 0.2778$; $P < 0.01$). Similarly, the common total PCB congener concentration decreased significantly ($R^2 = 0.1750$; $P < 0.01$) in eggs from Cootes Paradise between 1986 and 2003. These results contrast with Bishop et al. (1996), who reported a significant increase in total PCB concentrations in turtle eggs from Cootes Paradise between 1984 and 1991. The discrepancy in contaminant trends between our results and the results of Bishop and colleagues (1996) may be due to the construction of a barrier between Cootes Paradise and Hamilton Harbour in 1996. This barrier prevents large migratory carp (*Cyprinus carpio*), which possibly contain high contaminant burdens, from

entering Cootes Paradise. The decrease in carp in the diet of turtles may therefore explain the steady decrease in PCB and p,p'-DDE residue concentrations in snapping turtle eggs.

Snye Marsh/ St. Lawrence River

Turtle eggs, collected from the Snye Marsh located on the St. Lawrence River near Akwesasne, New York between 1990 and 2003, showed a significant decrease of 89% in PCB 1260 concentrations ($R^2 = 0.4153$; $P < 0.01$). Similarly, the concentrations of Aroclor equivalent (1260:1254) decreased by 83% ($R^2 = 0.3277$; $P < 0.01$), with a 81% decline in the sum of the common PCB congeners ($R^2 = 0.2949$; $P < 0.01$), and a 76% decline in p,p'-DDE concentrations ($R^2 = 0.2951$; $p < 0.01$).

Walpole Island/ St. Claire River

Turtle eggs from Walpole Island on the St. Clair River, were collected in 1992, 1995 and 1999. Discharge of chlorinated organic compounds, heavy metals, oils and greases, phenols, suspended solids from petroleum and chemical industries, spills, as well as historically contaminated sediments, are found in the St. Clair River and pose major community concerns (International Joint Commission 1999). No significant change in PCB or p,p'-DDE concentrations in eggs from Walpole Island were detected over this time span (1992, 1995, 1999).

7.4.4 *Temporal Variation in Chemical Concentrations at Hamilton Harbour: A Comparison of Contaminant Trends in Suspended Sediments, Herring Gull Eggs, and Snapping Turtle Eggs.*

An effective means to test the utility of the snapping turtle as an indicator of the bioavailability of persistent organic contaminants, is to compare contaminant concentrations in turtle eggs with other environmental media from the same site over time. Comparing contaminant trends in turtles with other indicator species is also useful, although caution should be exercised when interpreting the results since there are behavioral and dietary differences that will affect the exposure to chemicals.

Results from Marvin (2003) for sum PCB concentrations in suspended sediment collected from Hamilton Harbour were compared with sum PCB concentrations measured in snapping turtle and herring gull eggs collected from 1986 to 2002 (Canadian Wildlife Service Contaminants Database, 2003). The same number of PCB congeners (# 18, 44, 49, 52, 101, 105, 151, 138, 180, 183, 199, 149 and 118) were chosen based on the analytical results for suspended sediments (Fig. 7). Hamilton Harbour was chosen because of the extensive contaminants data collected for snapping turtles since 1996, and because of the high contaminant concentrations found at this location.

Declines in PCB concentrations in suspended sediment from Hamilton Harbour are most apparent from the mid-1980s to the early 1990s, with little change in concentrations in recent years. This pattern corresponds with the temporal variation of declining PCB concentrations in snapping turtle eggs collected from Cootes Paradise, a wetland contiguous with the Harbour. For the selected congener types, results from analysis of herring gull eggs also demonstrate decreasing contaminant burdens occurring mostly from the mid 1990s to present. This indicates that changes in contaminant concentrations in turtle eggs reflect changes measured in other environmental matrices from the same site, further substantiating the usefulness of the snapping turtle as an indicator of contaminant bioavailability.

7.4.5 *A New and Emerging Chemical of Concern: Polybrominated Diphenyl Ether (PBDE) Concentrations in Turtle Eggs*

Polybrominated diphenyl ethers enter the environment by leaching from plastics, textiles, and foams in which they are incorporated, and have generated substantial environmental concern. Once in the environment, penta-PBDEs are persistent, lipophilic, and readily bioaccumulate through the food chain (Hickey et al., 2002). Research and monitoring programs indicate that there is a global occurrence of PBDEs in wildlife, particularly the lower brominated congeners (BDE-47, - 99, -100, -153,- 154) (Sellstrom et al., 1993; Law et al., 2002; Luross et al., 2002). Retrospective analyses of wildlife tissues illustrate an exponential increase in total PBDEs (BDE-47, -99, -100) in herring gull (*Larus argentatus*) eggs collected from the Great Lakes between 1981 and 2000 (Norstrom

et al., 2002). This review provides insight into the occurrence of PBDEs in turtle eggs found in wetland systems in the Great Lakes basin.

The mean log transformed sum of PBDE congeners varied among sites ($F[8,43] = 12.07$, $P < 0.0001$). Sum PBDEs varied from a mean of 6.1 (Algonquin Provincial Park) to 107.0 (Humber River; Fig. 8). Although there were a number of differences among sites, generally levels were lowest at Algonquin Provincial Park, where airborne deposition is assumed to be the main contaminant source. Similar concentrations were found in eggs from Lyon's Creek, and the Upper Canada Bird Sanctuary. Consistent with reports that urban areas contain the highest PBDE concentrations, turtle eggs from Cootes Paradise in Hamilton Harbour and Humber River in Toronto, were the most contaminated among all sites.

We grouped each site into categories in relation to relative sum PBDE concentrations (low, medium, and high), and expressed each congener as a proportion of sum PBDEs, and log transformed Fig. 9). The congeners BDE - 47 and BDE - 99 contributed the most to the sum PBDEs, BDE – 28, -138, and -209 were detected in a limited number of samples. The proportion of BDE 47 increased with sum BDE concentrations ($F_{[2,49]} = 8.56$, $P < 0.0006$), whereas the proportion of BDE 153 and 183 decreased with sum BDE ($F_{[2,49]} = 6.03$, $P < 0.0046$; $F_{[2,49]} = 17.76$, $P < 0.0001$, respectively). Presently, the sum of PBDE concentrations in herring gull eggs are the third highest among groups of organohalogen compounds in the Great Lakes. PBDE concentrations in gull eggs are expected to reach current environmental concentrations of PCBs and DDE in as little as 10 years (Norstrom et al., 2002). Thus, we can expect similar trends in snapping turtle eggs, thereby warranting further use of this indicator to monitor trends in chemicals of recent concern.

8.0 The Usefulness of Snapping Turtles in a Monitoring Plan in Terms of Being Able to Set Endpoint(s) or Attainment Levels Relative to Contaminant Levels in Wetlands of the Great Lakes Basin.

The cumulative data that emerged from this literature review and validation study indicate that the common snapping turtle is indeed sensitive to contaminant exposure.

Furthermore, the geographic patterns and temporal changes in contaminant residues in eggs prove beyond doubt, its usefulness as an indicator model for environmental contaminant studies. A long-term, basin-wide contaminants monitoring program will be much stronger when it involves a large number of sites, greater frequency of sampling, and a longer time frame for data collection (years). Given the above conditions, the objective of providing information to policy makers on environmental contamination in coastal wetland systems in the Great Lakes basin can be met. Given the wide geographic distribution of the snapping turtle and its ability to live in close proximity to urban centers, chemicals of scientific and public concern, including newly emerging ones like polybrominated diphenyl ethers, can be characterized in wetland systems occurring in highly developed settings. Temporal and geographic trend information for these compounds could provide the necessary evidence needed to bring about the curtailment of their use.

The 1978 Great Lakes Water Quality Agreement has committed both Canada and the United States to the "virtual elimination of persistent toxic substances and restoring and maintaining the chemical, physical and biological integrity of the Great Lakes Basin Ecosystem" (International Joint Commission United States and Canada, 1988). With these objectives in mind, the data from this monitoring program are suitable measurement endpoints that could be used to verify attainment of this goal. In the short term, information can be used by policy makers and resource managers to substantiate progress on remediation measures at the local level in Areas of Concern via Remedial Action Plans (RAPs), or at the lake-level via lake wide management plans (LaMPs). The data generated as part of the program can also be used to increase public awareness of environmental contaminant levels in biota, report progress on reaching attainment levels, and facilitate assessment programs in both Canada and the United States.

According to Servos et al. (1999), defining virtual elimination is problematic, and the traditional approach using chemically defined detection limits or levels of quantification in routine sampling and analytical methods may be unrealistic. One problem inherent to this approach is improvements in analytical techniques resulting in an ever-diminishing chemical detection limits. Biological responses may also result from chemical concentrations currently not detectable when using current analytical techniques. It is suggested that an effects-based approach to establish biologically relevant endpoints in

sentinel species would be more useful to determine exposure and set targets for virtual elimination of substances of concern. In this regard, not only can the snapping turtle provide information on the types, levels, and bioavailability of pollutants, but also information on the biological effects of exposure to environmental pollutants. The results of studies on developmental toxicity, alterations in endocrine function, and functional immune response in snapping turtles could be used as a basis for decisions and policies regarding the effects of chemical exposures on wildlife inhabiting wetland ecosystems, as well as human populations.

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Table 1. Mean home ranges (ha) of snapping turtles from wetland sites within Canada and the United States.

Mean (SD) Home range (ha)	Method	Reference
1.84	Pennsylvania	Ernst 1968
0.65	Tennessee	Murphy and Sharber 1973
0.29 (0.27)	Tennessee	Froese 1974
3.44 (2.2)	Lake Sasajewun, Ontario	Obbard and Brooks 1981
0.71 (0.29)	Broadwing Lake, Ontario	Galbraith *et al*. 1987
8.14 (3.00)	Lake Sasajewun, Ontario	Brown 1992
8.64 (2.92)	Lake Sasajewun, Ontario	Brown *et al*. 1994
6.53 (6.15)	Cootes Paradise, Ontario	Brown *et al*. 1994
Male: 2.2-3.0, Female: 8.9-9.7	Cootes Paradise, Ontario	Pettit *et al*. 1995
5.13 (1.86)	Lynde Creek, Ontario	Brown *et al*. 1994

Table 2. A chronological overview of polychlorinated biphenyl (PCBs), dioxin (PCDDs), furan (PCDFs) and dichlorodiphenyl ethylene (p,p'-DDE) concentrations measured in snapping turtle eggs.

Location	# Clutches sampled	Total PCB µg/g [a]	Total PCDD ng/kg [a]	Total PCDF ng/kg [a]	p,p'-DDE µg/g [a]	Reference/Agency	Comments
Rondeau Provincial Park, Lake Erie	NM [b]	NM	NM	NM	0.21	Campbell, 1974, Canadian Wildlife Service	PCB Aroclor 1254:1260 1.92 :g/g w.w.
Hudson R, NY	6	28.9	NM	NM	NM	Stone et al. (1980)	Egg contents (w.w.)
Hudson R, NY	2	1.11-2.86	NM	NM	NM	Bryan et al. (1987), State University of New York at Albany	Egg yolks
Hudson R, NY	2	0.12-0.48	NM	NM	NM		Egg while and shells
Algonquin Provincial Park	7-14	0.025-0.076	ND	ND	0.0080	Bishop et al. (1991), Canadian Wildlife Service, Environment Canada	Data collected 1986-1989; 5 eggs pooled/ clutch
Cootes Paradise	8-21	0.947-2.854	46	17	0.877		
Lynde Creek	4-10	1.360-2.709	124	39	0.472		
Big Creek Marsh	7-18	0.223-0.690	8	5	0.044		
Cranberry Marsh	5-12	0.257-0.605	16	4	0.081		
Algonquin Provincial Park	6	0.187	NM	NM	0.027	Struger et al. (1993), Environmental Quality Branch, Environment Canada	Data collected 1981-1984; 5 – 10 eggs pooled /clutch
St. Lawrence River	5	0.537-0.914	NM	NM	0.010-0.180		Loon Island, Ingleside, Morrisburg
Bay of Quinte	5	0.271-2.751	NM	NM	0.020-0.350		South of Moira River, Sawguin Cr. Big Island
Murray Canal	5	1.324	NM	NM	0.090		
Lynde Shores C.A.	5	1.017	NM	NM	0.090		
Hamilton Harbour, Cootes Paradise	3	1.315	NM	NM	0.200		
Hamilton Harbour, Grindstone Creek	3	4.706	80	14	0.340		PCDDs and PCDFs; subsample from three clutches; 10 eggs pooled/clutch

Big Creek National Wildlife Area	4	1.006	NM	NM	0.097		
Rondeau Provincial Park	5	1.093	NM	NM	0.042		
Lake St. Clair	5	0.344-1.392	NM	NM	0.115-0.140		Thames River, St. Clair National Wildlife Area, Mitchell Bay
Port Franks	4	1.166-1.542	NM	NM	0.116		Pinery Provincial Park, Thedford
Hamilton Harbour, Cootes Paradise, Ontario	15	54.3	NM	NM	NM	Bishop et al. (1994), Canadian Wildlife Service, Environment Canada	5 eggs pooled/clutch; all data are % lipid basis
Hamilton Harbour, Cootes Paradise, Ontario	4	23.952	NM	NM	0.049	Bishop et al. (1995), Canadian Wildlife Service, Environment Canada	first 5 eggs oviposited in nest; data collected 1986
		28.574	NM	NM	NM		composite of 5 eggs per nest
		20.138	NM	NM	NM		last 5 eggs oviposited in nest)
Hoople Creek, Cornwall	4	0.678	NM	NM	0.055	Bonin et al. (1995), St. Lawrence Valley Natural Historic Society, Quebec	Data collected 1989-1990; egg contents; 5 eggs pooled /clutch
Ingleside, Cornwall	3	2.834	NM	NM	0.372		
Grays Creek, Cornwall	5	0.873	NM	NM	0.023		
Raquette R. Massena NY	5	5.094	NM	NM	0.075		
St. Regis R. Massena NY	1	0.942	NM	NM	0.0435		
Akwasasne Massena NY	2	1.575-5.073	NM	NM	0.035-0.047		
Dundee, St. Lawrence R.	7	1.862	NM	NM	0.219		
Beauharnois, St. Lawrence R.	3	1.837	NM	NM	0.068		
Boucherville, S. Lawrence R.	2	0.181-3.343	NM	NM	0.003-0.078		
Thurso, Ottawa R. Ontario	7	0.106	NM	NM	0.007		
Algonquin Provincial Park	15	0.32-3.38	16.8	ND	0.04-0.49	Bishop et al. (1996), Canadian Wildlife	Data collected 1981-1991;

						Service, Environment Canada	all data are % lipid basis
Cranberry Marsh	15	5.27-9.36	287.1	75.8	1.09-1.39		
Big Creek Marsh	12	6.23-14.25	58.1	54.4	0.74-1.44		
Rondeau Park	12	10.95-22.13	35.0	50.7	0.66-0.83		
Lynde Creek	26	20.50-37.64	4499.9-1898	732.1-1534.8	1.72-5.93		
Cootes Paradise	31	21.78-54.36	282.3-1230	206.5-273.7	4.52-10.65		
Algonquin Provincial Park	7	0.018	0.90	ND	0.0018	Bishop et al. (1998), Canadian Wildlife Service, Environment Canada	all data are on a wet weight basis
Cranberry Marsh	3	0.241	14.5	3.6	0.032		
Big Creek Marsh	5	0.388	3.1	2.9	0.0547		
Rondeau Park	6	0.617	2.0	2.9	0.0369		
Lynde Creek	8	1.430	107.8	81.5	0.232		
Cootes Paradise	7-12	2.082-3.574	19.7-39.7	14.4-16.7	0.312-0.389		
Trent River	4	0.835	68.0	6	0.071		
Akwesasne/USA	7	3.946	22.9	9.1	0.068		
Algonquin Provincial Park	9	0.024	NM	NM	0.006	Canadian Wildlife Service Database, 1999	
St. Lawrence River, Cooper Marsh	9	0.1604	NM	NM	0.003		
St. Lawrence River, Snye Marsh	9	1.9425	2.945-5.36	2.00-3.66	0.015		
Hamilton Harbour, Cootes Paradise	9	1.9287	3.61	2.26	0.069		
Raquette R., Akwesasne/ USA	5	4.9564	5.74 – 15.46	3.925-6.61	0.0436		
Cornwal, Grey's Creek	4	0.6197	8.385	6.09	0.0157		
Lake St. Clair, Walpole Island	5	0.187	2.44	1.18	0.00875		
Hosaic Creek	3	0.01166	1.67	0.94	0.00066	Canadian Wildlife Service Database, 2000	
Grey's Creek	2	0.5745	NM	NM	0.018		
Raquette R., Akwesasne/ USA	3	2.4736	14.56	5.083	0.024		

Akwesasne/St. Regis River, New York	1	6.785	9.65	76.15	0.011	De Solla et al. (2001), Department of Zoology, University of Guelph, Ontario	PCDD and PCDF concentrations were measured in a pool of 5 eggs/clutch
Akwesasne/Raquette River, New York	1	5.960	NM	NM	0.029		
AkwesasneSnye Marsh, New York	5	2.378	NM	NM	0.009		
Akwesasne/Turtle Creek, New York	1	737.683	NM	NM	0.852		
Algonquin Provincial Park	6	0.0157	3.165	0.744	0.0013	De Solla and Fernie, (2003, in prep), Canadian Wildlife Service, Environment Canada	
Tiny Marsh	9	0.0411	2.166	0.762	0.0049		
St. Clair National Wildlife Area	6	0.0742	NM	NM	0.0059		
Turkey Creek	8	0.9286	22.96	3.585	0.0244		
Canard River	4	0.2005	NM	NM	0.0047		
Wheatley Provincial Park	8	0.491	17.40	3.453	0.0579		
Algonquin Provincial Park	2	0.0175	NM	NM	0.0014	Canadian Wildlife Contaminant database/2002	
Tiny Marsh	4	0.030	NM	NM	0.0048		GLIER
Hamilton Harbour/ Cootes Paradise	5	1.306	8.593	6.487	0.0879		GLIER
Hamilton Harbour/ Grindstone Creek	5	1.706	15.599	9.908	0.1477		GLIER
Turkey Creek	4	1.074	NM	NM	0.0311		GLIER
Niagara River/ Lyons Creek		1.234	7.149	3.592	0.009		
Wheatley Park	5	0.589			0.0218		GLIER
Algonquin Provincial Park	4	0.0130	NM	NM	0.0032	Canadian Wildlife Contaminant database/2003	
Tiny Marsh	3	0.0075	NM	NM	0.0027		
Humber River, Toronto, Clairville	2	0.6211	NM	NM	0.0092		
Humber River, Toronto,	5	0.5635	NM	NM	0.0360		

Etobikoke Ingleside,	11	0.119	NM	NM	0.016
Cornwall Raisin River,	10	0.1348	NM	NM	0.0079
Cornwall Lyons Creek,	4		NM	NM	
Niagara River AOC		1.054			0.0029
Snye/ Akwesasne	4	0.2364	NM	NM	0.0051
Snye/ Quebec	4	0.8659	NM	NM	0.0193

[a] NM = not measured, ND = non-detectable
[b] wet weight unless otherwise stated

Figure 1. Study sites used to determine the levels of persistent, organic environmental contaminants in snapping turtle eggs.

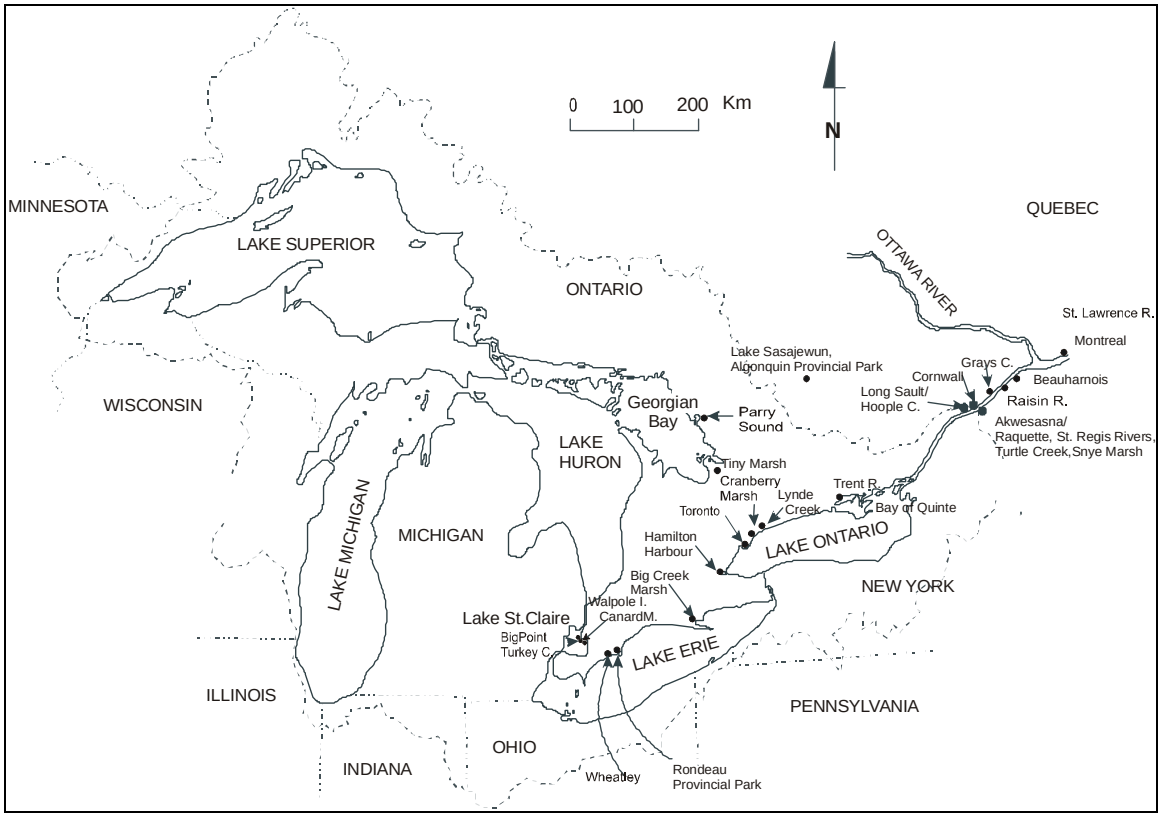
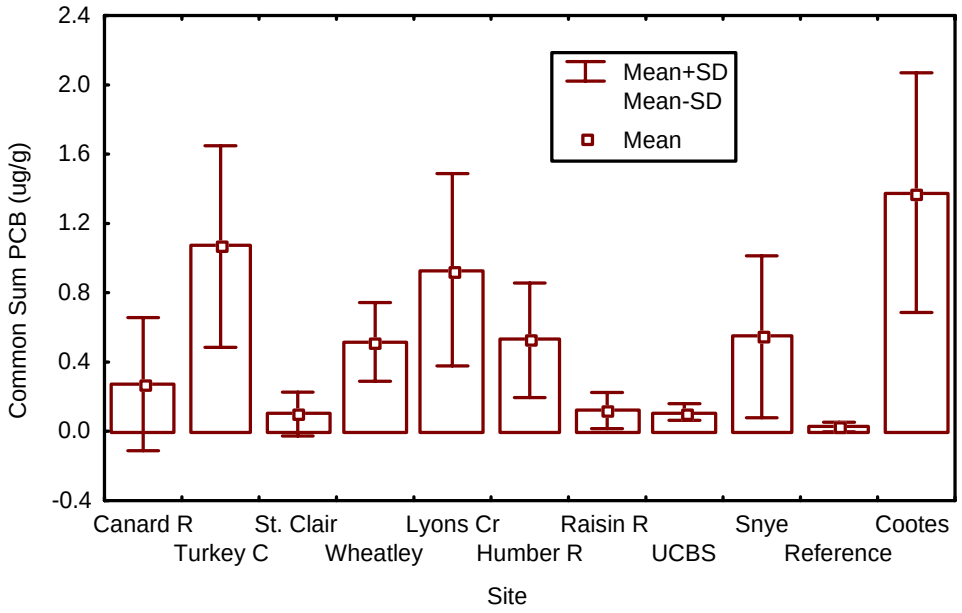


Figure 2. The spatial (geographic) pattern of total PCB concentrations in snapping turtle eggs collected from reference sites and Canadian Areas of Concern in the Great Lakes – St. Lawrence River Basin (2001-2003).



Canard R: The Canard River is located downstream of Windsor ON.

Turkey C: Turkey Creek is located within Windsor ON and runs into the Detroit River.

St. Clair: The St. Clair sites are located within one kilometer (by water) of the St. Clair Area of Concern (AOC) and Walpole Island. Both the St. Clair National Wildlife Area and one private property were sampled.

Wheatley: Clutches were collected from Wheatley Provincial Park and adjacent to the Hillman Marsh Conservation Area (2001 only).

Lyons Cr: Lyons Creek is located adjacent to the Welland Canal and is within the Niagara River AOC.

Humber R: This site is located at the Humber River Marshes at the mouth of the Humber River, Lake Ontario in Toronto ON.

Raisin R: Raisin River runs between Cornwall and Lancaster ON, exiting into the St. Lawrence River.

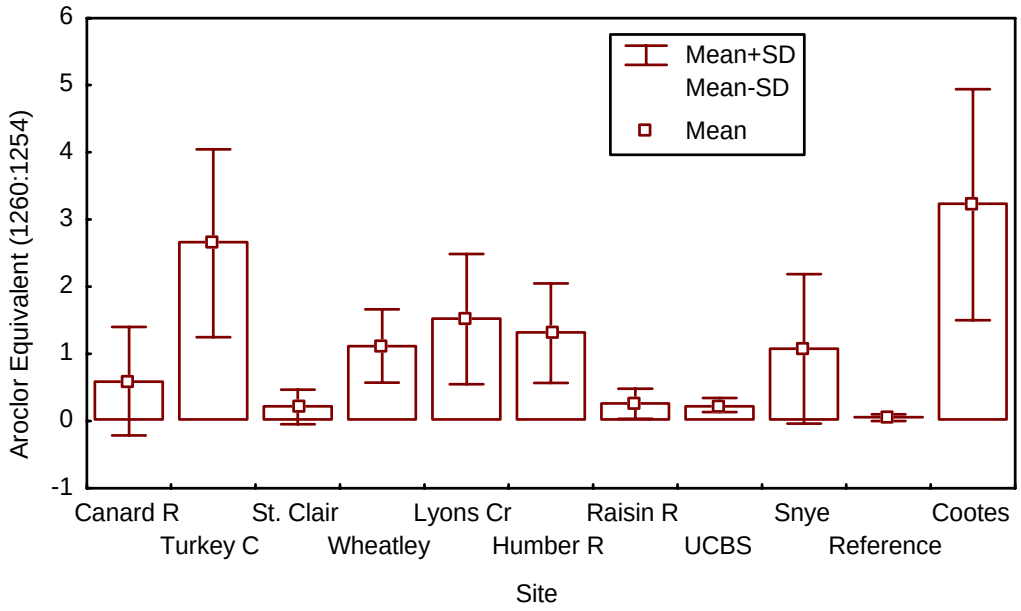
UCBS: The Upper Canada Bird Sanctuary is located within the St. Lawrence River upstream of that AOC near Ingleside ON.

Snye: Snye Marsh is located in Akwesasne and enters into the St. Lawrence River.

Reference: The reference site is Algonquin Park.

Cootes: This site is Cootes Paradise near Hamilton ON and located within the Hamilton Harbour AOC.

Figure 3. The spatial (geographic) pattern of the Aroclor equivalent (1260:1254) in snapping turtle eggs collected from wetlands at reference sites and Canadian Areas of Concern in the Great Lakes – St. Lawrence Basin (2001-2003).



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Cootes: This site is Cootes Paradise near Hamilton ON and located within the Hamilton Harbour AOC.

Figure 4a. Principal component loadings of PCB congeners in snapping turtle eggs from Great Lakes study sites used in 2001-2003. PC1 is dominated by higher chlorinated biphenyls associated with Aroclor 1260.

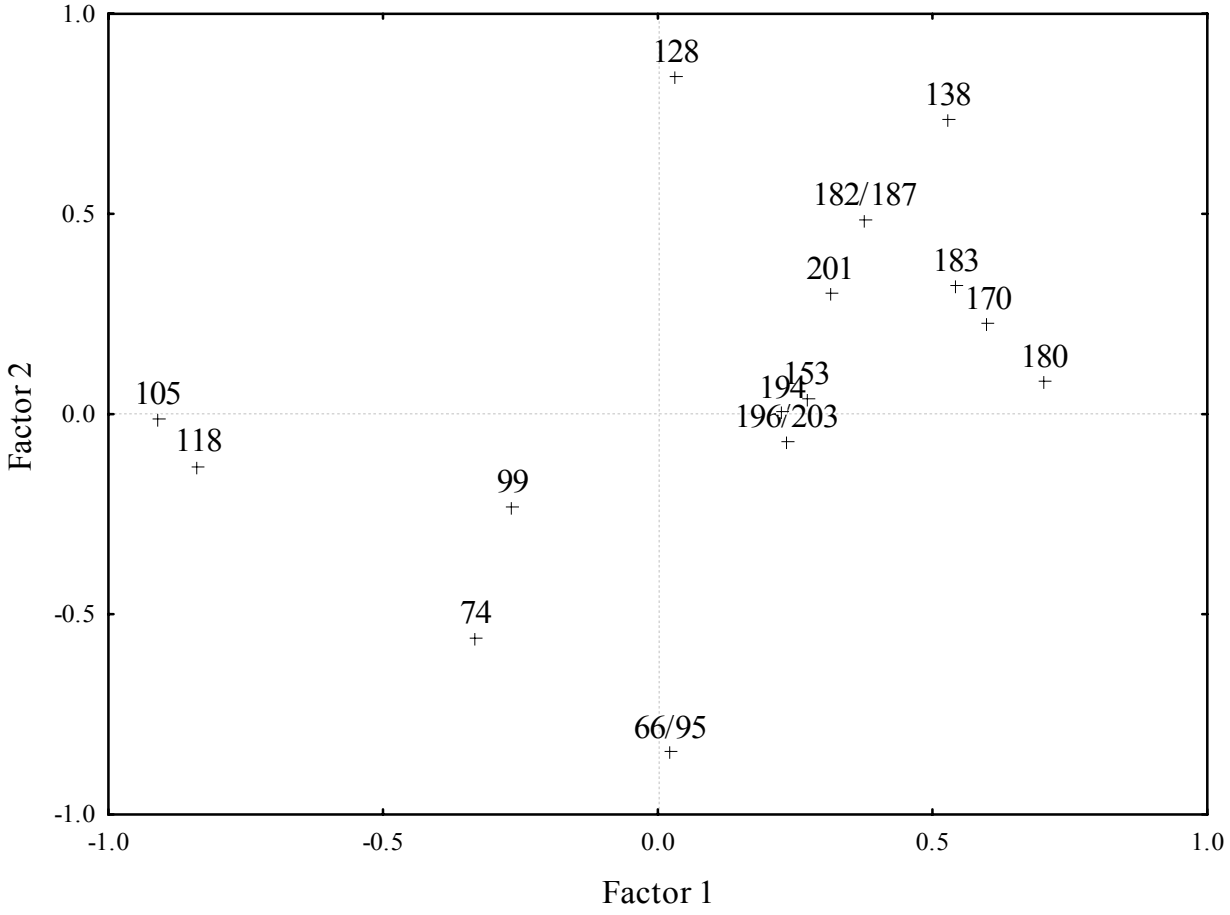


Figure 4b. Factor scores from egg samples for each location during 2001-2003 of the CWS Wildlife Health Effects Study. The boundary illustrates the clustering of different sites based upon the PCB burden in eggs.

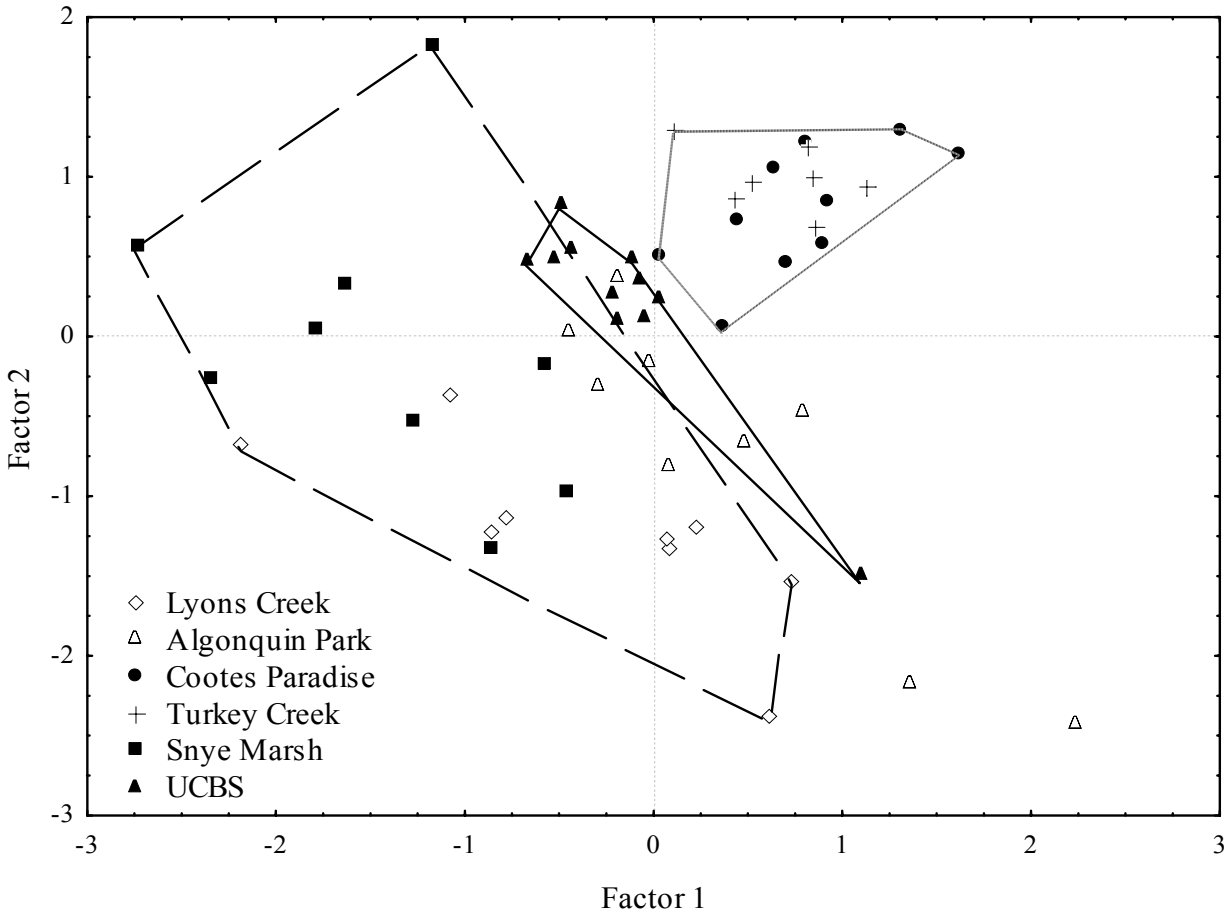


Figure 5. Temporal trends in PCB 1260 concentrations in snapping turtle eggs from a non-contaminated reference site in Algonquin Provincial Park, Ontario.

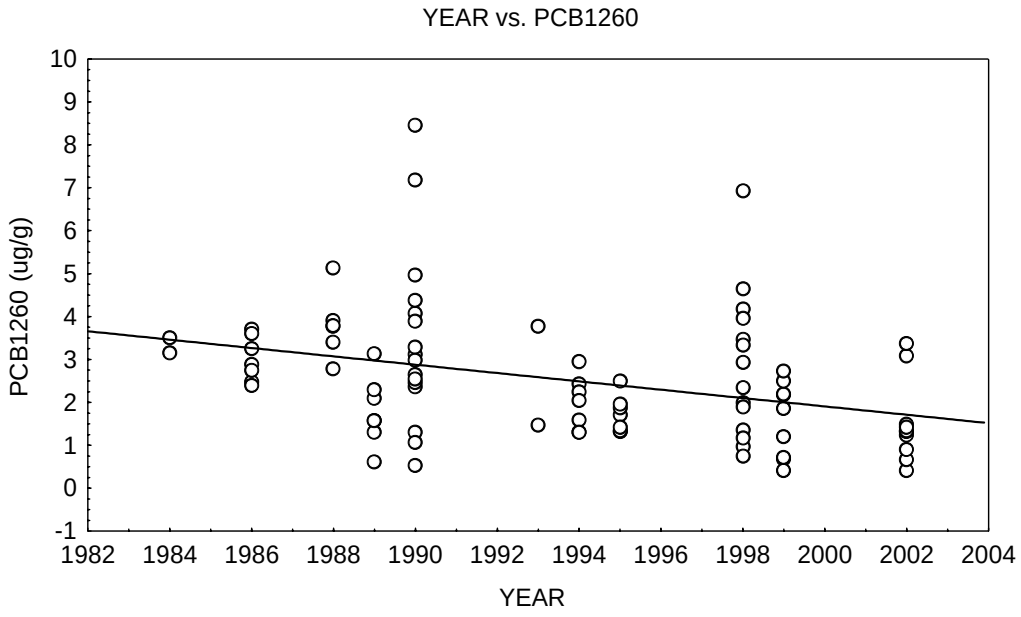


Figure 6. Temporal trends in PCB 1260 concentrations in snapping turtle eggs from Cootes Paradise, Hamilton Harbour AOC, Lake Ontario.

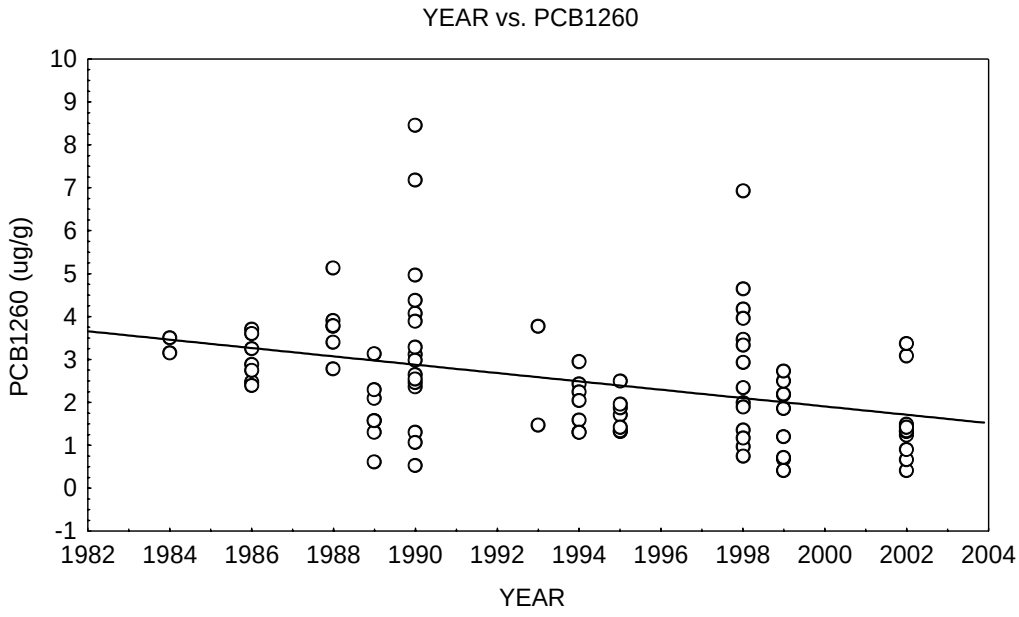


Figure 7. A comparison of mean sum polychlorinated biphenyl concentrations in suspended sediment, and eggs of herring gulls and snapping turtles collected from Hamilton Harbour from 1986 to 2002.

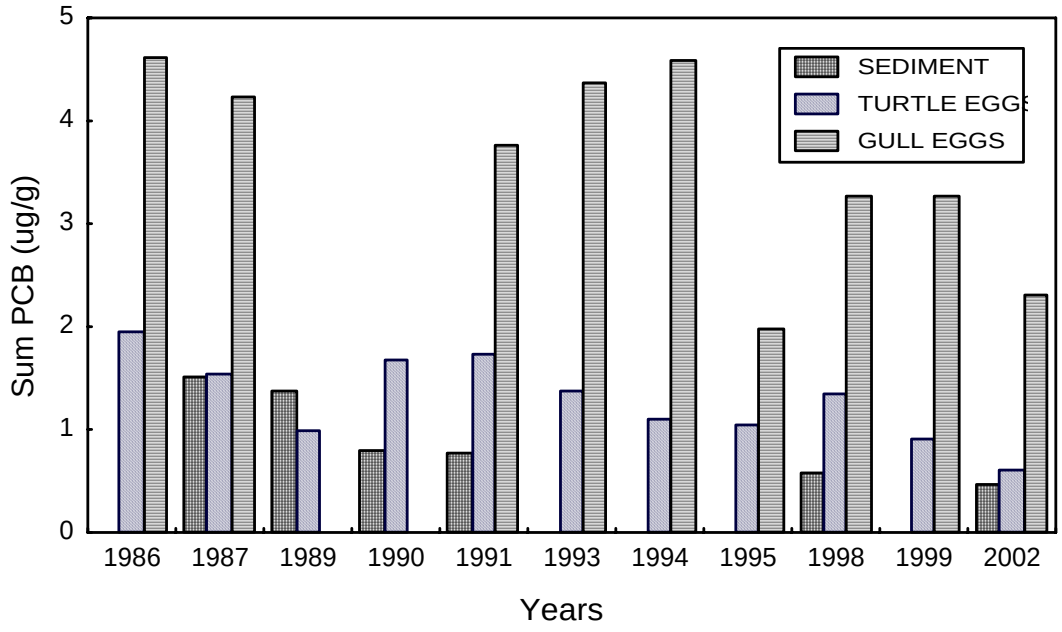
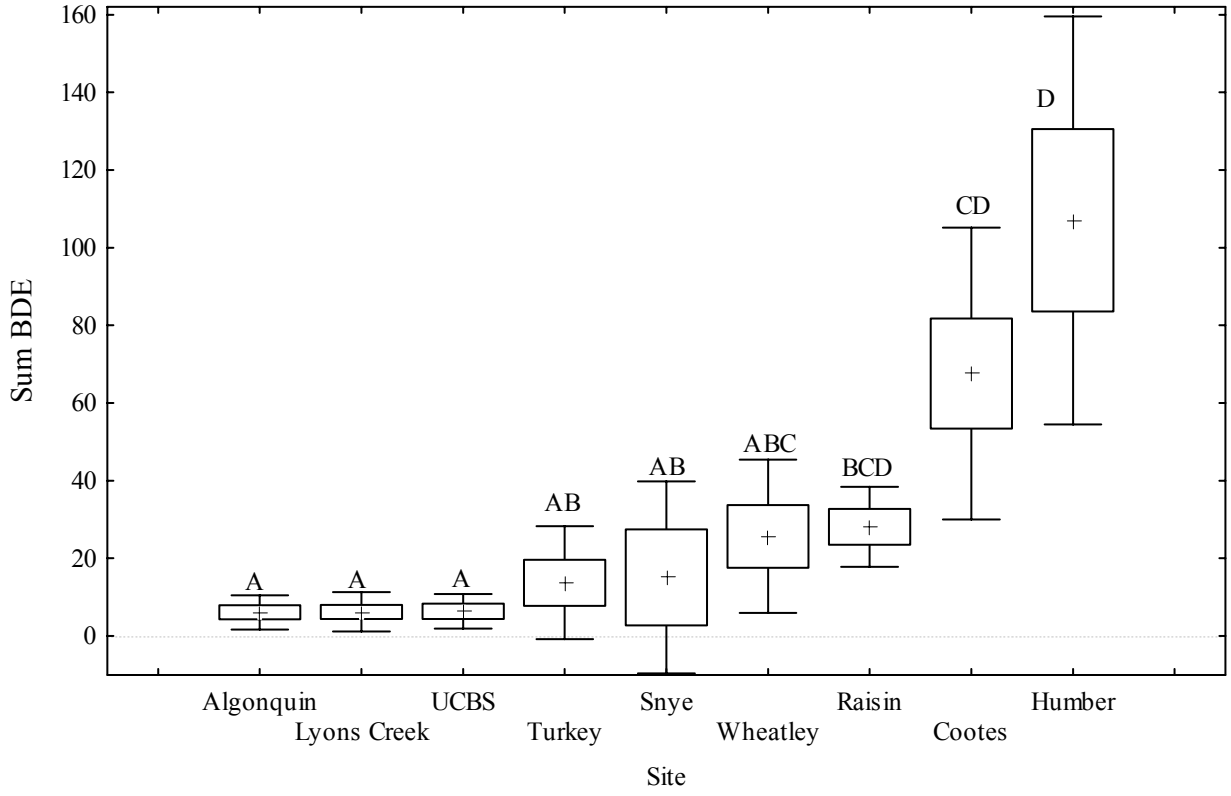


Figure 8. The spatial (geographic) pattern of polybrominated diphenyl ether (PBDE) concentrations in snapping turtle eggs collected from reference sites and Canadian Areas of Concern in the Great Lakes – St. Lawrence Basin (2001-2003).



Algonquin Park is the reference site.

Turkey: Turkey Creek is located within Windsor ON and runs into the Detroit River.

Wheatley: Clutches were collected from Wheatley Provincial Park and adjacent to the Hillman Marsh Conservation Area (2001 only).

Lyons Creek: Lyons Creek is located adjacent to the Welland Canal and is within the Niagara River AOC.

Humber: This site is located at the Humber River Marshes at the mouth of the Humber River, Lake Ontario in Toronto ON.

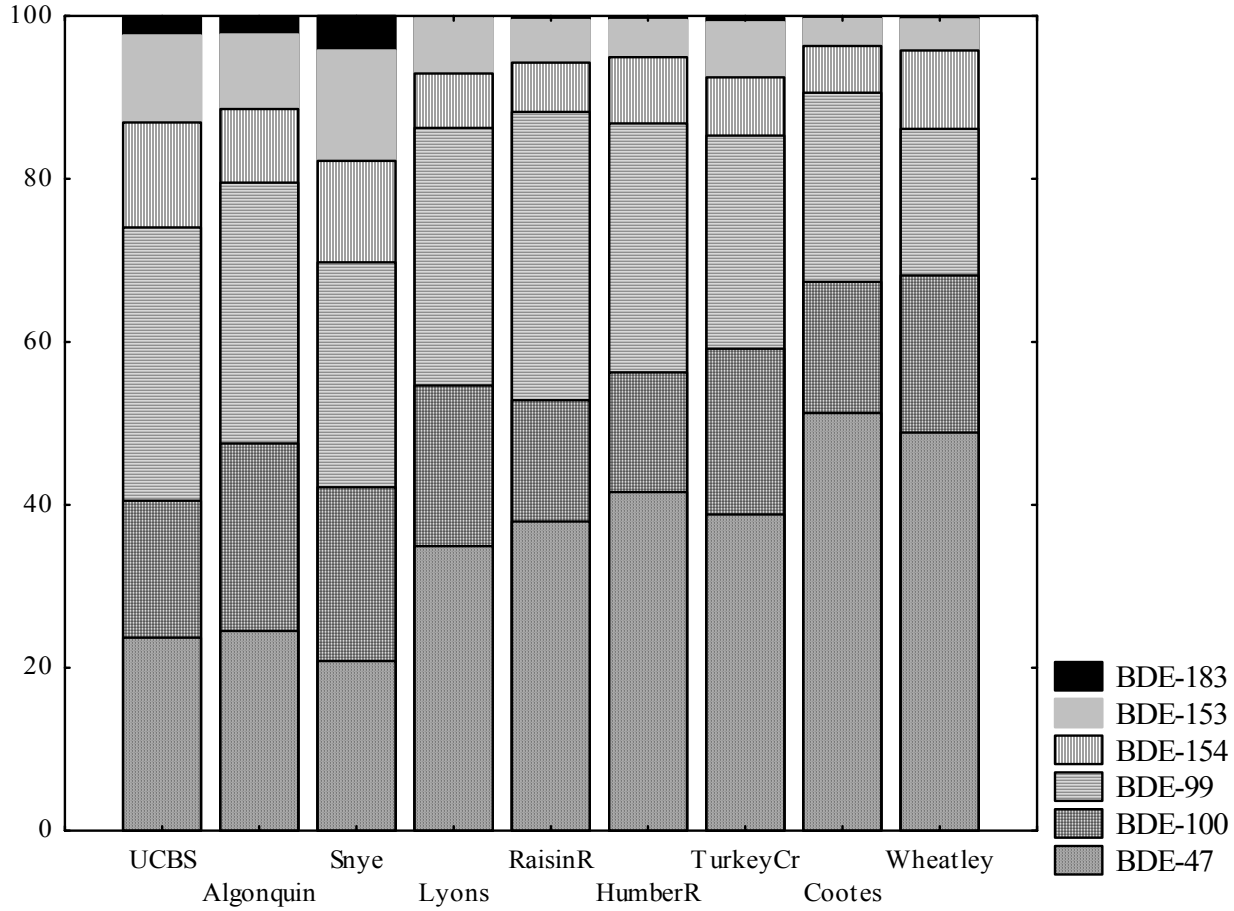
Raisin: Raisin River runs between Cornwall and Lancaster ON, exiting into the St. Lawrence River.

UCBS: The Upper Canada Bird Sanctuary is located within the St. Lawrence River upstream of that AOC near Ingleside ON.

Snye: Snye Marsh is located in Akwesasne and enters into the St. Lawrence River.

Cootes: This site is Cootes Paradise near Hamilton ON and located within the Hamilton Harbour AOC.

Figure 9. The contribution of individual polybrominated diphenyl ether (PBDE) congener concentrations (log transformed) relative to the total PBDE concentration measured in snapping turtle eggs collected from reference sites and Canadian Areas of Concern in the Great Lakes – St. Lawrence Basin (2001-2003).



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Cootes: This site is Cootes Paradise near Hamilton ON and located within the Hamilton Harbour AOC.

Appendix

A1 TITLE AND APPROVAL SHEET

QUALITY ASSURANCE PROJECT PLAN FOR

Measuring of Contaminants in Snapping Turtle Eggs
in Great Lakes Coastal Wetlands

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In Partnership with

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Ric Lawson
GLCWC Project Manager, Great Lakes Commission, rlawson@glc.org

Greg Mayne, Canadian Wildlife Service (Contractor) 125 Longwood Rd. N. Hamilton, Ontario. L8S 3V8 Tel. (905) 522-8534 greg.mayne@sympatico.ca

Signature		Date
……..	Kim Fernie	…….
……..	Chip Weseloh	…….
……..	Ric Lawson	…….
……..	Greg Mayne	…….

Project # WETLANDS2-EPA-05
Revision #3
December 22, 2003

A2 TABLE OF CONTENTS

A PROJECT MANAGEMENT

B MEASUREMENT/DATA ACQUISITION

C ASSESSMENT/OVERSIGHT

D DATA VALIDATION AND USABILITY

A3 DISTRIBUTION LIST

Ric Lawson, Great Lakes Wetlands Consortium, Great Lakes Commission
John Hummer, Great Lakes Wetlands Consortium, Great Lakes Commission
Kim Fernie, Canadian Wildlife Service, Ontario Region, Environment Canada
Chip Weseloh, Canadian Wildlife Service, Ontario Region, Environment Canada
Greg Mayne, Canadian Wildlife Service-contractor

A4 PROJECT/TASK ORGANIZATION

Kim Fernie, of the Canadian Wildlife Service (CWS-Ontario) is the Project Manager and is responsible for project development and implementation, data transfer and coordination issues between other collaborating investigators within the overall Great Lakes Coastal Wetlands Consortium. Kim Fernie will also maintain the official, and approved Quality Assurance Project Plan. Chip Weseloh (CWS-Ontario), will act as the Quality Assurance Manager. Kim Fernie and Chip Weseloh, will develop a detailed methodological framework that incorporates the use of snapping turtle eggs as an indicator of contaminant levels in coastal wetlands of the Great Lakes basin. When followed, this plan will yield information that can detect change and eventually establish basin-wide comparisons and temporal trends of contaminant levels in snapping turtle eggs collected from various wetland sites. As part of another Environment Canada project, Kim Fernie will oversee snapping turtle egg sample collection for contaminant analysis from the Toronto and St. Lawrence River Areas of Concern (AOCs). In addition, archived snapping turtle eggs collected from previously monitored coastal wetland sites will be analyzed by the National Wildlife Research Centre with the aim of detecting temporal and spatial differences in contaminant levels across multiple coastal wetland sites. Greg Mayne, a CWS-Ontario-contractor, will assist in writing the methodological framework. He will also

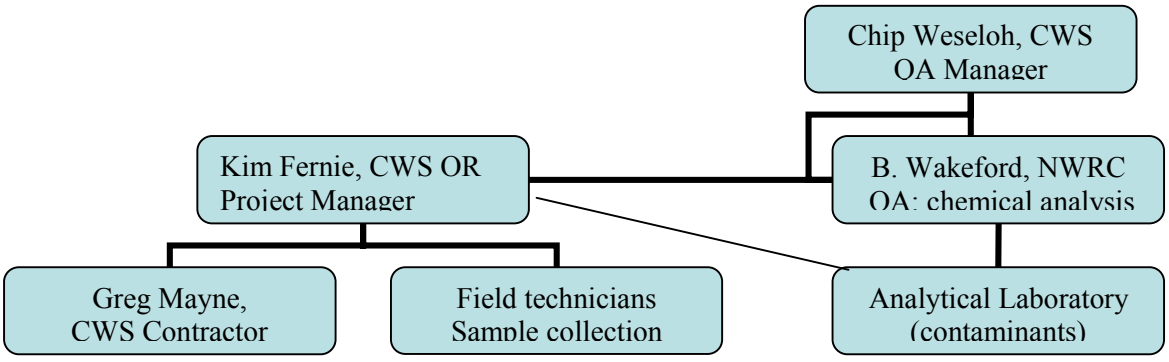
write a "White Paper" that reviews the scientific and government literature relevant to snapping turtles and contaminant levels in their eggs. As part of a sustainable monitoring program, Greg Mayne will contact the appropriate state and provincial agencies to determine the cooperation and willingness of these groups to collect snapping turtle eggs for contaminant monitoring purposes.

Collaborating Project Teams

To be decided following contact of appropriate individuals and agencies.

Project Organization

Dr. Chip Weseloh will provide the quality assurance for this project. Dr. Kim Fernie will report to Dr. Weseloh, providing him with final copies of all reports and seeking his advice when necessary; she reports to him as a wildlife biologist for the Canadian Wildlife Service. Greg Mayne as a contractor, will report to Kim Fernie; his services are contracted for other projects directed by her for the CWS. Dr. Fernie will coordinate the chemical analysis with appropriate labs and the QA manager (Bryan Wakeford). The chart below outlines the reporting structure of this group.



A5 PROBLEM DEFINITION/BACKGROUND

While progress has been made toward developing indicators that will lead to effective monitoring of coastal wetland quality, the consensus formulated at the State of

the Lakes Ecosystem Conference (SOLEC) indicated a need for a system that would consistently measure or monitor the status of coastal wetlands loss or degradation. Subsequent to this, wetland scientists identified indicators that would facilitate evaluation of wetland integrity. The Great Lakes Coastal Wetlands Consortium (GLCWC) was established to develop and implement a sustainable, long term basin-wide monitoring plan that would facilitate assessment programs and reporting capabilities of Canada and the U.S. under the Great Lakes Water Quality Agreement. As part of this long-term goal, the GLCWC has specified a set of metrics relevant to contaminant levels in wildlife that need to be validated for implementation within a long-term monitoring strategy. The ultimate goal of the present study is to validate the snapping turtle as a bioindicator of contaminant levels in Great Lakes coastal wetlands.

Floral and faunal assemblages have been used for centuries by humans as indicators of water quality or general environmental integrity (Landres et al., 1988). A particularly useful biosentinel of contaminant exposure is the common snapping turtle (*Chelydra serpentina serpentina*) (Bishop et al., 1994, 1995; 1996; Struger et al., 1993). The utility of the snapping turtle for biomonitoring purposes is based upon various life history traits. This ubiquitous species inhabits wetlands throughout eastern North America including the Great Lakes-St. Lawrence River basin (Weller and Oldham, 1988). They have a sedentary nature and a small home range and thus reflect local changes occurring in wetlands exposed to contaminants (Hammer, 1969; Congdon et al., 1987; Pettit et al. 1995). The snapping turtle is an omnivorous opportunist, basically consuming whatever is available. Because the snapping turtle occupies a high trophic position, it is subject to food chain biomagnification, and are consequently exposed to high concentrations of persistent organic contaminants (Ernst et al., 1994; Bishop and Gendron, 1998). In addition, there is evidence indicating that concentrations of hydrophobic organic chemicals in eggs reflect the concentration in maternal tissues of snapping turtles (Pagano et al., 1999; Russell et al., 1999). Female snapping turtles lay a single clutch of eggs each year and chemical analysis of a subsample of eggs provides a means to measure contaminant burdens in the body of the female turtle at the time and place of egg-laying (Bishop et al., 1994).

Canadian Wildlife Service researchers have been collecting snapping turtle eggs and measuring chlorinated hydrocarbon contaminant levels in wetland environments since the early 1980s (Struger et al., 1993; Bishop et al., 1994; 1995; 1996). To date, twenty organochlorine pesticides, total mercury, 59 polychlorinated biphenyl (PCBs) congeners, six non-*ortho* PCBs, approximately 10 polychlorinated dibenzodioxins (PCDDs), and 14 polychlorinated dibenzofurans (PCDFs) have been measured in snapping turtle eggs from the Great Lakes-St. Lawrence River basin (Bishop and Gendron, 1998; de Solla et al., 2001). This biomonitoring program has provided important spatial patterns of contaminant levels in the Great Lakes basin (Struger et al., 1993; Bishop et al., 1996). Monitoring efforts using the snapping turtle as a sentinel of wetland integrity continues to provide valuable information on contaminant levels of Great Lakes-St. Lawrence River wetlands (de Solla et al., 2001; K. Fernie, manuscripts in preparation, Environment Canada "Fish and Wildlife Health and Contaminant Concentrations in Selected, Canadian Areas of Concern").

The results from measurement of organic hydrocarbon contaminants in snapping turtles eggs collected from Great Lakes wetlands will eventually establish basin-wide temporal and spatial trends in contaminant levels in Great Lakes coastal wetlands. These

data will provide important contaminants trend data useful to resource managers and policy makers to facilitate the evaluation and effectiveness of clean-up actions. Participation from both U.S. and Canadian wildlife management agencies is important in evaluating the status of Great Lakes coastal wetlands. As such, development and implementation of a systematic, long-term, contaminants monitoring program with a binational focus will ensure that basin-wide information are available for regulatory purposes.

A6 PROJECT/TASK DESCRIPTION

This project is part of a three-year GLCWC initiative to develop a monitoring plan and data support system for Great Lakes coastal wetlands. The objective of this study is to create a methodological framework for the use of snapping turtle eggs as an indicator of contaminant exposure and levels within coastal wetlands of the Great Lakes basin. The use of snapping turtle eggs as a viable means to assess wetland contaminant status will be tested for incorporation within a long-term monitoring strategy. Snapping turtle eggs collected from the Toronto and St. Lawrence River Areas of Concern (AOCs) in 2003, and archived snapping turtle eggs collected from coastal wetland sites in previous years, will be analyzed to measure hydrophobic organic chemicals. Provincial and State wildlife agencies will be contacted to determine the cooperation and willingness of these groups to collect snapping turtle eggs for future monitoring purposes.

The framework includes a "White Paper" that provides a detailed methodological plan that utilizes snapping turtle eggs to measure and monitor contaminant levels in lacustrine, riverine and barrier-protected wetland systems of both upper and lower Great Lakes wetlands. This monitoring program, which when followed, will produce information that can detect change and eventually establish temporal trends and basin-wide comparisons for contaminant levels. The "White Paper" will review the scientific and government literature relevant to snapping turtles and their eggs, and how they may be used to measure contaminant exposure. In future years, investigators involved in the monitoring program will collect snapping turtle eggs from wetland sites within the Great Lakes basin and measure contaminant levels using standardized protocols.

Sampling Locations

Snapping turtles lay one clutch per season, typically in June in Southern Ontario. Archived egg samples will be chosen to maximize sample sizes so as to best represent wetland types and provide spatial and temporal data, while addressing financial constraints. To this end, the following 2003 study site locations are being considered:

Site Name	**Site Type**	**Hydrogeomorphic Type**	Provincial R.M./County	**Longitude**	**Latitude**
Humber River/ Toronto	AOC	Open, Drowned River-Mouth	Metropolitan Toronto, Ontario	43,38'10.21"	79,28'37.94"
Raisin River/ St. Lawrence	AOC	Open, Drowned River-Mouth	Stormont, Dundas & Glengarry; Ontario	45,07'44.21"	74,29'36.04"
Upper Canada Bird Sanctuary/	Up-stream AOC	Protected Embayment	Stormont, Dundas & Glengarry; Ontario	44,57'10.55"	75,02'40.69"

Cornwall					
Snye River/ St. Lawrence	AOC	Open, Drowned River-Mouth	St. Regis, Quebec	45,00'10.55"	74,31'45"

Criteria of the Great Lakes Coastal Wetlands Consortium

Six criteria that originate from the Request for Proposals (RFP) distributed by the Great Lakes Commission on behalf of the Great Lakes Coastal Wetlands Consortium will be addressed. These criteria fall under "Scope of Work" in the RFP as one of the goals "to test the feasibility of applying indicators in a monitoring plan."

The criteria are as follows:

1. Cost – The cost of implementing a program using snapping turtle eggs to measure routine organochlorine contamination and pesticides will be assessed. The cost and availability of analytical methods to measure other chemicals of concern (e.g., polyaromatic hydrocarbons (PAHs), polybrominated diphenyl ethers (PBDEs)), will be addressed;

2. Measurability – This section will provide detailed information regarding specific project design and methodology, including the selection of wetland sites that will provide necessary spatial and temporal data to assess contaminant trends in a Great Lakes coastal wetlands monitoring plan;

3. Applicability – Basin-wide applicability and reliability of snapping turtles to measure contaminants in various wetland types across the Great Lakes basin, including both the lower and upper basin, will be determined. The "White Paper" will identify other part(s) of the suite of indicator species for contaminants in tandem with the snapping turtle and provide advantages and disadvantages for this approach;

4. Complementary data – Availability of complementary existing research and data relevant to the use of snapping turtles to determine contaminant levels will be identified. A review of published materials will be used to identify previous researchers and organizations involved in the historical and current snapping turtle work of the CWS, what methodology was used to identify contaminant concentrations and the compounds targeted. Information relevant to contaminant levels in snapping turtle eggs and contaminant-induced health effects at possible sites for the monitoring plan will also be reviewed. In addition, the CWS currently has archived snapping turtle egg samples which would be analyzed to further establish contaminant levels and trends at possible monitoring sites;

5. Sensitivity – The sensitivity of snapping turtles will be assessed in terms of detecting changes in the contaminant conditions of wetlands over time as well as space. This task will be accomplished through a review of the published literature as well as analyses of archived and to-be-collected snapping turtle egg samples;

6. Endpoints – The "White Paper" will address the usefulness of snapping turtles for a monitoring plan in terms of being able to set endpoint(s) or attainment levels relative to contaminant levels and health effects in wetlands of the Great Lakes basin.

Work Schedule

June -August, 2003: Collect snapping turtle eggs at all 2003 study sites (see previous table). Transfer eggs to analytical laboratory at the National Wildlife Research Centre (NWRC, Ottawa, Ontario) or to certified, quality-controlled laboratories under contract to the NWRC for contaminant analysis.

September 2003 – April, 2004: Measurement of organochlorine levels in snapping turtle eggs collected from the Toronto and St. Lawrence River Areas of Concern (AOCs) in 2003. Measurement of organochlorine levels in archived snapping turtle egg samples collected from various coastal wetland types within the Great Lakes basin, including the Toronto and St. Lawrence River AOCs.

September 2003 – May, 2004: Write a comprehensive literature review of published materials relevant to snapping turtles and their eggs, and how this species will serve as a useful bioindicator model for contaminant exposure and effects. Identify researchers and organizations involved in the historical and current snapping turtle work of the CWS and elsewhere, what methodologies were used to identify contaminant concentrations and the compounds targeted.

A7 QUALITY OBJECTIVES AND CRITERIA

The primary quality objective of this study is to create a methodological framework for a long-term, basin-wide study which incorporates the use of snapping turtle eggs to detect temporal and spatial patterns of contaminants in specific types of Great Lakes coastal wetlands. A complete review of published materials will be conducted and this information will be provided along with the framework. As secondary materials are derived from numerous sources, primary importance will be placed on works published in peer-reviewed scientific journals and reports from scientific government sources. Snapping turtle eggs collected in the first year of study (2003) and archived egg samples will be analyzed in an effort to confirm the usefulness of the snapping turtle as an indicator of contaminant exposure. The contaminants targeted in routine chemical analysis include organochlorine pesticides, PCB congeners including non-*ortho* PCBs, PCDDs, and PCDFs. "Data acceptability" for chemical results will be contingent upon analytical methods following the Standard Operating Procedures established by Canadian Wildlife Service chemists and scientists. This approach will ensure that results of this project are comparable to (past and) future projects occurring around the Great Lakes and that data collected as part of this project can be integrated into centralized databases for to determine long-term trends in contaminant levels in snapping turtle eggs.

As part of the Quality Control criteria for chemical analysis of organochlorines and PCBs, a five-point initial standard curve is made with the organochlorines and PCBs standard mixtures to cover the range of interest. This established calibration curve is verified daily by analyzing a calibration verification standard having a mid-point concentration.

Reports from chemical analysis will include detection limits, which indicate the lowest quantifiable concentration using the associated method. A minimum detectable concentration is described as the concentration of analyte which produces a signal in an instrument three times the average noise level. In multi-residue analysis, such is the case of this project, it is not always practical to list the detection limits for each compound of interest. As a general rule, a detection limit of at least 0.0001 PPM is achievable for all compounds. In reporting the data, results having less than 0.0001 PPM are reported as NS (not detected) in the Laboratory Services analytical test report, and one half the detection limit is used in the statistical analysis. If a computed result falls in the range of 0.0001 and 0.0009 PPM, the compound is listed as TR (trace) and the median value of the trace range is used for statistical analysis.

Precision and recovery will be addressed by running an aliquot of the standard NWRC QA Reference Material (Herring gull eggs) along with each batch of samples. Concentrations of the major compounds (PCB-52, PCB-66, PCB-101, PCB-110, PCB-149, PCB-118, PCB-146, PCB-153, PCB-138, PCB-187, PCB-180, PCB-170, PCB-201, PCB-203, HCB, p,p'-DDE, photo-mirex, mirex, oxychlordane, cis-nonachlor, hetachlor epoxide and dieldrin) are determined and the results are compared to the previously established acceptance limits (i.e., ±2 SD of the long-term mean plotted in a Shewart chart). To determine the degree of analyte loss during sample cleanup, each sample is spiked with 13C-labelled chlorobenzenes/PCBs internal standard mixture.

Systematic biases in contaminant analysis are avoided through the proper preparation and analysis of method blanks. Method blanks ensure contamination of glassware or other equipment in the laboratory is accounted for. On each sampling date, one type of blank is prepared and analyzed. All three types of blanks should be below the prescribed method detection limit.

In the event of sample contamination or equipment failure, the data will be flagged accordingly. The use of these data will be restricted until an investigation resolves the issue of contamination or inaccurate results. Only values that meet the data quality objectives for accuracy, precision and bias will be used without caution. This ensures that the data reported are reliable, reproducible and accurate.

Representativeness of the entire snapping turtle clutch will be ensured by selecting and pooling five eggs collected from each clutch of eggs, and homogenizing this composite sample prior to chemical analysis. In an attempt to ensure that contaminant levels are representative of a particular wetland site, field biologist will attempt to collect eggs from approximately 10 clutches per site. This approach should provide the necessary means to represent contamination of each site, and then compare contaminant levels among various wetland study sites situated in the Great Lakes.

A8 SPECIAL TRAINING REQUIRMENTS

Kim Fernie and Greg Mayne will identify wetland study sites, and Kim will supervise collection, handling, labelling and storage protocols of snapping turtle eggs. Experienced chemists at the National Wildlife Research Centre in Ottawa, Ontario, will conduct the contaminant analysis of the snapping turtle eggs.

A9 DOCUMENTATION AND RECORD

Development and implementation of an integrated binational Great Lakes coastal wetland monitoring program using snapping turtle eggs as an indicator of contaminant exposure will require that participating researchers and organizations have the most current version of an approved Quality Assurance Project Plan (QAPP). If any changes in the QAPP occur, a new, updated version will be submitted to the Great Lakes Commission by Kim Fernie. The transfer of this QAPP would occur in the next stage rather than this current stage that only involves initial contacting of people and agencies.

Data obtained during field operations will be entered into field logs. Data will be reviewed for completeness each day by the field crew lead. All field logs will be stored at CWS-Ontario office and entered into the snapping turtle database. Contaminant analysis data will be provided by National Wildlife Research Centre chemists in hard copy and electronic file format. Original copies will be stored at the National Wildlife Research Centre in Ottawa, Ontario, Canada. Electronic data back ups will be completed regularly and copies of the data stored at the CWS- Burlington office. All records and reports generated from this study will be stored by CWS-Burlington and CWS-Downsview following study completion.

A "White Paper", detailing the methodological plan and including a review of the scientific and government literature significant to snapping turtles and their eggs will be produced as both hard copies and electronic files. Copies will be available to both the CWS and the Great Lakes Coastal Wetlands Consortium.

DATA GENERATION AND ACQUISITION

B1 SAMPLING PROCESS DESIGN (EXPERIMENTAL DESIGN)

Study and Design Rationale

Canadian Wildlife Service biologists and contractors will collect snapping turtle eggs from wetland sites in the Toronto and St. Lawrence Areas of Concern (AOCs), as well as traditional reference study sites located inland of the Great Lakes in Ontario in 2003. Eggs will be analyzed for contaminant levels along with archived egg samples (locations, dates, sample size to be determined) from various wetland types including the Toronto and St. Lawrence River AOCs. In addition, the CWS currently has historical contaminant data for snapping turtle eggs collected from inland reference sites, the Hamilton Harbour AOC and other St. Lawrence River AOC sites. All data being collected as part of this project are considered critical to meeting GLCWC and project objectives.

Taken together, our analytical results, combined with existing contaminant databases, will be beneficial in testing and validating the snapping turtle as an indicator of Great Lakes coastal wetland contamination by:

1. confirming the usefulness of the snapping turtle as an indicator of temporal and spatial contaminant trends in different hydrogeomorphical wetland types;

2. determining how well contaminants in snapping turtle eggs reflect environmental contaminants in sediment and/or water samples taken at these sites.

Wetland sites used in this study are known to have high contaminant levels; some of these sites are within IJC-designated Areas of Concern (AOCs). Other sites will be chosen because historical contaminant data already exists, they represent a specific type of wetland, and/or they are upstream of the AOCs for comparative purposes, or because they contain low contaminant levels and are useful as reference sites. Wetland sites for future monitoring efforts will be chosen based on their respective hydrogeomorphical characteristics, contaminant levels, and/or geographic location within the Great Lakes basin. The latter will be chosen based on information provided by wildlife managers with offices in the Great Lakes – St. Lawrence River basin. In the event that sampling sites become inaccessible, eggs will be collected from other, representative sites within the same wetland complex. This will be done by looking for evidence of previous nest sites, sites that offer optimal nesting habitat, or by actively searching for nesting females.

Although Bishop et al. (1995), reported a non-significant intra-clutch variation in contaminant levels among freshly laid eggs, the first five eggs contained the highest mean concentration of all chemicals on a wet-weight basis and the highest mean lipid values relative to the last five eggs collected. In order to estimate the "average" contaminant concentration of a nest, five eggs are typically selected from the clutch. The method suggested by Bishop et al. (1995), was to select one of the first few eggs laid, one of the last few eggs laid, and three eggs from the rest of the clutch. This pooled sample is assumed to approximate the median concentration of that clutch. More recently, we have selected eggs in a pseudo-random but stratified manner; eggs were ordered from first to the last egg laid, and each clutch was divided into five groups of approximately equal size. Within each group, an egg was selected haphazardly (de Solla and Fernie, submitted). Normally, five eggs were selected from each clutch for contaminant analysis, but if the clutch is to be used for other purposes, as few as one egg may be used.

There appears to be no literature reporting congener-specific PCB pattern/chlorination changes during embryonic turtle development. Nonetheless, the utilization of fresh eggs (< 48hours) removes the uncertainty of changes in contaminant concentrations by Phase I and Phase II metabolic enzymes (Bishop et al., 1995a). When possible, 10-15 clutches will be collected from each wetland study site in order to obtain a measure of variance of contaminant levels associated with each wetland. For further details, see Sampling Methods below.

The measurement parameters of interest include organochlorine pesticides and approximately 59 PCB congeners; oxy-, trans-, and cis-chlordanes; trans- and cis-nonachlor; p,p'-DDE, DDD, and DDT; octachlorostyrene; mirex; dieldrin; hexachlorobenzene and heptachlor epoxide. Pending cost constraints, polychlorinated

dibenzo-p-dioxins, polychlorinated dibenzofurans and non-ortho PCBs may also be measured.

B2 SAMPLING METHODS

A composite sample of five eggs will be collected from each clutch as outlined above. The remaining eggs in the clutch are immediately reburied without excessive rough handling. The five eggs selected for contaminant purposes are placed in a plastic container (e.g., sandwich container) and surrounded with moist vermiculite or sand to prevent breakage en route to the field base. If possible, 10-15 clutches per wetland site will be sampled. Egg samples will be identified by the site name, sample number, latitude and longitude of the collection site, the collection date, and the total number of eggs will be recorded for each clutch. Eggs will be cleaned of particulate matter, placed in foam-lined containers to prevent breakage and kept in coolers to prevent over-heating while in the field. Eggs will then be temporarily stored in a 5 ^{o}C walk-in refrigerator, or frozen in a – 20 ^{o}C chest freezer until the day of shipment to the Laboratory Service Section of the National Wildlife Research Centre in Ottawa, Ontario. The contents of five eggs will be pooled and stored in hexane rinsed jars at – 20 ^{o}C at the National Wildlife Research Centre, Ottawa, Ontario, Canada until the date of analysis following the Tissue Preparation Unit's standard operating procedure SOP-TP-PROC-07.

If problems are encountered during sample collection, transport, or storage, Kim Fernie will take the necessary corrective actions by reviewing each phase of sample handling with field personnel. If necessary (and/or possible), new samples will be collected from the same sites and reworked for analysis. Any problems, changes, or otherwise, will be reported to the GLCWC by Kim Fernie in quarterly reports or via email correspondence.

B3 SAMPLE HANDLING AND CUSTODY REQUIREMENTS

Canadian Wildlife Service biologists and contractors will collect snapping turtle eggs from the designated wetland sites in 2003, as well as selecting archived egg samples collected in previous years. Snapping turtle egg samples may be archived for extended periods of time (e.g., years) prior to contaminant analysis if they are stored under appropriate conditions (i.e., contents of eggs placed in solvent-rinsed glassware at –80^{o}C freezer). The NWRC currently manages a "tissue bank" that allows wildlife tissues to be stored until contaminant analysis occurs. This allows for historical contaminant analysis as well as the analysis of contaminants once suitable methodologies are developed (e.g., PBDE).

Personnel at the National Wildlife Research Centre responsible for registry of biological samples will be given at least one week advance notice of the date of arrival of samples at NWRC to ensure that appropriate materials are in place upon arrival of the shipment. If individuals other than CWS staff (i.e., air or courier) deliver samples to the National Wildlife Research Centre, a weighbill number is required so that the shipment can be traced. Examples of data collection sheets and custody forms are provided below.

PROJECT / PROJET :	CONTACT AND PH0NE NUMBER / PERSONNE RESSOURCE ET NO. DE TÉL
This form is used to complement the collection data sheet (FORM-TP-11). Please send one sheet for every shipment to NWRC	
Ce formulaire sert à compléter les données de collecte (FORM-TP-11). S.V.P. faire parvenir une lettre d'accompagnement pour chaque envoi au CNRF	
Part A - Collection data related to specimens / Données concernant les spécimens	
Source / Origine :	wild / sauvage
	other / autre
Collecting technique of whole specimen (e.g. shot, netted, picked up by hand, trapped, gaffed) / Technique de prélèvement (e.g. tiré, ramassé (oeuf), pêché, attrappé au filet):	
Condition when collected (e.g. fresh, dead-no info, dead-with info., sick) / État lors du prélèvement (ex. frais, mort/avec information, mort/pas d'info., malade) :	
Sacrifice :	

Part B - Data related to specimen preparation and preservation prior to shipment to NWRC /				
Tissue type / Type de tissu	Collecting technique, condition of tissues and remarks / Technique de collecte, condition des tissus et autres	Storage / Entreposage	Container and cap liner / Contenant et couvercle	Container treatment / Traitement
Tissu type: e.g. egg content, liver-left lobe, head, plasma …				
Collecting technique: e.g. biopsy, heparinized seringe, dissection with chemically cleaned instruments, homogenization (give details				
Container: e.g. glass jar, polyethylene (PE) bag, polypropylene (PP) scintillation vial, cryovial, egg carton, Teflon vial, etc				
Cap liner: metal foil, rubber, PE, Teflon				
Container treatment : rinsed with nitric acid (A); rinsed with organic solvents (S); not rinsed (N); unknown (U)				

Part C - Other comments / Autres commentaires
List exceptions, contamination problems, etc. / Énumérer les exceptions, les problèmes de contamination, etc.

Environment Canada / Environnement Canada
Canadian Wildlife Service / Service canadien de la faune
National Wildlife Research Centre / Centre national de la recherche faunique
Refer to SOP-TP-DOC-03 for explanatory notes / *Consulter la procédure SOP-TP-DOC-03 pour not*
PROJECT / *PROJET*
PROJECT LEADER / *AGENT DE PROJET*
List of abbreviations used / *Abbréviations utilisées* (e.g. K = kidney, LLL = liver left lobe, SNTU= snapping turtle)

USOX	**Specimen no**. / *No. d'échantillon*	**Type of tissue and number of containers**/ *de tissu et nombre de contenants*	**Species** / *Espèce* **(common name)** */(nom commun)*	**Age**	**Sex /Sexe**	**Collection** date / *Date de collecte (yyyy/mm/dd)*	**Collection site** / *Emplacement du prélèvement*		**Location** / *Enfroit*
							Latitude deg/min	**Longitude deg/min**	**Province**

B4 ANALYTICAL METHODS

Snapping turtle egg samples provided to the Trace Organic Chemistry Laboratory at the NWRC, Ottawa, are prepared as described in the Tissue Preparation Unit's standard operating procedure SOP-TP-PROC-07. The analytical method used for contaminant analysis of snapping turtle eggs is outlined in Technical Report Series Number 335 "Multiresidue Methods for the Determination of Chlorinated Pesticides and Polychlorinated Biphenyls (PCBs) in Wildlife Tissues by Gas Chromatography/ Mass Spectrometry" (Won et al., 2001).

EXTRACTION OF CONTAMINANTS FROM EGGS

Egg samples are homogenized and between 1.5 g to 3.0 g of the homogenate is treated with 25 g anhydrous Na_2SO_4 in a glass mortar and pestle until a free-flowing mixture is obtained. This mixture is then poured into a 2.1 cm x 35 cm glass column packed with treated glass wool and 1 cm Na_2SO_4. The mortar and pestle is rinsed three times with a dichloromethane/hexane (1:1) solution and transferred to the column and allowed to soak for 30 minutes. An additional 200ml of dichloromethane/hexane is added to the column and allowed to elute at 5-10 ml/min into a 500 ml flask. The eluate is evaporated to less than 5 ml on a rotary evaporator with a water bath (30^oC) then quantitatively transferred into a graduated centrifuge tube. Dichloromethane/hexane (1:1) is then added to obtain a final concentration of 0.2 g/ml (i.e., 3 g of tissue in 15 ml of Dichloromethane/hexane. An aliquot equivalent to 1.0 g of egg is transferred into a gel permeation chromatography (GPC) tube. The extract is spiked with 50 :l of ^{13}C-chlorobenzene internal standard spiking solution and diluted to 10 mL with dichloromethane/hexane. The GPC flow-rate is set at 5 ml/min of (1:1) dichloromethane/hexane. The eluate is evaporated to 3 ml on a rotary evaporator.

SAMPLE CLEANUP BY FLORISIL COLUMN

The florisil column is designed to isolate compounds of interest from any residual lipid. A Florisil column is packed with treated glass wool, saturated in 40 ml hexane, and 8g de-activated Florisil added, followed by approximately 1 cm Na_2SO_4. The solution is allowed to flow through the column until the solvent level is slightly above the Na_2SO_4 layer. The extract is loaded to the top of the Florisil column using a Pasteur pipet. A 150 ml flat-bottomed evaporating flask is rinsed with 3-4 small portions of dichloromethane/hexane and then added to the column and 95 ml of dichloromethane/hexane (1:1) is added and then eluted at 5 mL/min. The eluate is concentrated to less than 3 ml with rotary evaporator and quantitatively transferred to a 10 mL flask and further concentrated to 400 :l with rotary evaporator. The eluate is quantitatively transferred to autosampler vials, spiked with 20 :l of normalization standard and diluted to 570 :l. The autosampler vials are capped and thoroughly agitated.

CONTAMINANT ANALYSIS

Contaminant levels are determined by high-resolution gas chromatography coupled to a mass selective detector (GC/MSD) operated in selected ion monitoring mode for use in the analysis. Identification of contaminants is accomplished by comparing gas chromatography retention times and specific mass fragments known to be present in the spectra of authentic compounds. Quantification is accomplished by comparing the intensity of mass fragments of contaminants of interest in egg specimen extracts to the same compounds in a standard mixture, injected separately on the GC/MSD system.

In the event of problems occurring within the above mentioned methodologies, such as an instrumentation failure, the laboratory chemist will review all aspects of the analytical procedure and samples will be re-worked for analysis. All remaining samples from pooled extracts are archived in the Canadian Wildlife Service Specimen Bank, National Wildlife Research Centre, Ottawa, Ontario, Canada. Problems encountered during analysis of egg samples will be relayed to Kim Fernie by analytical chemists. These problems and the corrective actions taken will then be reported to the GLCWC by Kim Fernie in quarterly reports or via email correspondence.

B5 QUALITY CONTROL REQUIREMENTS

Compliance with the QA/QC program will be coordinated and monitored by the quality assurance manager and appropriate personnel at NWRC. The objectives of the QA/QC program are as follows: to ensure that all analytical procedures are documented, including any changes in administrative and/or technical procedures; to ensure that all field procedures are conducted according to sound scientific principles and have been validated; to ensure that all equipment is clean, calibrated and properly functioning; to monitor the performance of the sample collection procedures and provide for corrective action as necessary; and to ensure that all data are properly recorded and archived. Internal quality control procedures will be conducted by audits.

Quality control activities for contaminant analysis of tissues are outline in Technical Report Series Number 335 “Multiresidue Methods for the Determination of Chlorinated Pesticides and Polychlorinated Biphenyls (PCBs) in Wildlife Tissues by Gas Chromatography/ Mass Spectrometry” (Won et al., 2001).

Biweekly checks using certified reference standards will be performed to determine laboratory accuracy and equipment performance. A five-point calibration standard curve is made with the organochlorines and PCBs standard mixtures to cover the appropriate concentration range for the test. The calculated concentration of each compound must be within 20% of its actual known value. The final concentration of any reportable compounds must be within the demonstrated linearity of the detector. If necessary, samples are diluted with iso-octane to meet the calibration range. Laboratory accuracy should be within 80%-120% for all parameters tested (Won et al., 2001).

Detection Limits and Reporting Limits

A nominal or minimum detectable concentration is usually described as the concentration of analyte which produces a signal in an instrument three times the average

noise level. In this multi-residue method, it is not practical to list the detection limits for each compound of interest. Variability between compounds arises due to varying background noise and response factors for each compound due to the different mass ions being monitored. As a general rule, a detection limit of at least 0.001 PPM is achievable for all compounds.

Ongoing Precision and Recovery

An aliquot of the QA Reference Material (Herring gull eggs) is analyzed along with each batch of samples. The concentration of the major compounds (PCB-52, PCB-66, PCB-101, PCB-110, PCB-149, PCB-118, PCB-146, PCB-153, PCB-138, PCB-187, PCB-180, PCB-170, PCB-201, PCB-203, HCB, p,p'-DDE, photo-mirex, mirex, oxychlordane, cis-nonachlor, hetachlor epoxide and dieldrin) is determined and the results are compared to the previously established acceptance limits (i.e., ±2 SD of the long-term mean plotted in a Shewart chart).

To determine the degree of analyte loss during sample cleanup, each sample is spiked with 13C-labelled chlorobenzenes/PCBs internal standard mixture. Analysis is accepted when the % internal standard recoveries for most PCBs and OCs are between 80% and 110%, and for the highly volatile compounds are over 60%.

Accuracy

The accuracy of the quantitation standards is verified annually with a second source standard (containing most of the congeners of interest) as described in SOP-CHEM-PROC-13.

Method Blank

A method blank is run with each batch of samples to determine the levels of contamination associated with the processing and analysis of samples. If problems with the blank exist, associated data are carefully evaluated and appropriate corrective actions are applied. Blank values are not subtracted from reportable values. A compound found in a blank and also in an associated sample is flagged in the analytical test report when present at a ratio of at least 5/1, sample to blank.

Data Validation

Data validation is ensured by an internal quality assurance audit done by an independent reviewer (Head of the Laboratory Services Section), before the release of the analytical test report. If large discrepancies are noted in the analytical data between the specimens from close geographical areas, the raw data are examined and re-analysis of the sample aliquot may be indicated.

Systematic biases

Systematic biases in contaminant analysis are avoided through the proper preparation and analysis of method blanks. Method blanks ensure contamination of glassware or other equipment in the laboratory is accounted for. On each sampling date, one type of blank is prepared and analyzed. All three types of blanks should be below the prescribed method detection limit. The method detection limits indicate the lowest quantifiable concentration using the associated method. For the purpose of reporting data,

no results less than this concentration are reported and a result of NS (not detected) appears in the Laboratory Services Section analytical test report. If a computed result falls in the range of 0.0001 and 0.0009 PPM, the compound is defined as being detected but the result would be too variable to be reliable so a designation of TR (trace) is listed beside the compound in the final report. In the event of sample contamination or equipment failure, the data will be flagged accordingly. The use of these data will be restricted until an investigation resolves the issue of contamination or inaccurate results. Only values that meet the data quality objectives for accuracy, precision and bias will be used without caution. This ensures that the data reported are reliable, reproducible and accurate.

B6 INSTRUMENT/EQUIPMENT TESTING, INSPECTION, AND MAINTENANCE

No specialized equipment is required for collecting eggs samples in the field. Instrumentation required for chemical analysis consists of a GC/MSD, Hewlett-Packard gas chromatograph (GC) 5890 Series II equipped with an autosampler (7673A), a Galileo Channeltron electron multiplier (5778) and linked to a Hewlett-Packard 5970 (or 5971A) mass selective detector (MSD) with MS ChemStation,.

The Mass Selective Detector (MSD) is tuned weekly with the perfluorotributylamine (PFTBA) calibration standard using the Auto Tune program, and daily with the Quick Tune Program. The tuning of the instrument must meet the criteria for conformance outlined in SOP-CHEM-PROC-12 before sample analysis. Tune files are archived in a logbook at NWRC.

Laboratory technicians supervised by the chemist are responsible for testing, inspection and maintenance of laboratory instrumentation. Standard operating procedures for the maintenance of the GC/MSD are found in SOP-CHEM-MAIN-04 located in the trace organic analytical laboratory. The tuning of the mass selective detector (MSD) must meet the criteria for conformance outlined in SOP-CHEM-PROC-12 before sample analysis; certified technicians will be used to make the necessary repairs. Tune files are archived in the laboratory logbook.

B7 INSTRUMENT CALIBRATION AND FREQUENCY

A four-point initial calibration curve is generated every six months for the major compounds (e.g., oxychlordane, PCB-153, etc.) found in the control material to cover the range of interest. This established calibration curve is verified daily, by analyzing a calibration verification standard (quantitation standard) having a mid-point concentration. The calculated concentration of each compound must be within 20% of its actual known value. The final concentration of any reportable compounds must be within the demonstrated linearity of the detector. Calibration is documented daily in a laboratory log book by the technician or chemist performing the calibration. If problems are encountered, such as final concentrations of a reported compound falling outside the demonstrated linearity of the detector, the sample will be diluted with iso-octane to meet the calibration range.

B8 INSPECTION/ACCEPTANCE for SUPPLIES

The working standard solutions can be found in Table 1 – Supplier, catalogue number and concentration of PCBs and organochlorine standards of the Technical Report Series Number 335 "Multiresidue Methods for the Determination of Chlorinated Pesticides and Polychlorinated Biphenyls (PCBs) in Wildlife Tissues by Gas Chromatography/ Mass Spectrometry" (Won et al., 2001).

Chemists and technicians at the National Wildlife Research Centre in Ottawa, Ontario are responsible for inspection and acceptance of supplies. Acceptable supplies are those items that do not have any visual sign of defects/flaws and regents/chemicals that are not past expiry dates. Tracking records for supplies and consumables are kept in the trace organic analytical laboratory at the National Wildlife Research Centre, Ottawa, Ontario, Canada.

B9 NON-DIRECT MEASUREMENTS

Background information files will be accessed for all existing contaminant data in snapping turtle eggs, sediment samples and water samples, and this information will be incorporated into the project literature review. Background information will include researcher(s), organization(s), study locations, methodologies and contaminant data. As interest has been expressed in contaminant levels in tissues other than eggs, the results from chemical analysis of other liver, skeletal muscle and other tissues will be discussed. Sources of information will include published papers from peer-reviewed scientific journals as well as government reports and databases. These existing contaminant databases will be beneficial in testing and validating the snapping turtle as an indicator of temporal and spatial contaminant trends in different Great Lake coastal wetlands.

B10 DATA MANAGEMENT

All field data will be recorded in field logs and inspected at the end of each field day. All data will then be transferred to a central file at CWS-Ontario office in Burlington, Ontario where photocopies and electronic files will be made and stored. Original field logs and electronic files will be under the care of Kim Fernie. Contaminant data generated from snapping turtle egg analysis at the National Wildlife Research centre in Ottawa, Ontario, will be forwarded to Kim Fernie at the CWS- Burlington office in Burlington, Ontario, where it will be entered into a contaminants database by CWS technicians or contractors. The data are recorded electronically using Excel files on IBM-compatible computers. CWS technicians confirm and correct data entry to insure accuracy. CWS computers are back-up nightly using the Veritas program.

ASSESSMENT/OVERSIGHT

C1 ASSESSMENTS AND RESPONSE ACTIONS

As field operations are simple basic procedures, there are no expected sources of error in field sampling procedures. Similarly, chemists analyzing snapping turtle eggs at NWRC laboratories adhere to strict Good Laboratory Practice (GLP) principles. Kim Fernie will be responsible for supervising field staff with respect to appropriate and correct field sampling methods and oversight in data collection and review of field data logs for missing data daily while on site. Before the release of analytical reports, data validation is completed by the head of the Laboratory Services Section at the National Wildlife Research Centre in Ottawa, Ontario. Results of data verification are recorded on the "Data Validation Form for OC/PCBs Reports". The raw data is examined prior to release to CWS biologists and decisions are made by the head of the Laboratory Services Section regarding re-analysis of samples.

C2 REPORTS TO MANAGEMENT

Reports to the GLC will occur on a semi-annual basis and occur in December of 2003 and June of 2004 with a final report in June, 2004. These reports will include a brief narrative of progress to date and must detail any problems encountered as well as any changes to the project including personnel, schedule, and deliverable contents. The final report including all items as identified in the Project/Task Description and Data Quality Objectives sections of this project plan, and a financial report, will be submitted by Greg Mayne before June 30, 2004. All data collected as part of the project will be submitted in electronic format via electronic mail, or on CD or other compatible storage medium to Ric Lawson, Coordinator of Great Lakes Coastal Wetlands Consortium.

DATA VALIDATION AND USABILITY

D1 DATA REVIEW, VALIDATION, AND VERIFICATION REQUREMENTS

The project manager will review all documented field and laboratory operations including sample collection, handling, storage and analysis to ensure that methods conform to the specified QA/QC criteria. Analytical data will be examined for discrepancies (i.e., contaminant concentrations that fall far below or above the mean contaminant level for each site) upon delivery from testing laboratories.

D2 VALIDATION AND VERIFICATION METHODS

Data validation is ensured by an internal quality assurance audit done by an independent reviewer before the release of analytical reports. Results of this verification are recorded on a "Data Validation Form for OC/PCBs Reports". Analytical data on snapping turtle egg contaminant results will be examined for discrepancies by Laboratory Service technicians at NWRC. If large discrepancies are found in contaminant data for egg samples collected from the same site, analytical results will be re-examined. In instances

where data validity comes into question and cannot be resolved, the specimen will be re-analyzed by NWRC chemists. In the event that there is an omission of data, such omissions will be reported the project manager and conveyed to the GLCWC project manager and other collaborators identified in this QAPP. All analytical procedures and results will be fully documented; such documentation will reside in a file with the project manager.

D3 RECONCILIATION WITH DATA QUALITY OBJECTIVES

The framework for a sustainable basin-wide monitoring program using snapping turtles eggs as an indicator of contaminant exposure will be reviewed for completeness by the quality assurance manager and senior wildlife scientists within the Canadian Wildlife Service. Communication between field biologists and the quality assurance manager will be maintained on a daily basis throughout the data collection phase in the field to ensure a sufficient sample size for inter- and intra-site comparisons. Chemical reports will be provided by Canadian Wildlife Service chemists from the Laboratory Services Section in Ottawa, Ontario to the project biologist (Kim Fernie). Reports contain general information, methods, results, comments and detection limits on contaminants specific to snapping turtle eggs. Proper statistical methods will be used to analyze data for inter- and intra-site variation in contaminant levels in snapping turtle eggs. In the event that data quality objectives could not be attained for specific aspects of the sampling (i.e., insufficient sample size), the reason for not meeting the data quality objectives will be documented and reported in semi-annual progress reports and in the final report to the GLC.

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