# Changes in ornithological methods in the past 33 years

The Editors

The techniques used in ornithology have changed considerably since 1983. Most field researchers gathered data with notebooks and pencils, used low power scopes and took pictures with single lens reflex cameras and massive telephoto lens. Photography was a hit or miss exercise due to the need to develop the slides at a later date. Those wanting to track movements of birds with transmitters were limited to large birds due to the heavy weight of the batteries and the need to re-encounter the birds in real time. The internet was still a dream, computers were generally large mainframes or if they were desktops they were owned by only a few. Apple and PC computers were just starting and there was no home computer market. Most ornithologists still used typewriters to produce articles, but some were using mainframe computers for word processing and data analysis.

There is now a book on techniques for field ornithology (Sutherland *et al.* 2004) and an older book on techniques, which has been revised seven times (Silvy 2012). As techniques have developed greatly, we decided to give readers a feel for some of the new ones and how they

have changed ornithology. The next four papers examine several techniques now which weren't frequently used or didn't exist in 1983. Two new monitoring devices, one a transmitter (Motus) and the other a data logger (geolocator), provide types of information that were previously unobtainable for smaller birds. Information on stress hormones provides insight on natural behaviour and measurements of atomic isotopes show insights on changes in prey and ecological relationships. We hope these papers give you an appreciation for how new techniques, which were unimaginable a few years ago, have now expanded our knowledge of bird biology and ecology.

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## Cooperative automated radio telemetry: the Motus Wildlife Tracking System in Ontario

Stuart A. Mackenzie and Philip D. Taylor



Radio telemetry involves tracking animals using transmitters that emit pulses on very high radio frequencies. The technology allows researchers to track many different individual animals with high temporal and geographic precision. Radio-telemetry has played an important role in research and conservation on a wide variety of taxa for over 60 years (Adams 1965, Cochran et al. 1965). In recent decades, automation of receivers, miniaturization and digitization of tag signatures and coordination of monitoring efforts, have allowed researchers to simultaneously track larger numbers of individuals at broader scales than previously possible.

The Motus Wildlife Tracking System (Motus is Latin for 'movement') is a cooperative automated radio telemetry system that harnesses the collective power of many researchers and organizations into a globally coordinated effort that expands the scale, scope and impact of everyone's work. Motus is a not-for-profit program of Bird Studies Canada (BSC) in partnership with Acadia University and other collaborating researchers and organizations. It is funded through a combination of user fees and major support from various government agencies and private foundations. The core operations of Motus were initially supported by the Canada Foundation for Innovation, through a grant to Western University, Acadia University, BSC and the University of Guelph.

Figure 1. All Motus stations active for at least three months across all projects excluding Europe, 2014-2016. An up-to-date map of live stations is available at www.motus.org



Figure 2. Red Knot (*Calidris canutus*) outfitted with a radio transmitter (Lotek nano-tag) (see antenna extending from back) being released at Mingan, Quebec. *Photo: Yves Aubry* 

### Research in Ontario using Motus since 2014 has been comprehensive and diverse.

Researchers using Motus employ transmitters that weigh as little as 0.3 g. These transmitters emit a unique digital pulse every 5-40 seconds, which can be detected by automated receivers at distances of 15-20 km. Tags are fitted onto the backs of birds and bats, including small passerines such as warblers, or even large insects such as Monarchs (Danaus plexippu) and Green Darners (Anax junius). In July 2016, Motus comprised more than 300 receiving stations (Figure 1) throughout eastern North America, the Arctic and parts of Central and South America. The system has been used to track thousands of individuals of over 70 species of vertebrates. Unlike other lightweight tracking technologies like geolocators or global positioning system tags, location data are transmitted automatically to researchers and individual birds never need to be recaptured.

Research in Ontario using Motus since 2014 has been comprehensive and diverse. Perhaps the most ambitious project has been conducted by the James Bay Shorebird Monitoring Project led by Environment and Climate Change Canada (ECCC), Ontario Ministry of Natural Resources and Forestry, Trent University, the Moose Cree First Nation and BSC. This project aims to quantify habitat use and staging behaviour of migratory shorebirds on the western coast of James Bay (Friis *et al.* 2013, Friis and Peck 2014, Friis 2015). Since 2013, more than 400 individual shorebirds, primarily Semipalmated Sandpiper (*Calidris pusilla*), White-rumped Sandpiper (*C. fuscicollis*), Red Knot (*C. canuta*) and Dunlin (*C. alpina*), have been tagged in and around James Bay and tracked as they travel through the eastern seaboard and as far south as the Gulf of Mexico (Figure 2). These individuals are providing exciting new information about the importance of James Bay to migratory shorebirds as well as knowledge about their migratory pathways that aids in various conservation efforts.

Numerous studies on stopover and migration ecology of songbirds have been and are being conducted at Long Point Bird Observatory, Bruce Peninsula Bird Observatory and Thunder Cape Bird Observatory by Western University, University of Guelph, Trent University, Acadia University, BSC and ECCC. Specifically at Long Point, Black-throated Blue Warblers (Setophaga caerulescens), Magnolia Warblers (S. magnolia) and Yellowrumped Warblers (S. coronata) have been tagged to study protandry (differential habits of males and females during migration; Morbey 2001) and stopover (Taylor et al. 2011, Seewagen et al. 2013). Canada Warblers (Cardellina canadensis) and Blackpoll Warblers (S. striata) have also been tagged to study stopover habitat use, and regional and continental migratory movements (Brown and Taylor 2015).

In the field of species at risk research and recovery, researchers from ECCC, Trent University and BSC have been studying breeding movements and postbreeding dispersal of Bank Swallows (*Riparia riparia*) at natural and anthropogenic nesting sites, providing an eye-opening understanding of a wide use of landscapes throughout the province. In 2016, aerial insectivore work continued on Barn (*Hirundo rustica*) and Cliff swallows (*Petrochelidon pyrrhonota*) as well as Common Nighthawks (*Chordeiles minor*) (Greg Mitchell [ECCC], Myles Falconer [BSC], Stuart Mackenzie [BSC], Liam McGuire [Texas Tech University] and Mark Brigham [University of Regina]).

Wildlife Preservation Canada in collaboration with the Toronto Zoo, Mountsberg Raptor Centre, African Lion Safari and Smithsonian Conservation Biology Institute have also used Motus to assess survivorship and track early migratory movements of captiveraised Loggerhead Shrikes (*Lanius ludovicianus*) released into the wild in southern Ontario (Hazel Wheeler and Jessica Steiner).

Finally, in the depths of winter, researchers from the University of Windsor have been making strides to understand the winter ecology and spring migration of Snow Buntings (*Plectophenax nivalis*). A small number of individuals were tagged near Long Point in early winter 2016, and their movements have been tracked throughout Ontario and up the St. Lawrence River (Emily McKinnon and Oliver Love).

Motus stations have also contributed to many other national and international projects where individuals tagged elsewhere, such as the United States, Nunavut, Yukon or Colombia, have been detected migrating through Ontario. One example of how these data have expanded our knowledge of birds in

Ontario specifically is the discovery that the highest concentration of migratory flights for shorebirds originating at Delaware Bay (spring) and James Bay (fall) occurs through eastern Ontario (James Bay Shorebird Monitoring Project). Birders will also be interested to know that Red Knots have been detected migrating through every county in southern Ontario. We expect that they are not frequently observed by birders simply because many individuals choose to fly non-stop between the eastern seaboard and arctic staging areas and it is only during inclement or unusual weather that they are forced to land. Examples of the movement pathways from these and other projects around the hemisphere can be viewed at www.motus.org.

The cooperative application of this technology allows researchers to obtain significantly more data from their single studies and experiments, and likewise, allows researchers to contribute important data to other people's projects. As the network expands, we anticipate being able to supply low-cost receiving stations to members of the public, allowing birders and other citizen scientists to contribute to cooperative wildlife tracking at a global scale.

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## Stress hormones: assessing population health at the physiological level

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Figure 1: Extracting a small, 100ul, blood sample from an adult male Ovenbird. Blood is extracted from the brachial vein using a small gauge needle and heparinized capillary tube. Blood samples are centrifuged to separate blood cells from plasma, which is analyzed for corticosterone. *Photo: Rhiannon Pankratz*  With the emergence of the field of conservation physiology, there is increasing recognition that physiological metrics can provide critical information about the impact of environmental stressors on the flora and fauna of a region (Cooke et al. 2013). Of particular interest to us has been the measurement of the so-called "stress hormone", corticosterone, to infer the reaction of animals to environmental perturbations. Corticosterone is a glucocorticoid that circulates in the blood of birds and other taxa (cortisol in humans) and becomes elevated during periods of duress. Extended periods of exposure to a stressor can result in chronic elevation of glucocorticoids, and potential negative effects on health and survival (e.g., reduced body mass, reduction or cessation in reproduction) (Lattin et al. 2016). The measurement of corticosterone in a

> wild bird allows for the quantification of environmental or humaninduced stressors on an individual's health and ultimately fitness (Busch and Hayward 2009).

> In 2008, as a component of a larger project investigating the effects of group-selection silviculture on wildlife in Algonquin Provincial Park, we collaborated with Dawn Burke and Ken Elliott of the Ontario Ministry of Natural Resources and Forestry, to study the conservation physiology of

Ovenbirds (*Seiurus aurocapilla*). Groupselection silviculture is a form of logging that targets small patches of trees (ca. 0.5 ha) for removal, with the end goal of promoting the growth of typically shadeintolerant, but relatively rare, tree species (e.g., Yellow Birch, Betula alleghaniensis and Black Cherry, Prunus serotina) in the Algonquin landscape. In our study, there were two treatments: 'typical' which involved the removal of small (0.03 ha) or large (0.07 ha) groups of trees next to at least one mature shade sensitive tree species and 'intensive' which involved the removal of medium-sized (0.05 ha) groups of trees spaced 50m uniformly throughout a forest stand. Within an average 20 ha stand, 'typical' group selection resulted in 10-12 gaps and 'intensive' resulted in 80 gaps per forest stand. Our project was to complement a suite of studies looking at the potential effects of group-selection silviculture on various focal birds, including Yellow-bellied Sapsucker (Sphyrapicus varius), Rose-breasted Grosbeak (Pheucticus ludovicianus), Black-throated Blue Warbler (Setophaga caerulescens), and the bird community as a whole. The other studies of the larger project used traditional ecological metrics of success (e.g., monitoring nest success, fledgling survival, population estimates); our project additionally incorporated the quantification of glucocorticoid hormones. Our goal was to measure corticosterone levels in the blood of Ovenbirds across the different group-selection logging treatments and at undisturbed sites, to determine if group-selection silviculture was affecting Ovenbirds on a physiological level, perhaps in the

Our study was the first to document the effects of group-selection silviculture on stress physiology of birds... absence of other more commonly measured indices of health (e.g., body condition, reproductive success).

In our study, we measured baseline (immediately after capture) and stressinduced (30-minutes post-capture) corticosterone levels in blood samples extracted from adult male and nestling (baseline only) Ovenbirds (Figure 1). Baseline samples were extracted within three minutes of capture or disturbance of the nest site for nestlings. Blood was extracted within three minutes because it has been shown that there is insufficient time for corticosterone to increase as a result of the acute stress response and thus, this represents the best estimate of a baseline sample. Males were captured using mist nests and a territorial song lure; nestlings were sampled in their nests one to two days before fledging. These two measures of stress provide insight into the immediate response to stressors (baseline) and behavioural responses to stressors (stressinduced). We found that adults and nestlings responded differently to stress from different intensities of group-selection silviculture, with adult males showing elevated stress-induced levels in intensive sites (Figure 2) and nestlings showing elevated baseline levels in both intensive and typical sites (Figure 3) (Leshyk et al. 2012, 2013). We also measured body condition, nest success and ground insect abundance (Ovenbird food source) and found no difference across logging and control treatments (Leshyk 2011), highlighting the potential importance of measuring multiple indices of population health when assessing the effects of anthropogenic disturbance.

We attributed the hormonal responses of adult male Ovenbirds which showed elevated stress-induced levels to the changes in the perception of predator risk created by having to cross many relatively large openings in the canopy. We suspect that elevated baseline levels in nestlings were the result of chronic stressors in their environment, consistent with studies of other species (Wasser et al. 1997, Suorsa et al. 2003, Lucas et al. 2006). While we are uncertain of the specific mechanism responsible for the elevated baseline levels in nestling birds, we do not suspect reduced food abundance because of lack of a relationship between body condition and corticosterone levels (Leshyk et al. 2012). Taken together, our results suggest that the physiological response of Ovenbirds to group-selection silviculture is complex and additional research is needed to determine the specific mechanisms behind the observed responses.

Our study was the first to document the effects of group-selection silviculture on stress physiology of birds, and was one of only a handful of studies investigating glucocorticoid changes as a result of logging in general (Wasser et al. 1997, Suorsa et al. 2003, Lucas et al. 2006). Since the publication of our work, the field of conservation physiology, and in particular the application of stress hormones in evaluating wild populations has continued to advance, with a journal fully dedicated to this topic (Conservation Physiology) publishing its first articles in 2013. The development of noninvasive techniques to quantify stress in birds over longer time scales, including the use of feces (hours) or feathers (weeks



Figure 2: Baseline and stress-induced corticosterone levels in adult male Ovenbirds. Stress-induced levels were significantly higher in the intensive group selection treatment than either the typical group selection or control treatments. There was no difference in baseline corticosterone across the treatments. Means and standard error are presented. Redrawn from Leshyk *et al.* 2013.



Figure 3: Baseline corticosterone levels for nestling Ovenbirds were significantly higher in sites cut with the intensive group-selection treatment than the undisturbed (control) treatment. Means and standard error are presented. Redrawn from Leshyk *et al.* 2012. or months) may reduce the potential negative effects of capture, restraint and blood sampling (e.g., Hansen et al. 2016). At a regional level, a number of studies have quantified or experimentally manipulated corticosterone levels in Ontario bird populations (e.g., Hogle and Burness 2014, Ouyang et al. 2015, Madlinger and Love 2016). These studies contribute to the larger body of work exploring the physiology of free-living birds and the circumstances under which environmental variables may affect wild populations. Despite concerns that have been raised about the use of glucocorticoids to infer stress in wild animals (e.g., Dickens and Romero 2013), we argue that when combined with other metrics, measuring glucocorticoids can be an effective way to evaluate the health of wild bird populations, and can offer insight into the physiological and potential fitness effects of natural and anthropogenic stressors.

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Light-logging archival geolocators: opening the door to a new era of songbird migration science Samantha Knight and D. Ryan Norris



Figure 1a. (Above) Light-logging archival geolocator (0.8 g, M-series, British Antarctic Survey) with leg-loop backpack harness.

Figure 1b. (Opposite) Geolocator fitted onto the back of a Tree Swallow using the leg-loop backpack harness. *Photos: Dayna LeClair* 

Science is driven by the generation of new ideas and hypotheses, but technological developments can also open new doors and, in some cases, play a significant role in advancing our understanding of the natural world. The development of the light-logging, archival 'geolocator', we argue, is one such technology that has tremendously advanced our knowledge of songbird migration and will also be important in helping us understand the causes of songbird declines.

Because of their small size (typically < 30 g), songbirds have always presented a challenge for tracking long-distance migrations. Dating back over 100 years, attaching rings or bands to individuals has been the primary method for tracking bird movements (Bairlein and Becker 2011). However, not only are recapture rates of marked birds often <1% (USGS Bird Banding Laboratory 2016), but recovery of marked birds is restricted to a relatively small number of locations around the globe. Although the recent development of GPS-satellite tracking devices has provided the ability to remotely track yearround movements of a wide variety of animals with a high degree of precision (e.g., Bonfil et al. 2005, Alerstam et al. 2006, Mansfield et al. 2009), battery limitations have, thus far, prevented these devices from being light enough for most songbirds. The use of chemical markers, such as stable-isotopes, to estimate the geographic location of where tissue was grown in a previous period of the year has been revolutionary for linking the breeding and non-breeding sites of migratory birds (Chamberlain et al. 1997, Hobson and Wassenaar 1997, Hobson 1999). Stable isotope analysis requires only a small



amount of tissue (typically < 1mg) and, unlike band recovery, individuals only have to be captured once. However, stable isotopes have notable limitations, including providing relatively low spatial resolution and the inability to track continuous movements throughout the year.

By the beginning of the 21st century there was, therefore, a pressing need to find a solution to track the year-round movements of songbirds, and this was when geolocators were first introduced. Light-logging geolocators are based on a simple concept ingeniously engineered into a small archival device (Figure 1). To estimate daily locations, they record just two pieces of information: light levels (solar irradiance) taken at regular intervals (usually every 2 or 10 min) throughout the day, and time (Afanasyev 2004). Longitude is estimated by the time of solar noon or midnight (calculated as the midway point between sunrise and sunset) and latitude is estimated by day length (calculated as the length of time between sunrise and sunset; Afanasyev 2004). Geolocators are 'archival' because data are stored on board the device. Remote download via satellite would require too much power and, therefore, increase size and weight. This means that geolocators must be retrieved the following year to acquire the data and determine an individual's year-round migration. Depending on size, the devices can store approximately 8-12 months' worth of daily location data.

Vsevolod Afanasyev and James Fox, engineers with the British Antarctic Survey (BAS), originally developed geolocators as leg attachments for tracking seabirds (e.g., Weimerskirch and Wilson 2000). In 2006, after discussions with Bridget Stutchbury, a professor at York University, they began to modify geolocators for songbirds. To fit small songbirds, the geolocator needed to be fit as a 'backpack' with a leg-loop harness (Figure 1a; Rappole and Tipton 1991) instead of attached to the leg, as was done in heavier seabirds. A small stalk also had to be designed so back feathers would not cover the light-sensing device (Figure 1b, Figure 2). Using a 1.2 g version of this newlydesigned geolocator, Stutchbury and colleagues published the first study on songbirds that tracked the remarkable year-round migrations of both Wood Thrushes (Hylocichla mustelina) and Purple Martins (Progne subis) from their breeding grounds in northern Pennsylvania to their tropical wintering grounds (Central and South America, respectively) and back (Stutchbury et al. 2009). They showed that geolocators could be used to identify migratory routes, the timing and rate of migration, key stopover sites during fall and spring migration, as well as overwintering sites at a level of spatial resolution never before seen.

The Stutchbury *et al.* (2009) proof-ofconcept study opened the floodgates for new work on migratory songbirds in the following years, including a number of studies on species that breed in Ontario. For Gray Catbirds (*Dumetella carolinensis*; Ryder *et al.* 2011), Swainson's Thrushes (*Catharus ustulatus*; Delmore *et al.* 2012), Purple Martins (Fraser *et al.* 2012), Ovenbirds (*Seiurus aurocapilla*; Hallworth *et al.* 2015) and Barn Swallows (*Hirdundo rustica*; Hobson *et al.* 2015), geolocators have been used to reveal that breeding populations in western North America migrate to distinct, and often widely



Figure 2. A Blackpoll Warbler in Churchill, Manitoba, fitted with a 0.5 g light-logging geolocator (model ML6440, Lotek Wireless) in spring 2016. The 9 mm white light stalk can be seen on the bird's back. *Photo: Christian Artuso* 

separated, wintering areas compared to populations in the east. Using geolocators, it has also been discovered that several species, such as Swainson's Thrush (Delmore et al. 2012), Tree Swallow (Tachycineta bicolor; Bradley et al. 2014), and Barn Swallow (Hobson et al. 2015), cross the Gulf of Mexico during fall migration but migrate around the Gulf in the spring. This is likely due to wind patterns, where a tailwind assists the birds in a fall crossing, while less favourable wind conditions in the spring mean they must take a long detour (Bradley et al. 2014). Geolocators have also been used to demonstrate that male Savannah Sparrows (Passerculus sandwichensis) overwinter at higher latitudes in North America than females and that the further north a male overwinters the sooner he arrives on the breeding grounds to secure a territory (Woodworth et al. 2016). Many unusual and incredible migratory feats have also been uncovered using geolocators. Streby et al. (2015) used geolocators to show that Golden-winged Warblers (Vermivora chrysoptera) that had already reached their Tennessee breeding grounds travelled back south more than 1,500 km to avoid dozens of tornadoes that swept through the region only to return after the storms had passed. In another study, DeLuca et al. (2015) used geolocators to provide the first direct evidence that the 12g Blackpoll Warbler (*Setophaga striata*; Figure 2) flies over the Atlantic Ocean from the Maritimes to the Greater Antilles during fall migration, a distance of over 1,500 km that takes up to three days to complete.

The use of geolocators in the last few years has uncovered many fascinating aspects of songbird migration but what is next? One exciting application is the use of these data to understand songbird population dynamics. With migratory animals, one of the greatest challenges is identifying which period in the annual cycle is driving population declines. Geolocators can be used to help address this problem because they provide unique data on migration routes, stopover sites and wintering areas. Using geolocators, we are now able to extract relevant climate or habitat information from all periods of the year. However, the challenge is even more complicated because individuals from a given breeding population may go to different non-breeding sites, potentially sharing sites with birds from other breeding populations. Migratory birds can, therefore, form complex networks in which breeding and nonbreeding sites are linked through the mixing of individuals between seasons. Describing these migratory networks is of fundamental importance for understanding the causes of decline because events such has habitat loss that occur at one site may reverberate throughout the network (Sutherland and Dolman 1994, Taylor and Norris 2010). Geolocators will play a leading role in helping us describe these networks. For example, Stanley et al. (2015) used geolocators to describe the

connections between multiple breeding and wintering populations of Wood Thrush and to then make recommendations about where conservation efforts would be most effectively focused for this species. Our current work using geolocators to describe the migratory network of Tree Swallows involves a collaboration of over 25 researchers across Canada and the U.S. We now have data from 137 geolocators deployed at 12 breeding sites ranging from Alaska to Nova Scotia, which will provide us with the most comprehensive description of a migratory network of any species to date.

The adoption of light-logging archival geolocators for songbirds has resulted in an incredible opportunity to track individual songbird movements throughout the annual cycle. However, like any method, there are drawbacks. Some, but not all, studies have shown that geolocators can result in reduced survival (Arlt et al. 2013, Gomez et al. 2014, Scandolara et al. 2014) and lower reproductive success (Arlt et al. 2013, Scandolara et al. 2014). Furthermore, because geolocators are archival, we can only obtain data from birds that have survived the entire annual cycle, which means that we gain no information from geolocators on the causes of mortality. New technologies, such as the recently established Motus automated radio telemetry array (see Mackenzie and Taylor article in this issue) and ICARUS (Wikelski et al. 2007), offer promise for tracking both movements and mortality, although their development is still in its infancy.

Regardless of what comes next, there is no doubt that light-logging geolocators have contributed enormously to our understanding of bird migration and will play a central role in helping us determine the causes of songbird declines.

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How biochemical indicators can be used to detect changes in food webs of gulls *Craig Hebert* 

A Herring Gull on colony. Photo: Craig Hebert

In the Laurentian Great Lakes, many factors act together to alter biological communities. These changes can affect the structure of the food web which regulates the flow of energy, nutrients, disease and through contaminants ecosystems. Changes in the amounts and pathways of transfer of these things are important regulators of wildlife populations. For example, changes in bird diets resulting from food web change can affect exposure to biomagnifying environmental contaminants (Hebert et al. 1997) and impact diet quality with resultant effects on bird reproductive success (Hebert et al. 2002). Food web change also appears to be playing a role in the increased mortality of Great Lakes waterbirds due to botulism type E (Hebert et al. 2014).



Figure 1. Location of Herring Gull colonies historically monitored as part of the Great Lakes Herring Gull Monitoring Program. Data shown in Figure 2 were generated from the analysis of egg samples collected from the two sites in red on Lake Huron (Site 5: Double Island, Site 6: Chantry Island).

To fully appreciate the importance of food web change to wildlife, it is essential to have tools that help detect it and determine its ecological significance. One way to do that is through regular monitoring of the diets of predators near the top of the food web. These species reflect change across lower trophic level food web components. Here, I focus on the use of a high trophic level colonial waterbird, the Herring Gull (Larus argentatus), as an indicator of changes in Great Lakes food webs. This species has been monitored annually across all of the Great Lakes since the 1970s as part of the Great Lakes Herring Gull Monitoring Program (GLHGMP) (Figure 1) (Mineau et al. 1984. Hebert et al. 1999a). Biochemical markers of diet were first added to the suite of measurements made as part of this program post-1990 (Hebert *et al.* 1997, 1999b, 2006) but it has been possible to generate data for earlier years using archived samples.

Eggs are collected annually and archived in a frozen state in the National Wildlife Specimen Bank (NWSB) at the National Wildlife Research Centre in Ottawa, Canada. Originally, these eggs were used to track levels of chemical contaminants in the environment and that continues to be an important aspect of the program. However, because portions of these egg samples have been archived in the NWSB since the beginning of the GLHGMP, they also provide a unique resource for evaluating temporal changes in Herring Gull diet. Changes in Great

Lakes food webs that change the availability of prey will be reflected in gull diets. In turn, the diet of gulls can be evaluated by measuring biochemical markers in eggs. These biochemical markers reflect the diet of female Herring Gulls during egg formation as females form their eggs from resources obtained near their breeding colonies. Hence, egg biochemical measurements reflect the availability of different food types in the vicinity of breeding sites and, by measuring these markers, we can gain insights into how and why Great Lakes food webs are changing. We routinely measure two types of dietary markers in eggs: 1) stable isotopes of nitrogen and carbon and 2) fatty acids.

Elements can exist in various stable (i.e., non-radioactive) forms. These are termed isotopes. Stable isotopes of an element have the same number of protons but differ in their number of neutrons. creating differences in the atomic mass of isotopes. Different isotopes of an element are chemically identical but their mass differences cause them to have different kinetic properties. These differences result in fractionation of isotopes during biochemical reactions that are useful in understanding the diets of higher trophic level organisms. For example, the ratio of the heavier <sup>15</sup>N isotope to the lighter <sup>14</sup>N isotope increases with trophic position (e.g., fish occupy higher trophic positions than most alternative foods that gulls may consume) so higher <sup>15</sup>N/<sup>14</sup>N ratios in eggs may reflect a greater proportion of fish in the gull diet. Examining the ratio of the heavier <sup>13</sup>C carbon isotope to the lighter <sup>12</sup>C isotope provides further insights into gull food sources. For example, prey obtained from food webs based on aquatic primary producers, such as phytoplankton, will have lower  ${}^{13}C/{}^{12}C$  ratios whereas food obtained from terrestrial sources will have relatively more of the  ${}^{13}C$  isotope.

Fatty acid composition of eggs provides another way to evaluate changes in gull diets through time. Fatty acids are required for normal growth and development; however, some fatty acids either cannot be synthesized at all or cannot be synthesized with high efficiency in higher trophic level predators. Instead, these "essential" fatty acids are formed by primary producers and are passed up the food chain through consumption. During trophic transfers, prey fatty acid signatures are largely retained in higher trophic level species. In general, aquatic organisms such as fish contain greater amounts of the Omega-3 polyunsaturated fatty acids (PUFAs). In terrestrial organisms, Omega-6 PUFAs are relatively more abundant. Thus, the ratio of Omega-3 to Omega-6 PUFAs can be a useful indicator of the amount of aquatic versus terrestrial food in an organism's diet.

Examining temporal changes in egg nitrogen and carbon isotopes and in egg fatty acid patterns has provided us with the means to detect changes in gull diets over the past four decades and have allowed us to examine the reasons for those changes along with their biological significance. I illustrate these aspects using data from Lake Huron but similar information has been generated for all the sites shown on Figure 1. Biochemical data generated from eggs collected on Lake Huron revealed significant changes in the diets of Herring Gulls through time. Egg stable isotope values are expressed in delta notation ( $\delta$ ) which reflects the ratio of <sup>15</sup>N to <sup>14</sup>N in the egg relative to a standard. Declining  $\delta^{15}$ N values reflected a decrease in gull trophic position, i.e., gulls are not feeding as high in the food web as they used to (Figure 2a) likely indicating a reduction in the amount of fish in the gull diet. Egg carbon isotope values ( $\delta^{13}$ C) also changed through time indicating an increased reliance on terrestrial foods in

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recent years (Figure 2b). Egg fatty acid signatures summarized as egg Omega-3/ Omega-6 ratios also declined indicating an increase in the proportion of terrestrial food in the gull diet through time (Figure 2c). All of these biochemical markers provided corroborating evidence of significant shifts in gull diets through time with an increasing reliance on terrestrial food.

Fish management practices, changes in system productivity and exotic species introductions may be underlying the

Figure 2. Temporal trends in dietary biochemical markers measured in eggs collected from two Herring Gull colonies on Lake Huron:

- a) stable nitrogen isotope  $(\delta^{15}N)$  trends
- b) stable carbon isotope  $(\delta^{13}C)$  trends
- c) Omega 3/Omega 6 fatty acid ratio trends
- d) relationship between annual Omega 3/Omega 6 fatty acid ratios and estimates of prev fish abundance.

Each point represents the annual value generated for one colony. Annual estimates of prey fish relative abundance for Lake Huron provided by the United States Geological Survey. All correlations are significant at p<0.05.

\_\_\_\_\_ 0.95 Conf Int



changes observed in gull diets from Lake Huron (Hebert *et al.* 2008, Paterson *et al.* 2014). For example, the introduction of exotic dreissenid mussels (zebra (*Dreissena polymorpha*) and quagga (*D. bugensis*) mussels) and round goby (*Neogobius melanostomus*) have greatly altered nutrient dynamics, the composition of lower trophic level communities and the spatial distribution of prey. Declines in the availability of prey fish in surface waters of the lake may be largely responsible for shifts in gull diet through time (Figure 2d).

These dietary shifts may be limiting the availability of resources for reproduction in gulls possibly contributing to population declines. Similar factors are likely at work in other lakes (e.g., Lake Superior), where gull populations have decreased substantially. Further research is being conducted to examine this issue but biochemical markers will continue to play an important part in providing the means to assess further dietary change and connect it to larger ecosystem-scale processes.



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