MANAGEMENT BRIEF

**Size and age of Stonecat in Lake Champlain; estimating growth at the margin of their range to aid in population management**

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*Abstract*

Little is known about Stonecat *Noturus flavus* populations, especially in the Northeastern United States, where they are at the edge of their range. In Lake Champlain tributaries, Stonecat are listed as endangered in Vermont, but not in New York. Here we describe the growth of Stonecat in two tributaries to Lake Champlain, one in Vermont (LaPlatte River), which was our primary interest, and one in New York (Great Chazy River), with von Bertalanffy growth models fit to lengths at the times of marking and recapture and to observed length and age data. We also compared growth of Stonecat in these waters to results from other locations near the middle of their distribution. Stonecat in the Great Chazy River were larger at ages 1-3, but smaller at age 5, than Stonecat from the LaPlatte River. Stonecat in Lake Champlain tributaries were generally larger at age than those from the middle of their range, except for those from Lake Erie. From our mean length-at-age results and previous literature estimates of length at maturity for Stonecat, it appears that Stonecat in Lake Champlain reach maturity by age 3, though future research that directly estimates age at maturity would be more informative. These results will help managers assess the effect of various environmental and human stressors that have affected Stonecat in the Lake Champlain basin in recent years. Furthermore, our results expand the literature that lacks information about growth of this species. Finally, our mark-recapture approach to estimating growth of Stonecat can be applied to other species, especially where data are limited because of their status, and in other systems.

Stonecat *Noturus flavus* are widely distributed in the interior of North America, with populations in Vermont at the northeastern edge of their range (Langdon et al. 2006). In 1994, the Vermont Agency of Natural Resources listed the Stonecat as endangered because its known distribution within the state was limited to two tributaries of Lake Champlain: the LaPlatte and Missisquoi rivers (Langdon et al. 2006). There is concern over the continued survival of these populations of Stonecat, especially given the modeling results that support a slightly decreasing population size in the LaPlatte River (Puchala et al. 2016) and only a small population in the Missisquoi River (Puchala 2015). Understanding the life history of a species is important to improve management of that species, particularly when their population abundance is low or is decreasing.

Quist and Isermann (2017) stated that “age and growth investigations are critical for providing information on the basic ecology of a species and guiding management and conservation actions.” This is especially important for species such as Stonecat that are of conservation concern and often understudied (Burr and Stoeckel 2000). We are aware of only five studies, none of which were from the Lake Champlain drainage, that examined growth of Stonecat (Gilbert 1953; Carlson 1966; Paruch 1979; Walsh and Burr 1985; Tzilkowski and Stauffer 2004). The utility of these studies for better understanding the dynamics of Stonecat in Lake Champlain or other populations is limited because they are either quite dated, from populations near the middle of the distribution of Stonecat, or have other concerns such as small sample size, varied methods to estimate age (e.g., pectoral spines, dorsal spines, and vertebrae), and specimens combined across multiple populations.

Our primary objective is to describe the growth of Stonecat in the LaPlatte River. In doing so, we demonstrate an underutilized modeling approach to estimate growth for species where lengths at the times of marking and recapture are available from throughout the growth season, but estimates of age (e.g., from calcified structures) are not available. Secondarily, we compare these results to results from other Stonecat populations to better understand the growth dynamics of LaPlatte River Stonecat. To augment previously published results, we also describe growth of Stonecat from the Great Chazy River, which is a tributary to Lake Champlain in New York. Inclusion of these results allows us to compare the LaPlatte River results to a contemporary population in the same watershed. Our results, along with estimates of survival and population change provided by Puchala et al. (2016), will be an important consideration in the continued management of Stonecat populations in Lake Champlain for long-term stability. These results also contribute significantly to the literature that lacks information about growth of this species, especially from throughout its geographic range. Perhaps more importantly, the approach we use in this data-limited situation, which is not novel but is underutilized, can be applied to other species and systems.

<A>Methods

*Study sites*.— The LaPlatte River is 24 km long, drains a 138 km2 watershed (Pelton et al. 1998), and enters Lake Champlain in Shelburne Bay, Vermont (44.39959N; 73.23385W). The Great Chazy River originates near Ellenburg, New York, and empties into northern Lake Champlain (44.93236N; 73.38537W), is approximately 86 km long, and drains a watershed of 790 km2.

*Data Collection*.— Stonecat were collected from the LaPlatte River from June to October 2012, May to October 2013, and June to October 2014 using backpack electrofishing (DC) and minnow traps. Backpack electrofishing generally used 200 volts, 20-30 Hz, and a 20-40% duty cycle and, because Stonecat are nocturnal, began no earlier than 0.5 h after sunset. Electrofishing effort depended on the length of stream section and ranged from 26 to 247 minutes, with a mean effort of 86 minutes (SD = 49.4). We repeatedly sampled two 200-m long sections over the three-year period, and on two occasions, we sampled the entire 1.2 km of river between them. Minnow traps were 42 cm long and 23 cm diameter with 2.5 cm openings at each end and 0.6 cm square meshed sides. Minnow traps were set overnight (18-24 h soak time) in gangs of three or four attached to a single weight. Details of the study sections are in Puchala et al. (2016).

Captured Stonecat, not experiencing obvious distress (i.e., swimming normally), were anesthetized in a 100 mg/L concentration of tricaine methanesulfonate (MS-222). Each individual was measured for TL to the nearest mm and all Stonecat approximately 90 mm TL and greater had a passive integrated transponder (PIT) tag (134-kHz, 8.4 x 1.4 mm; Biomark, Boise, Idaho) inserted into the peritoneal cavity through a 2-mm incision in the upper abdominal wall. The slit was then treated with iodine. Individuals were examined for the presence of a PIT tag after the first sampling event. Spines were not removed from these fish to minimize the traumatic impact of removal on other aspects of our overall study (Puchala et al. 2016).

Stonecat were collected from the lower 33 km of the Great Chazy River on 17-19 October 2012 as mortalities from a 3-trifluoromethyl-4-nitrophenol (TFM) lampricide treatment conducted on 16-18 October 2012. During the post-treatment assessment, teams of two biologists each visually scanned the banks, shallows, and portions of the river where the bottom was visible to collect non-target mortalities, including Stonecat. Additional Stonecat were collected from the Great Chazy River on 8-9 August 2011 and 15 November 2011 as part of a bioassay study (Calloway 2012) and frozen as quickly as possible. After XX months, Stonecat were thawed and measured for standard (SL) and total lengths (TL) to the nearest mm. For aging purposes, the dorsal spine was removed from each individual by snipping it just above the articulation point (Buckmeier et al. 2002; Manny et al. 2014; Fischer and Koch 2017).

Spines were placed in boiling water to remove excess skin and flesh and allowed to dry before being set in epoxy, largely following the procedures of Koch and Quist (2007) but with plastic straws similar to the procedure of Bauerlien et al. (2018). One or two 0.5-mm sections were cut from the spine using a BuehlerTM low-speed isomet saw (Buehler, Lake Bluff, Illinois). Thin sections were glued to slides for viewing under an Olympus SZX9TM dissecting microscope using fiber optic transmitted light. Mineral oil was used to help with clarity of the structure. Three readers blind to fish size independently estimated age by identifying annuli in the patterns of translucent and opaque zones of the sectioned spine. The three readers attempted to reach a consensus age if there were discrepancies among their estimated ages. If a consensus could not be reached then the fish was removed from further analysis.

*Data analysis.*— Growth of Stonecats collected from the LaPlatte River could not be summarized with a typical growth model because age for these fish could not be estimated. Rather we summarized growth of Stonecats from the LaPlatte River with a von Bertalanffy growth function (VBGF) modified by Francis (1988a) for use with mark-recapture data and including a seasonal component:

where

and *ΔL* is the change in TL between marking and recapture, *tm* and *tr* are the marking and recapture times (years), *Δt* is the change in time (years) between marking and recapture, *Lm* is the TL at *tm*, *g1* and *g2* are parameters that represent the mean annual growth rate or increment at *L1* and *L2* (which we chose to be 100 and 150 mm, respectively), *w* is a parameter that represents the time of year when the growth rate is maximum, and *u* is a parameter that describes the extent of the seasonal variation in growth (i.e., *u* = 0 represents no seasonal variability in growth). For fish that were recaptured multiple times, we treated each interval between recaptures as independent mark-recapture (M-R) events (Ogle et al. 2017). For example, if a fish was captured three times then we considered the interval from marking to the first recapture as one M-R event and the interval from the first to second recapture as a separate M-R event. Mark-recapture events based on observations within 7 d of each other were excluded from further analysis under the assumption that any growth that occurred in this short period was minimal and likely less than measurement error. We modeled a seasonal component to growth with these data because fish were collected on many dates within each year such that times-at-large might span different parts of the growing season.

Growth of Stonecat collected from the Great Chazy River was summarized with the traditional VBGF (Beverton and Holt 1957):

where *Lt* is the observed TL at time (or age) *t*, *L*∞ is the asymptotic mean TL, *K* is the Brody growth coefficient, and *t0* is the theoretical time when the mean TL is zero (Ogle et al. 2017). We used fractional ages in this model to adjust for our fish being collected at various times throughout the growing season (Ogle et al. 2017). We assumed that annual growth on the spine commenced on June 1, as shown for vertebrae by Carlson (1966), and was completed by November 1. Thus, the adjusted age was equal to the number of observed annuli for fish collected before June 1, was one more than the number of observed annuli for fish collected after November 1, and was the number of observed annuli plus the fraction of the growing season completed for fish captured between June 1 and November 1. We chose not to use a growth function with a seasonal component (e.g., Somers 1988) for fish collected from the Great Chazy River because sampling dates were concentrated on only a few days in a year such that a seasonal component would not be well estimated.

Both growth models were fit with the nls() function in R v3.5.1 (R Core Team 2018) using the “port” algorithm. The *g1*, *g2*, and *u* parameters were constrained to be positive and the *w* parameter was constrained to be between 0 and 1. All other parameters were unconstrained in model fitting. Three different starting values and two other algorithms (Gauss-Newton in the nls() function and Levenburg-Marquardt in the nlsLM() function from the minpack.lm package v1.2-1 [Elzhov et al. 2016]) were used to determine the robustness of parameter estimates to starting values and model fitting algorithms (Ogle et al. 2017). Bootstrap confidence intervals for model parameters were estimated from 999 bootstrapped samples using the nlsBoot() function from the nlsTools package v1.0-2 (Baty et al. 2015) as described in Ogle (2016).

Parameter estimates could not be compared between the LaPlatte and Great Chazy rivers because different growth models were required for each location. Thus, we compared growth between locations by predicting mean lengths-at-age. Mean lengths-at-age were predicted directly from the traditional VBGF for Stonecat from the Great Chazy River. However, mean lengths-at-age cannot be predicted directly from the Francis model because it does not use age as a variable. In this case, we estimated the mean length at age 1 from monthly length frequency histograms of all Stonecat captured in the LaPlatte River. We then used the results from the Francis model to predict the annual growth increment for fish of this length. This predicted annual growth increment was added to the mean length at age 1 to predict the mean length at age 2. This process was repeated until mean lengths for all ages up to age 6 had been predicted. We also compared predicted mean lengths-at-age for Stonecat from the LaPlatte and Great Chazy rivers to mean lengths-at-age reported for Stonecat in the literature (Gilbert 1953; Carlson 1966; Paruch 1979). Some of the literature results were converted from SL to TL using results from a linear model fit to our measurements of SL and TL on fish collected from the Great Chazy River.

<A>Results

A total of 1,469 Stonecat were collected in the LaPlatte River, of which 1,311 were PIT tagged. These fish ranged in length from 54 to 205 mm, with a mean of 131 (SD = 24.3) mm. Our gears collected few fish under 90 mm, but some collections of these fish provided insight into first year growth of Stonecat in the LaPlatte River (Figure 1). The mode of small fish in September 2012 indicates that LaPlatte River Stonecat are approximately 55-80 mm near the end of their first year. The mode of small fish in May 2013 and the lower mode of the bimodal distribution in June 2012 suggested that age-1 fish in the LaPlatte River begin their second growing season at approximately 70-80 mm TL.

Of the 133 Stonecat recaptured from the LaPlatte River, 111 fish (83%) were recaptured once, 20 fish (15%) were recaptured twice, and 2 fish (2%) were recaptured three times. Thus, 157 paired M-R events were observed, though 9 (6%) of these were within 7 days of each other and were removed from further analysis. Of the remaining 148 M-R events, 61% of recaptures were in the same year as the original capture, 39% were in the following year, and 1% were two years later (Figure 2). Stonecat recaptured from the LaPlatte River ranged from 87 to 185 mm TL at marking, with a mean of 131 (SD = 20.5) mm. Parameter estimates (with 95% confidence intervals) from fitting the modified VBGF to the LaPlatte River Stonecat (N=177) are 34.2 (32.6 – 35.7) mm for *g1* at *L1* = 100 mm, 18.0 (16.6 – 19.4) mm for *g2* at *L2* = 150 mm, 0.55 (0.52 – 0.58) y for *w*, and 2.52 (2.25 – 2.87) for *u*.

Of the 183 Stonecat from the Great Chazy River aged from spines, six (3%) were removed from further analysis because the three readers could not agree on a consensus age. Age-classes ranged from 0 (young-of-the-year) to 5 with most fish age 0 (49%) and only five fish (3%) age 4 or older. Stonecat from the Great Chazy River ranged from 44 to 193 mm TL, with a mean TL of 114 (SD = 41.5) mm. The SL-TL relationship was TL=1.239+1.166SL (r2 = 0.996). Parameter estimates (with 95% confidence intervals) from fitting the traditional VBGF to the Great Chazy River Stonecat were 172 (160 - 193) mm for *L*∞, 0.79 (0.52 - 1.16) y-1 for *K*, and 0.13 (-0.15 – 0.34) y for *t0* (Figure 3).

Stonecat from the LaPlatte River were slightly smaller than those from the Great Chazy River for the first three years, similar for the fourth year, and slightly larger for the fifth year of life. Stonecat from the Lake Champlain tributaries were approximately the same size as Stonecat from Lake Erie at age 1 (Figure 4), but substantially smaller for LaPlatte River fish by age 2 and fish from both tributaries after age 2. Stonecat from the Lake Champlain tributaries were longer at all ages than Stonecat from other populations reported in the literature (Figure 4).

<A>Discussion

Stonecat in the Lake Champlain tributaries, at the northeast margin of their distributional range, may grow faster than Stonecat from streams in the middle part of their distribution, but not from those in Lake Erie. Gilbert (1953) suggested that Lake Erie Stonecat may exhibit exceptional growth because of the availability of mayfly *Ephemeroptera sp.* nymphs as prey. We do not know why Stonecat in Lake Champlain grow faster than many of those in the middle of their range, but it is plausible that density dependence has a role; i.e., lower abundance of Stonecat in Vermont especially would contribute to individual fish growing faster. Regardless, knowing how fast individuals grow is important for agencies to be able to predict and manage populations for sustainability.

We found it difficult to identify annuli, especially near the central lumen, on sectioned spines from the Great Chazy River fish. Other authors have noted similar difficulties. Gilbert (1953) commented on difficulties identifying the first annulus on Stonecat vertebrae and Tzilkowski and Stauffer, Jr. (2004) noted that annular rings were often not discernible on Stonecat pectoral spines. Given similar growth curves between the LaPlatte River (derived independent of any calcified structure) and the Great Chazy River (derived from spines) Stonecat, we feel that our age estimates from spines are reasonable. Similarly, our growth estimates from capture-recapture data appear reasonable, which demonstrates that this method may be used to assess growth for populations of Stonecat where calcified structures cannot be collected. Nevertheless, future Stonecat age and growth studies would benefit from understanding the precision and accuracy (i.e., validity) of various methods for assessing age of Stonecat.

Our results contribute to a better understanding of the general growth dynamics of Stonecat. However, they are also immediately relevant to management of fish populations in these Lake Champlain tributaries for several reasons. For example, in 2012, the single area known Stonecat below the Swanton became dewatered and several dead Stonecat were recovered from under rocks (Puchala 2015). Very low water levels and high temperatures have occurred in the LaPlatte River after our study was completed. Another consideration is that the LaPlatte River was first treated with TFM in 2016, two years after our last sampling, to control larval Sea Lamprey *Petromyzon marinus* numbers. Bioassay results on Stonecat between 122 and 200 mm TL indicated that 10% mortality could occur at a TFM concentration of 1.2 times that needed to kill 99% of the larval Sea Lamprey present (Calloway 2012). The predominance of age-0 fish in our Great Chazy River samples, which largely came from mortalities collected following a TFM treatment, suggests that this mortality may primarily affect age-0 fish. Tributaries to Lake Champlain are treated with TFM on a rotating basis every four or more years, which could pose a risk for Stonecat populations if most fish matured after age 4. In Pennsylvania, female Stonecat matured at 102-141 mm SL, or approximately 120-166 mm TL (Tzilkowski and Stauffer, Jr. 2004). These lengths correspond to age-2 to age-4 Stonecat in the LaPlatte River. Thus, it appears that most female Stonecat in the LaPlatte River would mature within the minimum TFM treatment interval. While this result is useful to managers planning TFM treatments in Lake Champlain tributaries, future research that directly estimates age at maturity would be informative.

Stonecat in the Lake Champlain drainage have been subjected to a variety of environmental and human stressors in recent years. For example, several Stonecat were found dead after a dewatering event in Swanton (Puchala 2015) and the endangered population of Stonecat in the LaPlatte River were exposed to very low water levels and high temperatures in 201X-201X, the xxx years immediately after our sampling was completed. Our results contribute to a better understanding of the growth dynamics of Stonecat, which may aid managers in understanding the effects of these stressors on Stonecat populations. As a specific example, the LaPlatte River was first treated with TFM in 2016, two years after our last sampling, to control larval Sea Lamprey *Petromyzon marinus* abundance. Bioassay results on Stonecat between 122 and 200 mm TL indicated that 10% mortality could occur at a TFM concentration of 1.2 times that needed to kill 99% of the larval Sea Lamprey present (Calloway 2012). The predominance of age-0 fish in our Great Chazy River samples, which largely came from mortalities collected following a TFM treatment, suggests that this mortality may primarily affect age-0 fish. Tributaries to Lake Champlain are treated with TFM on a rotating basis every four or more years, which could pose a risk for Stonecat populations if most fish matured after age 4. In Pennsylvania, female Stonecat matured at 102-141 mm SL, or approximately 120-166 mm TL (Tzilkowski and Stauffer, Jr. 2004). These lengths correspond to age-2 to age-4 Stonecat in the LaPlatte River. Thus, it appears that most female Stonecat in the LaPlatte River would mature within the minimum TFM treatment interval. While this result is useful to managers planning TFM treatments in Lake Champlain tributaries, future research that directly estimates age at maturity would be informative.

Here, with Stonecat from the LaPlatte River, we demonstrated the use of a growth model with mark-recapture data collected across much of the growing season. While this model has been used in other studies (e.g., Francis 1988b; Wilde and Sawynok 2005; Afeworki et al. 2014), it is used rarely and those studies have been primarily with marine species that were largely of commercial or recreational interest. We showed that the model can be used to derive useful estimates of growth rates and growth model parameters for an imperiled species for which individuals cannot be legally or ethically sacrificed to remove calcified structures for estimating age. This approach may be useful to others studying imperiled species under similarly constraining conditions.

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**Figure headings**

Figure 1. Histograms of total length of Stonecat captured in the LaPlatte River by month during 2012-2014. Note that no sampling occurred in May or August 2012, June 2013, or May 2014. Collections from October are not shown because sample sizes were small.

Figure 2. Histogram of times-at-large for Stonecats captured and recaptured from the LaPlatte River, Vermont in 2012-2014. Each bar in the histogram is fourteen days wide. Note that nine mark-recapture events where the time between marking and recapture was less than seven days are not included.

Figure 3. Fit (solid line), with 95% confidence bounds (dashed lines), of the traditional von Bertalanffy growth function to total lengths and ages estimated from spines of Stonecat collected from the Great Chazy River, New York in 2011 and 2012. Ages have been adjusted to represent the number of observed annuli on the spine plus the fraction of growth completed in the year the fish was collected. Observations are plotted with a semi-transparent color such that darker points represent more observations.

Figure 4. Mean total lengths-at-age for the two locations of this study (LaPlatte R. [VT)] and Great Chazy R. [NY]) and for four previous studies (Lake Erie (OH) [Gilbert 1953], Wisconsin streams [Paruch 1979], Vermillion River (SD) [Carlson 1966], and Ohio streams [Gilbert 1953]). The LaPlatte River results were predicted from the fit of the von Bertalanffy growth function modified by Francis (1988) assuming a mean length at age 1 of 75 mm. The Great Chazy River results were predicted from the fit of the traditional von Bertalanffy growth function. The results from the other locations were either observed or back-calculated lengths-at-age.



Figure 1.



Figure 2.



Figure 3.



Figure 4.