Dietary Niche and Growth Rate of the Nonnative Tubenose Goby
(Proterorhinus semilunaris)

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This thesis is dedicated two ways:

To my mother, who taught me the importance of self-sacrifice for a worthy cause;

And to my father, who taught me to be the man in the arena.

Abstract

Basic life history knowledge is essential to determine if a nonnative species is potentially invasive. The nonnative tubenose goby *(Proterorhinus semilunaris)* arrived in the Great Lakes in the 1990s via ship ballast water, but remains poorly studied within North America, making it difficult to predict its effects on native ecosystems.

Diet and growth patterns were examined from age-0 tubenose gobies within the estuary of the St. Louis River, a tributary to Lake Superior near Duluth, Minnesota. Dietary breadth and growth rates have important ramifications for survival, competitiveness, and dispersal ability of a fish species, influencing its potential success as an invasive species. I sampled tubenose gobies from shallow vegetated habitat via beach seine during summer and fall periods, and removed otoliths and aged fish on daily increments for growth modelling. Furthermore, stomach contents were identified and weighed to provide measures of fitness and dietary breadth between seasons (fall vs. summer) and between several locations within the estuary.

Results suggest a low dietary breadth that overlaps with tadpole madtom (*Noturus gyrinus*); tubenose goby diet was mostly comprised of Crustacea, regardless of location or season. Growth modelling demonstrates that tubenose gobies reach a small maximum size and are short-lived, indicating an *r*-selected life history. The Gompertz function is preferred for modelling the growth of this species.

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<u>Introduction</u>

Invasive species are considered one of the most significant ecological threats of the 21st century (Kohler and Courtenay 1986; Burr et al. 1996). As of 2016, over 180 aquatic nonnative species have been documented in the Laurentian Great Lakes, and the introduction rate is estimated as 1.3-1.8 new species per year (Sturtevant et al. 2016). Many of these nonnative species have caused extensive ecological and economic damage, such as declines in biodiversity and shifts in primary productivity (Mills et al. 1993; Vanderploeg et al. 2002; Pagnucco et al. 2015). The Great Lakes display high connectivity and have multiple vectors of introduction for new species, such as through ballast water from international shipping vessels or via constructed canals such as the Saint Lawrence Seaway (Mills et al. 1993). Among the most recent nonnative fishes to be introduced to the Great Lakes is tubenose goby, a small (<130 mm) demersal fish from the Black and Caspian Seas of Europe.

Tubenose goby is one of 24 neogobiine species from the Ponto-Caspian region; the subfamily includes four genera (Neilson and Stepien 2009a). Several other neogobiine fish – monkey goby *(Neogobius fluviatilis)*, racer goby *(N. gymnotrachelus)*, bighead goby *(N. kessleri)*, and round goby (*N. melanostomus*) – have successfully invaded freshwater systems in eastern and central Europe. To date, only round goby and tubenose goby are known to inhabit the Great Lakes region. Round goby demonstrates an opportunistic diet and rapid rate of growth, allowing it to reproduce quickly and interact aggressively with other species (Bergstrom and Mensinger 2009). These traits have been

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attributed as primary causes for its success (MacInnis and Corkum 2000), and have resulted in competition with several demersal species for food and habitat resources, particularly mottled sculpin (*Cottus bairdi*), logperch (*Percina caprodes*), and northern madtom (*Noturus stigmosus*) (French and Jude 2001). Round goby also predates upon native fauna (Jude et al. 1995) and exhibits nest predation upon species such as smallmouth bass (*Micropterus dolomieu*), walleye (*Sander vitreus*), and lake sturgeon (*Acipenser fulvescens*) (Corkum et al. 2004; Steinhart et al. 2004; Roseman et al. 2006).

Tubenose goby were first discovered in 1990 in Lake St. Clair (Jude et al. 1992) and subsequently spread to Lake Erie, Lake Superior, Lake Huron, and Lake Ontario (United States Geological Survey 2018). They likely spread from the lower Great Lakes to Lake Superior via shipping vessels (Hensler and Jude 2007; Dopazo et al. 2008). A resident population exists at the mouth of the St. Louis River (Kocovsky et al. 2011; Grant et al. 2012), a tributary to Lake Superior at its southwestern corner, near Duluth, MN. The St. Louis River population possesses low genetic variability (Neilson and Stepien 2009b), indicating a high probability that this population was introduced from a single event (Kocovsky et al. 2011). Among tubenose goby populations throughout the Great Lakes, however, there is higher genetic diversity than within the St. Louis River population, suggesting multiple founding sources (Stepien and Tumeo 2006). Furthermore, like round goby, tubenose goby larvae exhibit diel vertical migration, a significant factor affecting dispersal success for both species (Hensler and Jude 2007; Janáč et al. 2013).

It remains unclear why the tubenose goby has played a relatively minor role as an "invasive" species compared to the round goby (Dopazo et al. 2008), although it has generally been regarded as a lesser threat to native species than round goby due to its smaller size, lower rate of aggression and dispersal, more localized distribution, and confinement to macrophytic habitat (Jude et al. 1995; Vanderploeg et al. 2002; Dopazo et al. 2008). The two species share a close phylogenetic relationship, and both are widespread in their native Ponto-Caspian range (Pinchuk et al. 2003), although the tubenose goby is more restricted than the round goby (Dillon and Stepien 2001). Round goby and tubenose goby have often been found to inhabit similar habitats in European systems (Vašek et al. 2014; Mikl et al. 2017); however, it appears that within the Great Lakes tubenose goby prefers shallow vegetated habitat in coastal wetlands (Peterson et al. 2011; Ramage 2017), unlike the round goby, which prefers rocky rip-rap habitat. This habitat distinction is significant because coastal wetlands in the Great Lakes are essential spawning and nursery areas for native fish (Sierszen et al. 2012).

Wide dietary breadth is a characteristic of most invasive vertebrates (Sakai et al. 2001), and is a top predictor of success and range during the establishment phase of invasive fish species (García-Berthou 2007; Pettitt-Wade et al. 2015). Predation by invasive fish can additionally have cascading trophic consequences, leading to the decrease of benthic invertebrates or zooplankton (Gallardo et al. 2015) and the out-competition of native fishes, even when invasive fish are non-piscivorous (Pyke 2008; Strayer 2010). Ponto-Caspian gobiids (racer goby, monkey goby and round goby) have a broad diet and opportunistic feeding strategy in invaded areas of Central Europe

(Grabowska and Przybylski 2015). A broad diet and aggressive feeding strategy are also key aspects of successful round goby dispersal in the Great Lakes (Leino and Mensinger 2016). Knowledge of diet breadth and composition for the tubenose goby is essential for predicting its impacts on native species; however, little research exists from the Great Lakes to show whether the tubenose goby shares the round goby's opportunistic diet within this system (French and Jude 2001).

Published studies characterizing the diet of tubenose goby are primarily from European waterbodies. Among studies, both the diet composition and the degree of opportunism varies. Adámek et al. (2010) found that tubenose goby diet was dominated by Chironomidae larvae and a species of Isopoda, with minor representation of various zooplankton. Other groups of invertebrates, including insects (Trichoptera, Ephemeroptera), are of varying importance and tubenose goby diet is generally characterized as opportunistic (Adámek et al. 2010; Vašek et al. 2014; Všetičková et al. 2014). Tubenose goby also exhibit diet plasticity between seasons and locations, but fish eggs have not been identified as a significant diet component despite occasional occurrence (Vašek et al. 2014; Všetičková et al. 2014). Within the Great Lakes, tubenose goby consume small insects and crustaceans (Kocovsky et al. 2012) as well as small amounts of round goby eggs (French and Jude 2001). Their diet overlaps with rainbow darter (*Etheostoma caeruleum*) and northern madtom; furthermore, tubenose goby may also compete with mottled sculpin, logperch, and Johnny darter for food resources. No diet studies have been conducted for this species within Lake Superior.

In addition to diet, rapid somatic growth rate is another important factor for the successful establishment of nonnative fishes in the Great Lakes (García-Berthou 2007) and is characteristic of most successful invasive species (Sakai et al. 2001). A rapid growth rate is associated with early reproductive maturity and high fecundity, increased prey accessibility, predator avoidance, and increased ease of mobility and dispersal (Beverton and Holt 1957; Jones 2002). Therefore, estimates of growth rates for nonnative species are essential for determining their potential as an invasive species. Despite extensive research examining the growth rates for the closely-related round goby (MacInnis and Corkum 2000; Gruľa et al. 2012; Thompson and Simon 2015), to date there have been no growth rate estimates for tubenose goby. In some studies from European waterbodies, researchers examined tubenose goby cohort structure; however, it is important to note that these analyses were based on extrapolating age from size, in which tubenose goby of 1+ year were assigned to fish >40 mm standard length (Janáč et al. 2012; Všetičková et al. 2014). Estimates of maximum lifespan vary within the literature, ranging from 2 years (Všetičková et al. 2014) to 4-5 years (Leslie et al. 2002).

Age-at-length data can be obtained from individual fish via the examination of otolith increments. Otoliths are crystalline structures that are components of the hearing mechanism in fishes, formed by the periodic deposition of aragonite (inorganic) calcium carbonate upon an organic protein matrix. The sequential deposition of these "growth rings" in an otolith corresponds with the rhythmic growth pattern of the individual fish on both a daily and an annual basis (Gauldie and Nelson 1990; Morales-Nin 2000). Upon removal, the examination of these otoliths can be used to provide age-at-length data of

individual fish. Age-at-length data can then be used in modelling approaches to describe somatic growth. The Von Bertalanffy and Gompertz functions are two of the most popular and common growth models for fish.

Diet composition and breadth is an essential aspect of life history that influences both establishment success and impact from a potentially invasive species. Growth rate is also an essential aspect of life history for the establishment of nonnative fish species, influencing aspects of survival and dispersal. However, literature regarding tubenose goby diet has produced inconsistent results, and is limited within the Great Lakes. Growth of this species has also been studied in a limited capacity, and age-based analyses have relied upon categorization by size. Therefore, the purpose of this study is to characterize tubenose goby diet composition and growth rate in the St. Louis River estuary. I hypothesize that tubenose goby is an invasive species which displays an opportunistic diet and a high likelihood of dietary competition with native species in the Lake Superior region. Furthermore, I hypothesize that tubenose goby will display high spatial and seasonal diet plasticity, which are traits common to invasive fish species. Finally, I hypothesize that tubenose goby will demonstrate aspects of growth that are characteristic of an *r*-selected species, such as a rapid growth rate and a relatively small maximum size. These characteristics are commonly attributed to successful nonnative species. I will utilize field collections and laboratory processes to examine these hypotheses.

Methods

Study and Site Locations

The St. Louis River estuary (SLRE) is a coastal wetland that is situated at the mouth of the second-largest tributary to Lake Superior. Within the SLRE is the Duluth-Superior harbor, which is the largest port by cargo tonnage in the Great Lakes (O'Malia et al. 2018). The SLRE is home to over 40 resident fishes (Peterson et al. 2011) and is also an "invasion hotspot" due to a high amount of shipping traffic (Grigorovich et al. 2003); in 2011, it was home to at least 10 invasive species of fish (Peterson et al. 2011). The SLRE is over 4,850 hectares in size and contains many wetlands that provide essential nursery areas for a variety of fish species (Breneman et al. 2000; Hoffman et al. 2010; Peterson et al. 2011). Habitats that occur within the estuary include shallow flats (<3 m; either vegetated or unvegetated), as well as dredged and undredged channels (Leino and Mensinger 2016). Bottom substrates are both soft (clay, sand, and organic material) and hard (rip-rap and sheet-pile). Shoreline development is variable: in the lower estuary, around the harbor, the shoreline is extensively developed, whereas in the upper estuary, much of the shoreline remains unmodified (Leino and Mensinger 2016). Clarity is generally low throughout the estuary owing to high concentrations of colored dissolved organic matter and local inputs and resuspension of clay (Peterson et al. 2011).

Tubenose goby were first discovered in the Great Lakes in 1990 and in the SLRE in 2001 (United States Geological Survey 2018). By 2006, they had become abundant and widespread in littoral habitat (Peterson et al. 2011) of the system, where they

dominate the assemblages of fish young-of-year (Ramage 2017). However, they remain uncommon in bottom trawl surveys conducted in the river channel (Leino and Mensinger 2016). Tubenose goby have been shown to associate strongly with shallow areas of abundant and diverse submergent vegetation within the SLRE (Ramage 2017).

I selected several locations within the SLRE for sampling based on habitat and prior tubenose goby occurrence (Ramage 2017). Subsequent sampling found consistent occurrence of tubenose goby at only two of the proposed locations, and we selected these locations for continued targeted sampling. Indian Point Campground (IPC) and Lower Pokegama (LPK) are similar embayments that are both found within tributary-influenced vicinities (Kingsbury Creek and the Little Pokegama River). Both are sheltered bay areas with low current, have substrates composed of both organic material and clay, and have shallow (<3 m) heavily-vegetated flats dominated by yellow pond lily (*Nuphar variegata*), coontail (*Ceratophyllum demersum*), pondweed (*Potamogeton sps.*), milfoil (*Myriophyllum sps.*), water celery (*Vallisneria americana*), and waterweed (*Elodea sps.*).

Field Sampling and Preservation

We sampled fish via beach seine (7.6 m long by 1.2 m tall; 0.48 cm mesh) equipped with a bag (1.2 m deep by 1.8 m wide by 1.2 m tall) and weighted bottom rope. We targeted shallow (<1 m) flats with dense, submergent vegetation for sampling because this habitat has the highest density of tubenose goby within the SLRE (Ramage 2017). We used an active sampling gear to minimize digestion of prey items but recognize that beach seine efficiency can be reduced when sampling in vegetation.

Electrofishing is impractical in the SLRE owing to high turbidity and because tubenose

goby lacks a swim bladder. The transect distance was approximately 20 meters, with

flexibility for complex underwater obstructions and variable depth structure. We

conducted seine pulls counter to wind direction and processed the net contents on the

boat. Summer sampling (July 30-Sep. 7) began after the emergence of visible aquatic

vegetation and concluded prior to the first frost; fall sampling (Oct. 1-Oct. 17) occurred

after the first frost of the year and concluded prior to complete senescence of aquatic

vegetation. We sampled each of our two sites three times for each season, and during the

morning for diet consistency (08:00-12:00). We allowed at least five days to pass

between sampling at the same site to allow for fish recolonization and to ensure diet

independence among sampling events.

We visually sorted seine contents and identified and counted all individual fish

(*Lepomis* spp. were not differentiated); tubenose goby and tadpole madtom were

euthanized via MS-222 and retained. I made a small incision in the body cavity of each

euthanized fish and preserved specimens in 95% ethanol (EtOH). We did not collect

mottled sculpin ($n = 0$), Johnny darter ($n = 1$), logperch ($n = 0$), or round goby ($n = 0$) in

sufficient numbers to enable dietary comparisons.

We collected surface water and habitat quality data at the beginning of transects.

Turbidity (NTU), pH, dissolved oxygen (mg/L and % saturation), temperature (ºC), and

specific conductivity (μS/cm) were quantitatively measured at 0.5 m depth (Hydrolab

HL4 multiparameter sonde, OTT Hydromet, Loveland, CO). Prior to the field season,

EPA personnel calibrated the sonde following the step-wise instructions of the operating manual (2014).

At each seine pull, the field crew visually assessed the vegetation cover. Visible cover density was assigned a value ranging from 0 (no vegetation present) to 4 (dense; plants cover most of the sampling area), and we noted predominant taxa present. We sampled substrate via PONAR dredge and assigned a primary class from gravel, sand, silt, clay or organic composition. At each site, we deployed HOBO Pendant (UA-002-08) loggers attached to fenceposts at 2 feet below the surface of the water, which recorded water temperature over the course of the season (measurements taken every hour and averaged for a daily temperature).

Gut Content Processing

In the laboratory, I measured whole preserved fish for total length ($\pm$ 0.5 mm) and wet weight ($\pm$ 0.001 g). Tubenose gobies >20 mm TL (n = 144) were sexed (Guellard et al. 2015; Valová et al. 2015), used for dietary analysis, and dissected for otolith removal. I identified sex via visual gonadal examination (n = 142). For both tubenose goby and tadpole madtom, I removed the cardiac stomach. I weighed full cardiac stomachs ("guts"; $\pm$ 0.001 g) prior to and following content removal and determined eviscerated fish weight by subtracting the weight of the full gut from that of the undissected fish. Prior to weighing, I blotted fish and guts, and allowed them to dry for thirty seconds. I removed stomach contents and sorted them into 11 possible groups consistent with European literature (Všetičková et al. 2014); I then derived wet weight of gut contents for each fish

by subtracting the weight of the empty gut from that of the undissected gut. Finally, I removed sagittae and lapilli otoliths from tubenose goby for growth analysis.

I assessed dietary composition proportion by dry weight. All tubenose goby or tadpole madtom from each site- and date-specific sample were pooled for analysis; the number of individual fishes in each diet pool ranged from 7 to 15. I examined pooled gut contents under a dissecting scope and separated them into 11 categories: Annelida, zooplankton (primarily Cladocera), benthic Crustacea (Amphipoda, Isopoda, and Ostracoda), Ephemeroptera, Trichoptera, Chironomidae larvae, Chironomidae pupae, other Diptera, terrestrial insects, fish eggs and fry, 'other' invertebrates, and detritus (not included in dietary analysis). I placed categorized gut contents in small single-use tin trays (pre-weighed; handled with forceps only) and dried them at 55°C for 24 hours prior to weighing (± 0.001 mg). Diet analysis followed identical procedure for tubenose goby and tadpole madtom.

Fragments of Isopod and Amphipod remains, the dominant forage item, were DNA sequenced to determine species. I preserved samples in 95% non-denatured ethanol and shipped them to the United States Environmental Protection Agency laboratory in Cincinnati, Ohio. DNA was extracted using a DNeasy Blood and Tissue Kit and eluted with 100 µL of sterile water. Mitochondrial DNA genetic marker COI locus (B/R5) was PCR amplified with forward and reverse primers; PCR thermal cycling was then applied, and products were dual indexed and then metabarcoded on the Illumina Miseq sequencing platform.

I used tadpole madtoms ($n = 36$) and a subset of tubenose gobies ($n = 98$) for diet rarefaction. I visually estimated the number of individual prey items per category for each dissected stomach, and used EstimateS software to produce a rarefaction curve for each fish species with a Chao1 95% Upper Boundary Confidence Interval (Colwell 2013). A rarefaction curve is the relationship between the average number of cumulative species captured for a given sampling effort. The curve is usually linear when sampling effort is low, and reaches an asymptote as increasingly rare species require increasing effort to capture. The attainment of an asymptote in such a curve indicates thorough sampling of the prey species community. Chao1 is a nonparametric abundance-based estimator that provides an upper boundary 95% confidence interval of the species richness (assuming that the lower confidence bound cannot be less than the observed number of species); a relatively small value of Chao1 further indicates adequate sampling for diet representation.

Lengths and weights of fish change when preserved with EtOH (Leslie and Moore 1986). To account for this, I conducted a preservation experiment: ten tubenose gobies in a range of sizes (28.0-59.0 mm) were collected and measured for length ($\pm$ 0.5 mm) and weight ($\pm$ 0.001 g) prior to preservation. I then immersed individuals in 95% EtOH for 32 days and remeasured them, giving an average percent change of weight and length. Tubenose gobies retained 95.68% ($\pm$ 1.44% SD, $n = 10$) of their original length and 67.38% ($\pm$ 4.29% SD, $n = 10$) of original weight after preservation (this large weight difference is likely due to the loss of water content found in fresh fish). Calculated fresh

length and weight data were used for growth analyses, while preserved length and weight were used for the Gut Fullness Index.

Gut Content Analysis

For each digestive tract, I assessed food bulk with an index of gut fullness (GFI). This was calculated as a ratio between preserved wet diet weight (w; calculated by subtracting the weight of the empty gut lining from the weight of the full gut) and eviscerated preserved fish weight (W_{evi}; calculated by subtracting the weight of the full gut from the weight of the undissected fish):

$$GFI = 10^4 * (w/W_{evi})$$

Welch's Two-Sample t-test, which is robust with respect to unequal variance, was used to compare GFI between groups (species, season, site, size). Fish with empty guts were included in the analysis. I first power-transformed GFI data by 0.6 to better meet assumptions of normality. For analysis based on size, I sorted tubenose goby into two size categories. Previous literature has used 40.0 mm SL to differentiate between small and large tubenose goby (Janáč et al. 2012; Všetičková et al. 2014; Valová et al. 2015); I used the same length for consistency (40 mm SL was found to approximate 50 mm TL).

I calculated percent representation of each dietary item (pooled; $\%W_i$) as a relative percentage dry weight (Hyslop 1980). W_i is the weight of diet item i from all tracts and $\sum W_i$ represents the total weight of all dietary items:

$$\%W_i = 100 * (W_i/\sum W_i)$$

I used Levins' Index (B) to quantify dietary niche breadth (Levins 1968):

$$B = 1/\sum p_i^2$$

where p_i represents the proportion of fish diet (pooled for each sampled date-site combination) made up of diet category i. This index produces a value between 1 and n (for this study, $n = 11$ categories). Low values indicate a low level of dietary opportunism (diet being dominated by few prey categories), while high values indicate a high level of opportunism and a more diverse diet. I used a nonparametric Kruskal-Wallace test to compare between groups (species, site, season) for significant differences.

I used Schoener's Index (S) to quantify dietary overlap between species, seasons, and locations (Wallace 1981):

$$S = 1 - 0.5 \sum |p_{xi} - p_{yi}|$$

where p_{xi} and p_{yi} represent the proportions by weight of prey category i in the diets of species x and y. Values range from 0 (complete dissimilarity) to 1 (identical composition of diet). Values of $S > 0.6$ are considered to indicate biological significance and likely food competition between species (Wallace 1981; Vašek et al. 2014) or, alternatively, high similarity of diets between sites or seasons.

Weight-Length Relationship

As increasing variance in weight occurred with an increase in length, heteroscedasticity exists within the data. I used a power function with a multiplicative error term to model the length-weight relationship for tubenose goby:

$$W = a(L^b)(e^\varepsilon)$$

where W = average weight, a and b are constants, L = average length, and e^{ε} is the multiplicative error term. I also transformed this model to a linear model by taking the natural logarithms of both sides:

$$\ln(W) = \ln(a) + b * \ln(L) + \varepsilon$$

Otolith removal, polishing, and aging

Round and tubenose goby are closely related species that share similar otolith structure (Neilson and Stepien 2009b), and the occurrence of daily otolith increments and their use in aging round goby has been validated (MacInnis 1997). Otoliths have been used to successfully age round goby in the Great Lakes (MacInnis and Corkum 2000; Huo et al. 2014; Duan et al. 2016).

I removed sagittae and lapilli pairs of otoliths from tubenose goby and used lapilli for aging after I determined that they provided more discernible increments. This concurred with MacInnis and Corkum (2000), who found that lapilli provided clearer increments than sagittae in round goby. I polished and examined otoliths from a subset of tubenose goby (n = 52) that encompassed the range of sizes of fish sampled. After removal, I affixed lapilli on a glass slide and polished them on one side with lapping paper (0.01-3.0 micron) to reveal daily growth increments. I then coated the polished otolith in immersion oil and examined it via transmitted light microscopy before photographing for aging.

Two readers independently assigned an age to each fish, and we averaged those that were within 5 days (n = 22); those individuals that differed >5 days (n = 36) were

mutually discussed for the assignment of an agreed-upon age consensus. Fish that could not be reconciled between readers ($n = 6$) were removed from the dataset, so that I used a total of 52 tubenose goby for growth modeling.

Growth Modeling

The Von Bertalanffy Growth Function (VBGF) and the Gompertz Growth Function are two of the most common models used for fish somatic growth. I used both functions to model tubenose goby length-at-age, using the FSA and nlstools packages in Rstudio (Ogle et al. 2018). Residual plots suggested an increase in variability with increasing fitted values. This growth depensation is a cause of data heteroscedasticity (that the variability increases with age of fish), a common phenomenon with fish length-at-age data. Therefore, I modified the typical model parameterizations to assume a multiplicative error structure rather than an additive error structure.

I obtained starting values for the VBGF using polynomial regression and then applied them via nonlinear least squares to fit the model. I then used typical non-linear estimation techniques with the data on the natural log scale to estimate model parameters:

$$L_t = L_\infty\left(1 - e^{-K(t-t_0)}\right)e^\varepsilon$$

where L_t = average length-at-age (days), L_∞ = asymptotic average length, K = the exponential rate of approach to the asymptotic length ("Brody Growth coefficient"), t_0 = a model artifact that is said to represent the "average age when the average length is equal to zero", and ε = the error of the model.

I also fit the Gompertz growth function using the FSA package in R (Ogle et al. 2018). I found starting values by iteratively superimposing a curve of the function at chosen parameter values onto a scatterplot of the data, and then applied these via nonlinear least squares to parameterize the model. I modified the typical Gompertz parameterization to assume multiplicative error structure rather than additive error structure, and used typical non-linear estimation techniques with the data on the natural log scale to estimate model parameters:

$$L_t = L_\infty e(-e^{-g_i(t-t_i)})e^\varepsilon$$

where L_t = average length-at-age (days), L_∞ = asymptotic average length, g_i = the instantaneous growth rate at the inflection point, t_i = the age (days) at the inflection point, and ε = the error of the model. I calculated 95% confidence intervals via bootstrapping (1000 iterations).

Because all tubenose goby aged were age-0, I also used a simple linear regression to illustrate growth, as has been used elsewhere for age-0 fish (Steinhart et al. 2004; Oyadomari and Auer 2008):

$$L_t = (age\ at\ hatching) + G*(days) + \varepsilon$$

where L_t = average length-at-time and G = overall daily growth rate (mm/day). Finally, to account for heteroscedasticity, I performed a simple linear regression on the natural log-transformed data:

$$\ln(L_t) = \ln(a) + b*\ln(days) + \varepsilon$$

where L_t = average length-at-time (days), $\ln(a)$ = the intercept, and b = slope. This model can also be simplified to its exponential form:

$$L_t = e^{(a + b(days) + \varepsilon)}$$

I allowed the three models based upon natural-log transformed data (VBGF, Gompertz, linear) to compete using Akaike's Information Criterion (AIC) in RStudio; this model selection approach balances the goodness of fit with model simplicity for selection of the most favorable model, indicated by the lowest AIC value.

Mortality

I estimated tubenose goby mortality within the first year of life via catch curve regression, using the number of individuals captured at various daily ages. For each captured fish with length data ($n = 209$), I estimated age using the Gompertz growth function. I then plotted the estimated age (in five-day increments) against the natural log of catch-at-age. The ascending left limb of the plot represents age-classes that are not yet fully vulnerable to the gear; these age classes are not useful for mortality estimation. The descending right limb represents the decline of fully-recruited individuals in the population; thus, I calculated instantaneous total mortality (Z) by applying linear regression to all age classes older than and including the age with the maximum catch (Ogle 2016). This is illustrated via the following linear equation:

$$\ln(C_t) = \ln(vN_0) - Zt$$

where C_t = catch-at-age, v = a constant proportion of the population that is "vulnerable" to the fishery, N_0 = initial population size, and Z = instantaneous total mortality. Therefore, the negative slope of the linear regression between t (x-axis) and $\ln(C_t)$ is a measure of the instantaneous total mortality rate. However, practical interpretation of

instantaneous mortality rate is difficult; therefore, I converted Z to daily mortality rate (*D*) via the equation:

$$D = 1 - e^{-Z}$$

and to an estimated annual mortality rate (*A*) via:

$$A = 1 - e^{-Z(365)}$$

Results

Fish Community

I captured a total of 3,592 fish at the two sites, of which 216 were tubenose goby (5.99% of total fish; Fig. 1). Other common fish groups included sunfishes (bluegill, pumpkinseed, and black crappie; 81.49%), golden shiner (3.48%), yellow perch (3.06%), and spottail shiner (2.53%). Tubenose goby was the second most common fish and the most common demersal species. Tadpole madtom comprised 1.03% of total fish ($n = 37$), while only one Johnny darter and one bullhead (*Ameiurus* sps.) were sampled. Other sparsely represented fishes (all <1.5% dominance) included rock bass, largemouth bass, northern pike, walleye, common carp, and white sucker. No round goby was captured at either location.

Diet Between Species

143 tubenose gobies were used for diet composition analysis. The most common prey item was benthic Crustacea, which composed 94.62% of the diet by dry weight (Table 1, Fig. 2). I analyzed a subset of samples ($n = 5$ pools; 58 fish) and found that

among the benthic Crustacea, Isopoda comprised 65.07% of the dry weight, and

Amphipoda and Ostracoda comprised 32.44% and 0.06% respectively. A subsample of

benthic Crustacea tissue was sequenced, revealing the genera *Gammarus*, *Proasellus*, and

Caecidotea to occur in the diet. Ephemeroptera, Trichoptera, Chironomidae larvae, and

zooplankton (mostly Cladocera) were also present, although at considerably lower

proportions than benthic Crustacea. Chironomidae pupae (1 individual; 0.15% of total

diet weight) and other Diptera (1 individual; 0.16% of total diet weight) were the least

commonly consumed prey items, while fish eggs or fry, terrestrial insects, Annelida, and

other invertebrates were not found in any guts. Detritus (sand, wood) was not included in

diet proportions; overall, detritus was uncommon and composed only 0.24% of total

stomach material. Overall Levins' Index (*B*) was 1.12 (of a maximum of 11), and average

overall Gut Fullness Index was 194.41 (range: 0.00-656.93, Fig. 3). A two-tailed, two-

sample Welch's t-test on power-transformed data did show a significant difference

between large ($\hat{y}$ = 160.50) and small ($\hat{y}$ = 205.30) tubenose goby for GFI (p = 0.03, α =

0.05; Fig. 4).

 Tadpole madtom were less numerous than tubenose goby, with 37 collected over

the course of the study (36 used for diet analysis). This native species displayed similar

diet proportions to tubenose goby: benthic Crustacea formed the majority of the diet

(84.36%), while Ephemeroptera (1.52%), Trichoptera (3.84%), Chironomidae larvae

(3.93%), and zooplankton (6.26%) contributed small amounts (Table 1, Fig. 2). Although

an overall Levins' Index of 1.39 indicates tadpole madtom has a broader diet niche than

tubenose goby, statistical significance is precluded due to an insufficient number of

sample pools containing tadpole madtom. Dietary overlap between tadpole madtom and tubenose goby was high ($S = 0.891$), and a two-tailed, two-sample Welch's t-test on power-transformed data showed no significant difference for average GFI between species ($\alpha = 0.05$, p = 0.08; Fig. 4).

A rarefaction curve from tadpole madtom guts ($n = 36$) and a subset of tubenose goby guts ($n = 98$) estimated 6 prey categories to be present for each fish species (Fig. 5), with a Chao1 95% Upper Boundary Confidence Interval of ~8 prey groups (tubenose goby) and ~7 prey groups (tadpole madtom). Both curves demonstrated approach to an asymptote and a small confidence interval via Chao1, indicating thorough sampling of the prey community.

Diet Between Seasons

I was unable to locate and capture tubenose goby in the spring, precluding inclusion in seasonal dietary comparison. Tubenose goby summer and fall diets were each primarily composed of benthic Crustacea, and Schoener's Index returned a high degree of overlap between seasons ($S = 0.885$; Table 1). However, this dependence on benthic Crustacea increased from 86.32% of diet in summer to 97.65% in fall, with an accompanying decrease in percentage of other prey categories (Table 1; Fig. 2). Levins' Index differed between summer ($B = 1.36 \pm 0.22$; $n = 6$) and fall ($B = 1.05 \pm 0.07$; $n = 6$) via nonparametric Kruskal-Wallace (p = 0.01, $\alpha = 0.05$, df = 1), indicating a greater dietary breadth in the fall. Furthermore, Welch's t-test indicates that overall GFI decreased from summer to fall (p = 0.02; Fig. 4, Fig. 6).

Diet Between Sites

Indian Point Campground (IPC) and Lower Pokegama (LPK) displayed high similarity and overlap in diet between sites ($S = 0.972$), with benthic Crustacea composing >93% of diet at both locations (Table 1, Fig. 2). Levins' Index values of dietary breadth did not differ (p = 0.26, $\alpha = 0.05$, df = 1) between IPC ($B = 1.25 \pm 0.20$; $n = 6$) and LPK ($B = 1.16 \pm 0.26$; $n = 6$), and a Welch's t-test returned no significant difference ($\alpha = 0.05$, p = 0.17) in GFI between IPC ($\hat{y} = 174.26$) and LPK ($\hat{y} = 211.50$).

Growth and Age Characteristics

Tubenose goby total length ranged from 11.0 mm to 74.0 mm, with a median size of 39.0 mm TL ($n = 209$). Of the fish with visible gonads, 59.86% were female and 40.14% were male (Fig. 7). Average size was significantly different between summer ($\hat{y} = 30.6 \pm 10.7$; $n = 133$) and fall ($\hat{y} = 51.9 \pm 7.7$; $n = 76$) via Welch's t-test (p < 2.2e-16, $\alpha = 0.05$). Fresh wet weight ranged from 0.012-4.711 g with a median of 0.530 g. Aged >20 mm fish ranged from 34 to 111 days ($n = 52$), demonstrating that all tubenose goby sampled were age-0 fish. I subtracted the estimated ages from capture dates to find a median hatch date of June 19 for the 2018 season, with a hatch date range from June 2 to July 29 (Fig. 8).

Fresh total length (± 0.5 mm) was strongly correlated with wet weight (± 0.001 g) of tubenose goby ($n = 209$). As is commonly observed, variability in weight

(heteroscedasticity) increased as fish length increased. Transformation was used to stabilize this variance, and following natural log transformation the data showed a significant linear relationship (Fig. 9; $r^2 = 0.99$, p < 0.00001):

$$\ln(W) = -12.724 + 3.297 \ln(L) + \varepsilon$$

which was then used to produce parameters for the weight-length allometric relationship:

$$W = (2.979 * 10^{-6}) L^{3.297}$$

with the constant a equal to $2.979*10^{-6}$ and the constant b equal to 3.297 (± 0.02 SE; 95% confidence intervals of 3.252-3.342), indicating allometric growth for this species (Fig. 10; df = 205; p < 0.00001).

Von Bertalanffy Growth Function

The best-fit Von Bertalanffy Growth Function had the following form:

$$L_t = 109.90 \, (1 - e^{-0.01(t-13.72)}) \, e^{\varepsilon}$$

where $L_\infty = 109.90$ (± 31.18 SE; 95% conf. int. = 77.72-641.95), $K = 0.01$ (± 0.005 SE; 95% conf. int. = 0.001-0.02), and $t_0 = 13.72$ (± 4.71 SE; 95% conf. int. = 0.77-21.30). 95% confidence intervals were calculated via bootstrap methods (1000 iterations). Therefore, average asymptotic length is estimated to be 109.9 mm, the Brody growth rate coefficient is estimated as 0.01 days^{-1}, and the modeling artifact "age at length zero" is estimated as 13.7 days (Fig. 11).

Gompertz Growth Function

The best-fit Gompertz growth function was as follows:

$$L_t = 78.84e\,(-e^{\,-0.03(t\,-\,43.77)})\,e^{\,\varepsilon}$$

where L_t = length (TL; mm) of the animal at age t; L_∞ = 78.84 ($\pm$7.76 SE; 95% conf. int.

= 67.97-101.58); g_i = 0.03 ($\pm$0.01 SE; 95% conf. int. = 0.02-0.04); t_i = 43.77 ($\pm$ 3.49 SE;

95% conf. int. = 39.00-54.62); e = natural logarithm base; and ε = random error

associated with each length. 95% Confidence Intervals were calculated via bootstrapping

(1000 iterations). Therefore, the maximum average asymptotic length is estimated as 78.8

mm; the rate of maturity (per day) is estimated at 0.03; and the number of days of

maximal growth rate (i.e., age at the inflection point) is estimated as 43.8 days (Fig. 12).

Linear Growth Function

The simple linear regression of overall daily growth rate (Oyadomari and Auer

2008) is as follows:

$$L_t = 4.06 + 0.618t$$

where L_t = length-at-age (days), 4.06 = size at hatching (mm), 0.618 = the overall daily

growth rate (mm/day), and t = the age of the fish in days (Fig. 13; r^2 = 0.84; n = 52).

However, because of heteroscedasticity within the length-age data, a linear regression

was also performed on the natural log-transformed data to account for unequal variance,

and parameterized as:

$$\ln(L_t) = 2.82 + 0.014\ln(t) + \varepsilon$$

where L_t = length at time (days), 2.82 = the intercept, and 0.014 = the slope (Fig. 13; r^2 = 0.82; n = 52). This model can also be simplified to its exponential form:

$$L_t = e^{(2.82 + 0.014(t) + \varepsilon)}$$

The three natural log transformed models were allowed to compete for best fit of the data via AIC and were selected in the order of Gompertz (-75.83), VBGF (-72.28), and natural log-linear regression (-49.75). Simple linear regression should not be compared via AIC due to its lack of transformation.

Mortality

The relationship between the natural log of catch-at-age and time (daily age) is shown as a longitudinal catch curve (Fig. 15). This linear relationship (r^2 = 0.86) is represented by:

$$\ln(C_t) = 4.73 - 0.036t$$

which gives an instantaneous mortality rate of 0.036 (±0.003 SE). This then gives a daily mortality rate (D) of 0.0350 and an estimated first-year mortality rate (A) of 0.9999.

Discussion

Tubenose goby displays a Crustacea-dominated diet that differs from European systems and that overlaps with at least one native species. A young age structure within the St. Louis River population suggests high mortality within the first year of life.

This is the first study to quantitatively model tubenose goby growth, and one of few studies in the Great Lakes to evaluate its diet. This study reveals considerable dietary and growth differences from what have been recorded in European literature, indicating lifestyle flexibility for this species; combined with an *r*-selected growth pattern and a diet that heavily overlaps with at least one native species, the tubenose goby should be considered as potentially invasive. System-specific research is important for evaluating impacts on new locations from potentially invasive species. This discussion will examine the findings and implications of tubenose goby diet, and then growth patterns, in the St. Louis River estuary.

Diet

Tubenose goby exhibits high dietary overlap with at least one native species, a common characteristic of invasive species. Tubenose goby also exhibited seasonal diet plasticity, a common attribute of invasive fish. However, I did not find locational diet plasticity or an opportunistic diet, and egg predation was not observed in any sample.

Excluding the minor occurrence of some sand and wood (likely incidentally consumed with prey), tubenose goby diet was exclusively composed of invertebrate animals, and predominantly composed of benthic Crustacea (Isopoda and Amphipoda).

Annelida (Hirudinea and Oligochaeta; 0% of diet), Chironomidae larvae, Trichoptera and

Ephemeroptera were by comparison much less common in stomach contents, despite

being relatively common within the St. Louis River estuary area (Breneman et al. 2000)

and observed in tubenose goby diets in other areas. Literature from Europe has previously

documented consumption of benthic Crustacea by tubenose goby (Shorygin 1939), but

usually of lesser importance than Chironomidae (Adámek et al. 2007; Adámek et al.

2010), Trichoptera, Ephemeroptera (Vašek et al. 2014), or zooplankton (Všetičková et al.

2014). Benthic Crustacea, especially Amphipoda, have been found to be important in

tubenose goby diets in the St. Clair River in Michigan (French and Jude 2001) as well,

indicating regional diet flexibility and opportunism for this species. This is unsurprising

given the habitat differences between European and Great Lakes populations of this

species, as habitat type is strongly related to the available prey types.

Tubenose goby and tadpole madtom both had a low Levins' Index (Table 1),

indicating a low degree of opportunism and a relatively narrow prey selection. The high

value of Schoener's Index between tadpole madtom and tubenose goby ($S = 0.891$)

indicates that dietary competition is likely between species, although this likelihood may

be reduced if these species display different foraging strategies (French and Jude 2001).

The lack of significant difference in gut fullness between species (Fig. 2) may indicate

that food availability is not yet a limiting factor or that neither species displays a clear

competitive advantage. Slimy sculpin (*Cottus cognatus*), spoonhead sculpin (*C. ricei*),

logperch, Johnny darter, bullheads (*Ictalurus* spp.) and channel catfish (*I. puncatus*) are

other native benthic species in the St. Louis River estuary (Leino and Mensinger 2016).

Sculpins, darters, and logperch have all been implicated as likely to be affected by resource competition from tubenose goby within the Great Lakes (Jude et al. 1995; French and Jude 2001; Kocovsky et al. 2011; Ramage 2017), but their lack of detection in this study suggests that this threat may be reduced in the St. Louis River due to different habitat preferences or life history strategies. Slimy sculpin numbers have been at historically low levels in the SLRE since 1989 (Leino and Mensinger 2016), and although logperch and Johnny darters have been variably abundant in the soft substrate of the SLRE in past years, they have been recorded to prefer less-vegetated and sandier habitat than tubenose goby in the Great Lakes (Lane et al. 1996).

Seasonally, diet overlap was high between summer and fall ($S = 0.885$) and benthic Crustacea dominated tubenose goby diet in both seasons (Table 1, Fig. 1). However, both gut fullness and dietary breadth did significantly decrease from summer to fall as benthic Crustacea increased in importance. The St. Louis River estuary is a northern system relative to the majority of the tubenose goby's range, and water temperature was drastically lower during the fall sampling period than that of the summer (Fig. 4). It is possible that tubenose goby may have had a more difficult time foraging as vegetation began to senesce and vegetation-dependent prey items become less available, although these prey items were also likely easier to target as vegetation cover decreased. Isopoda increases in importance to tubenose goby diet during cold portions of the year (Adámek et al. 2010), although the same study found no significant difference for gut fullness during the summer and winter.

Sampling locations within the St. Louis River estuary displayed very similar dietary composition, with a high overlap ($S = 0.972$) and no significant difference for dietary niche breadth or gut fullness. This similarity between sites does support specialist tendencies of tubenose goby, which contrasts with European literature that establishes a strong locational effect indicating high feeding opportunism (Všetičková et al. 2014). However, it should be noted that both sites were relatively similar in habitat and possibly in prey availability.

Although tubenose goby diets were dominated by benthic Crustacea at both of my sampled locations, a lack of ambient prey density data prevents the ability to distinguish whether benthic Crustacea were preferentially selected or more abundant within the environment. Crustacea were anecdotally observed at high abundance during sampling, and several species of Amphipoda and Isopoda (including several species from DNA-identified Gammaridae and Assellidae) are highly abundant within high-macrophyte cover habitat (Tall et al. 2008). Combined withF the relatively wide range of preferred prey that is documented within existing literature, tubenose goby diets may be opportunistic despite my results, and this species may be exploiting a highly abundant prey source within coastal wetlands of the St. Louis River estuary.

It is possible that diet composition results were biased by variable rates of digestion: hard tissues (i.e., Crustacea exoskeleton and Chironomidae heads) may persist in guts longer than predominantly soft-tissue organisms (i.e., eggs and zooplankton). To minimize bias, fish sampling was conducted with active gear and consistently during morning hours. Additionally, feeding experiments have shown that even soft items such

as fish eggs remain identifiable in deepwater sculpin (*Myoxocephalus thompsonii)* and slimy sculpin stomachs within 3-5 days after feeding (Mychek-Londer et al. 2013). I did not attempt to examine 24-hour feeding within the scope of this study; data from sample collection consistently represents feeding during the night and morning, rather than assessing differences between diurnal versus nocturnal feeding.

Fish eggs were not present in any sampled stomachs. This concurs with European literature, which has found egg predation by tubenose goby to be insignificant (Adámek et al. 2010; Vašek et al. 2014; Všetičková et al. 2014). However, it should be noted that each of these studies focused on sampling tubenose goby from stony rip-rap habitat with only rare vegetation. There are many fish species within the Great Lakes that make use of vegetated coastal wetland habitat for spawning and nursery areas (Sierszen et al. 2012), and tubenose goby have been shown to strongly associate with these vegetated areas in the Great Lakes. Furthermore, tubenose goby in the St. Clair River, Michigan were found to consume eggs (French and Jude 2001). I was unable to capture tubenose goby prior to July 30, and therefore did not sample during the spring time period when a majority of fish spawning occurs.

Growth

Hatch dates ranging from June 2-July 29 (Fig. 8) concur with literature that has observed a protracted spawning period for this species in the Great Lakes (Leslie et al. 2002). However, the absence of age 1+ fish within samples indicates that older fish are

rare in this system and lifespan is relatively short; this is supported by an estimated daily mortality rate of 0.035 and a mortality rate of 0.9999 in the first year of life (Fig. 15).

Sampling in mid-channel habitat of the estuary via trawl has not historically produced large numbers of tubenose goby (Peterson et al. 2011; Leino and Mensinger 2016); furthermore, post-establishment sampling did not capture tubenose goby via electrofishing or trawling gear (although some were sampled via fyke net), and found that tubenose goby were confined to shallow littoral habitat (Peterson et al. 2011). Thus, it is unlikely that the lack of older fish is due to differential habitat preferences or gear type bias. Seasonal migrations and predation have been suggested in other systems as causes for a decrease in winter abundance of this species (Kocovsky et al. 2011; Valová et al. 2015). Given the persistence of tubenose goby in the St. Louis River estuary despite a scarcity of age 1+ fish, it is likely that tubenose goby reaches sexual maturity within one year; indeed, visible gonads were distinguished in tubenose goby of 2-3 months old. Rapid growth and attainment of sexual maturity are important characteristics of successful invasive fish species and are also traits of an *r*-selected life history.

Several aspects of growth differed between pre-existing literature conclusions and the results of this study. European literature (Janáč et al. 2012; Všetičková et al. 2014) has assigned fish of >40 mm SL (approximately 50 mm TL) an age of 1+; however, otolith analysis from this study has aged fish of that size as age 0+. Furthermore, the lack of age 1+ fish found during sampling indicates that maximum lifespan may be less than in other systems, where it has been estimated to be as high as 4-5 years (Leslie et al. 2002).

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Tubenose gobies displayed allometric growth ($b = 3.297$; Fig. 9), indicating that weight increases disproportionally to length as fish age. This is unsurprising because strictly isometric growth is relatively rare in fish, and because the round goby has been observed to display a similar growth coefficient of $b = 3.261$ (MacInnis 1997).

Although the Gompertz Growth Function was selected as more favorable than the Von Bertalanffy Growth Function, the difference in AIC score was relatively small. Average maximum size was estimated as 78.84 mm (Gompertz) and 109.90 mm (VBGF); however, no obvious asymptote is apparent in the data due to the lack of adult fish in the system. The L_∞ parameter is meaningful only in populations with a sufficiently low mortality to enable fish to reach maximum size (Francis 1988), and this parameter may thus be underestimated by the models. These estimates of L_∞ better represent the seasonal average maximum size after the first growing season in the St. Louis River estuary.

Although the transformed linear growth function (Fig. 13) was selected as the least favorable via AIC, the untransformed model still had an $r^2 = 0.84$. This model is useful because it provides estimates of several important biological parameters: a length of 4.06 mm ($\pm$ 2.71 SE) at hatch, and an overall growth rate of 0.62 mm/day during the growing season for age-0 tubenose goby. This size at hatch is similar to reported appearances of just-hatched tubenose goby (5-6 mm) in the St. Clair River system (Leslie et al. 2002). This model also allows for comparison with growth rates of age-0 fish of other species, which are often derived from simple linear regression and measured as mm per day (Steinhart et al. 2004; Höök et al. 2007; Oyadomari and Auer 2008). However, it

should be noted that many of these studies continue sampling only into the end of the summer growing season. This makes direct comparison difficult with tubenose goby from this study; unsurprisingly, growth rate decreased during the latter part of the growing season as water temperature decreased and tubenose goby approached the seasonal growth asymptote (Fig. 14). Overall, age-0 tubenose goby displays a growth rate that falls within the range of other sampled species within the Great Lakes (Table 3).

The Gompertz model is the most useful for predicting length-at-age of age-0 tubenose goby. This is unsurprising; although linear and log-linear models are helpful for assessing biological parameters of larval fish, these models are primarily useful for the earliest stage of life prior to the inflection point in growth rates as fish age. The Von Bertalanffy Growth Function, although it has an output similar to the Gompertz Growth Function, is not often a suitable model for age-0 fish (Jones 2002). Furthermore, the Von Bertalanffy Growth Function was determined unsuitable for modeling growth of the closely related round goby (Gruľa et al. 2012) and monkey goby (Plachá et al. 2010) in European systems.

The tubenose goby displays characteristics of an *r*-selected species in that it reaches a small maximum size, grows quickly, experiences high annual mortality, and likely reaches sexual maturity within the first year of life. Many of these attributes are important factors for successful establishment of introduced species (Sakai et al. 2001; García-Berthou 2007), and suggests that tubenose goby has a high chance of establishment if introduced to new suitable environments. Vegetation corridors have been hypothesized to act as avenues of dispersal for this species (Kocovsky et al. 2011) and the

lack of such vegetation in many areas of the Great Lakes may explain the hitherto limited dispersal of this species from areas of initial introduction.

Fisheries managers may wish to consider the potential effect of an invasive benthic fish that prefers shallow wetland areas, which are vital for recruitment and production of many native fish species throughout the Great Lakes. In many areas of the Great Lakes, including the St. Louis River estuary, restoration of coastal wetland areas is a priority for multiple agencies and stakeholders. Such restoration projects are rarely evaluated for their effects on the dispersal of nonnative species, and the ecology of nonnative species is rarely a significant factor in the decision-making process of habitat modification (Jude and Deboe 1996). Facilitation of tubenose goby dispersal – and the resulting trophic effects of dietary competition and potential egg predation - should be considered by agencies as an unintended consequence of increasing shallow wetland habitat connectivity within the Great Lakes. In addition to exercising caution with the implementation of wetland restoration projects, managers should also consider pre- and post-restoration monitoring to determine further effects of such projects on the dispersal and proliferation of tubenose goby.

Conclusions

The tubenose goby demonstrates life history traits (rapid growth and early maturity) that indicate it is likely to establish widely throughout the wetland habitat of a new system if introduced. Previous literature has documented that the tubenose goby displays different habitat preferences between Europe and in the Great Lakes, where it is

found exclusively in shallow vegetated areas (Kocovsky et al. 2011; Peterson et al. 2011; Ramage 2017). This study demonstrates that tubenose goby also displays dietary differences, with a much greater dependence on benthic Crustacea than in European systems (Adámek et al. 2010; Vašek et al. 2014). These differences make it difficult to extrapolate characteristics of invasive fish, and demonstrate the necessity for system-specific research to accurately assess the threat of a new species where it has established.

Tables

Table 1. Mean ± SD total length (mm; calculated fresh), wet weight (g; calculated fresh), total number of tubenose goby (TNG) sampled, and number of individuals that were used for sex, size (small = 20-50 mm TL, large = >50 mm TL), and site (IPC = Indian Point Campground, LPK = Lower Pokegama). Only tubenose gobies >20 mm were used for categorical analysis (n = 144; two were damaged and not used for sex or size analysis).

Season	TL (mm)	Weight (g)	TNG	Sex		Size		Site	
				Male	Female	Small	Large	IPC	LPK
Summer	30.6 ±10.7 (n = 133)	0.36 ±0.46 (n = 133)	133	36	30	57	9	26	42
Fall	51.9 ±7.7 (n = 76)	1.5 ±0.92 (n = 76)	83	21	55	40	36	40	36
Total	38.3 ±14.1 (n = 209)	0.785±0.871 (n = 209)	216	57	85	97	45	66	78

Table 2. Diet category proportions (%W_i), Levins' Index (B; average from replicates with ± SD), and Schoener's Index (S). %W_i was calculated as percent of total dry weight (dried at 55°C for 24 hrs.) after weighing (± 0.001 mg). P-values for Levin's Index were calculated via nonparametric Kruskal–Wallis test from replicates of site-date samples (n = 6 / category for site & season).

Prey Category	Tubenose Goby	Tadpole Madtom	Summer TNG	Fall TNG	IPC TNG	LPK TNG
Crustacea	94.62	84.36	86.32	97.65	93.92	95.29
Ephemeroptera	1.96	1.52	5.06	0.82	1.45	2.44
Trichoptera	1.24	3.84	3.10	0.56	1.76	0.74
Chiron. larvae	0.68	3.93	1.43	0.40	0.79	0.58
Chiron. pupae	0.15	0.08	0.00	0.20	0.30	0.00
Other Diptera	0.16	0.00	0.61	0.00	0.00	0.32
Zooplankton	1.19	6.26	3.48	0.36	1.78	0.63
Annelida	0.00	0.00	0.00	0.00	0.00	0.00
Terrestrials	0.00	0.00	0.00	0.00	0.00	0.00
Eggs	0.00	0.00	0.00	0.00	0.00	0.00
Other Invert.	0.00	0.00	0.00	0.00	0.00	0.00
Levins' (B)	1.12	1.39	1.36 ±0.22	1.05 ±0.07	1.25 ±0.20	1.16 ±0.26
B p-value	insufficient n		0.01		0.26	
Schoener's (S)	0.891		0.885		0.972	
n of fish used	144	36	68	76	66	78

Table 3. Growth rates of age-0 tubenose goby, alewife, and three native species from within the Great Lakes region. All growth rates were calculated as mm/day from wild-caught fish; however, discrepancies in environmental conditions and sampling dates between studies demand a general, rather than direct, comparison.

[1](Höök et al. 2007) [2](Jude, unpublished data) [3](Oyadomari and Auer 2008) [4](Steinhart et al. 2004)   [5](Ney and Smith 1975)

Species	Growth Rate (mm/day)	Study system	Sampling season
Tubenose Goby	0.62	St. Louis River, MN	30 July – 17 Oct. 2018
Alewife (*A. pseudoharengus*)	[1]0.85-0.91	Lake Michigan	15 June – 15 July 2001 - 2002
	[2]0.80-0.90	Lake Michigan	1989 – 1992
Cisco (*C. artedi*)	[3]0.18	Lake Superior	15 May – 20 June 2000
Smallmouth Bass (*M. dolomieu*)	[4]0.58-1.20	Lake Erie	July – September 1940's – 2001
Yellow Perch (*P. flavescens*)	[5]0.72	Red Lake, MN	1 July – 20 August 1952 - 1967
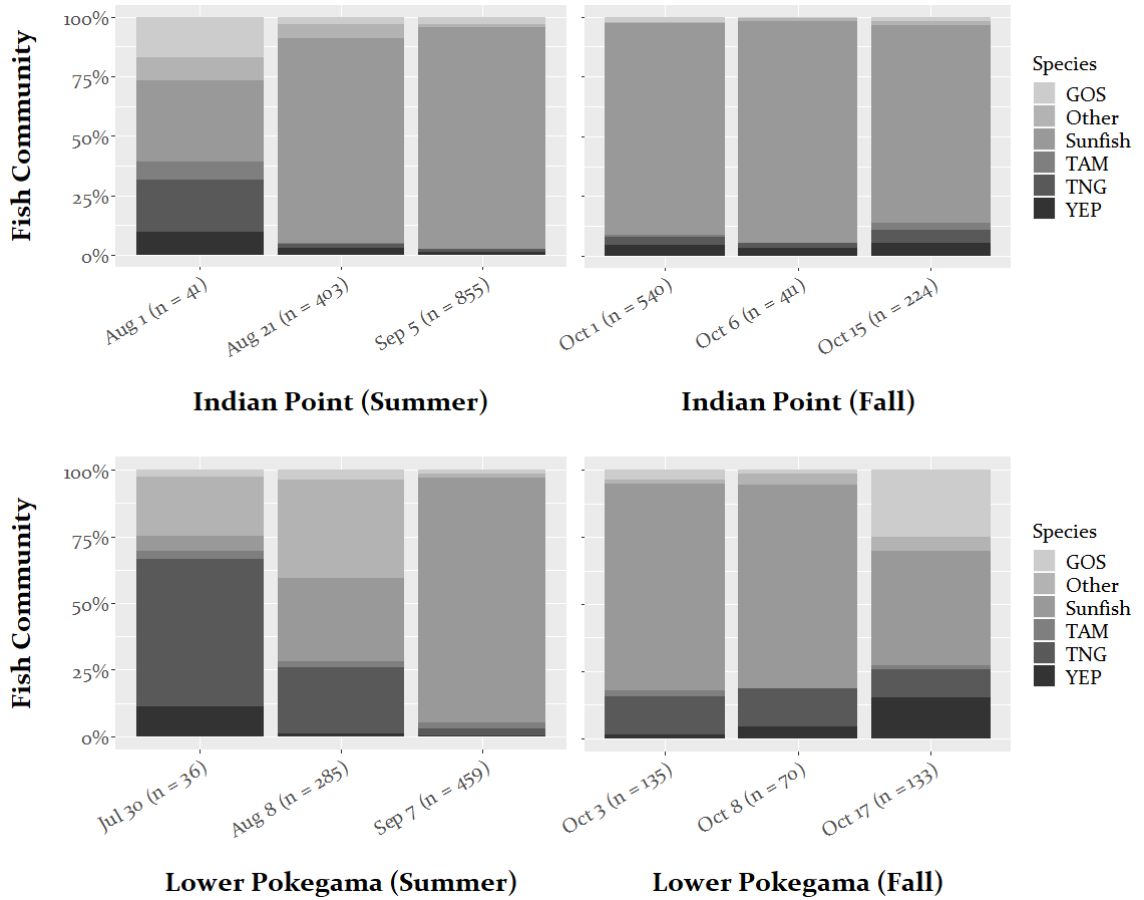
Figure 1. Fish community per season at each location (three sampling dates for each category). Percentages were calculated by number of individuals. Major groups included sunfish (*Lepomis* & *Pomoxis*), golden shiner (GOS), tadpole madtom (TAM), tubenose goby (TNG), yellow perch (YEP), and Other (rock bass, largemouth bass, northern pike, walleye, spottail shiner, common carp, Johnny darter, and bullhead *sps*).
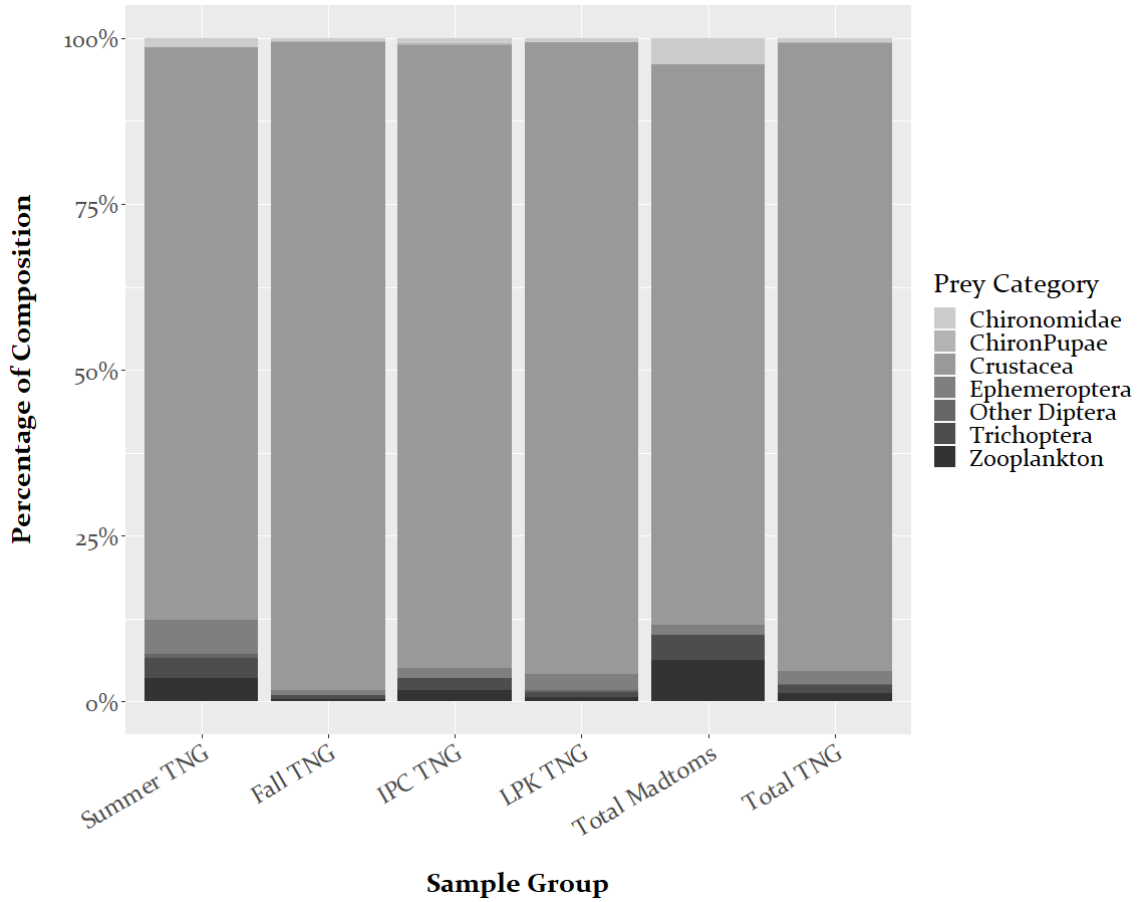
Figure 2. Weight percentage of diet composition by season, site, and species. Prey items were categorized and dried at 55°C for 24 hours prior to weighing (±0.001 mg). Benthic Crustacea (non-planktonic: Isopoda, Amphipoda, Ostracoda) dominated diets of all pools; only seven prey categories were represented. Detritus (sand, wood) was not included.
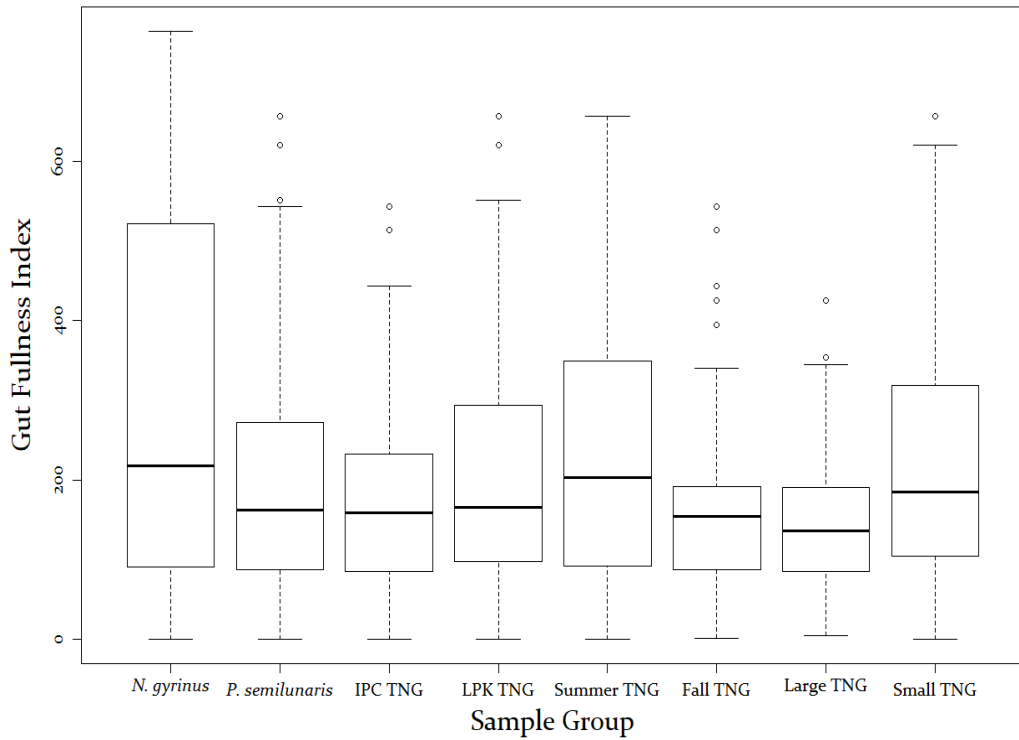
Figure 3. Gut fullness (GFI) for species, site, season and size class during the 2018 season in the St. Louis River estuary, Duluth MN. GFI was calculated for each fish as a proportion of total gut content weight to eviscerated fish weight (EtOH preserved; wet weight; ± 0.001 grams). Empty guts were included in analysis & detritus weight was not included. Heavy line represents the median, solid lines represent the quartiles (75th and 25th percentile), dashed lines represent the minimum and maximum, and open circles represent outliers. For individuals used per group, see Table 2.
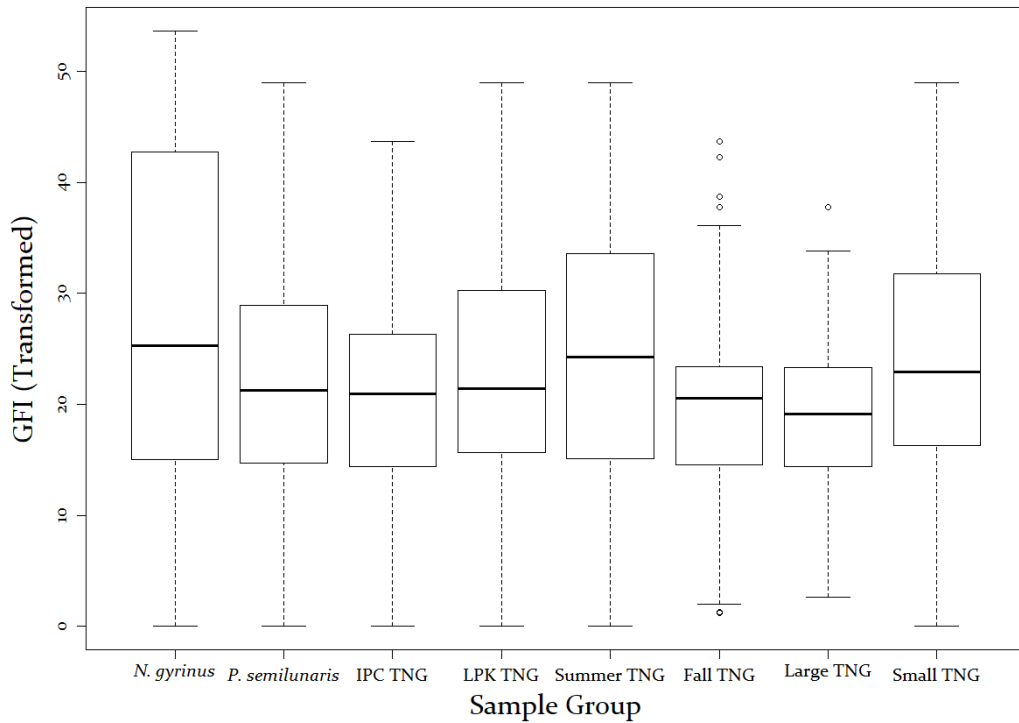
Figure 4. Gut fullness for sample groups (species, site, season, size) after power transformation (GFI $^{0.6}$) to meet assumptions of normal distribution (Shapiro-Wilk test). Significant differences from Welch's two-sample, two-tailed t-test (robust to violations of equal variance) were found between season (summer vs fall; p = 0.02) and size of tubenose goby (large vs small; p = 0.03). GFI was calculated for each fish as a proportion of total gut content weight to eviscerated fish weight (EtOH preserved; wet weight; ± 0.001 grams). Empty guts were included in analysis & detritus was not included in diet weights. Data collected during the 2018 season in the St. Louis River estuary, Duluth MN.
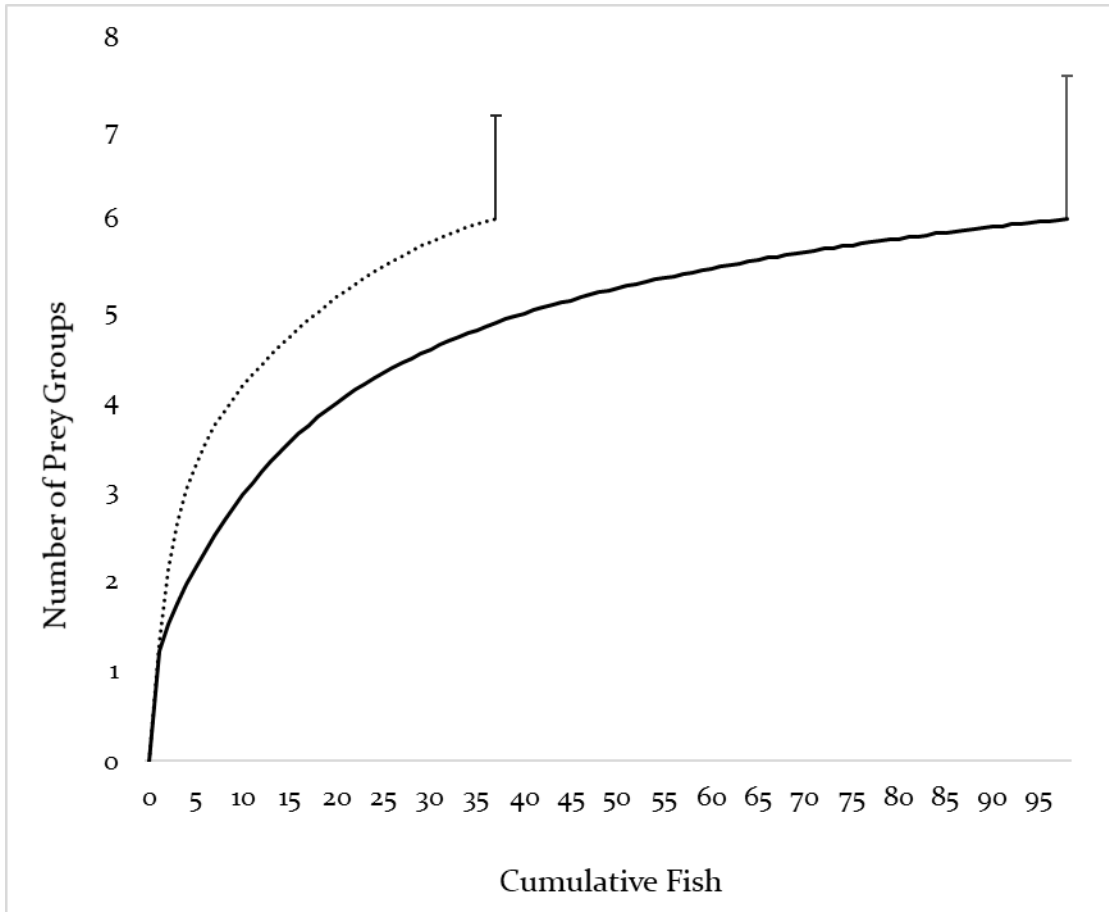
Figure 5. Rarefaction curves for tubenose goby (solid line; *n* = 98) and tadpole madtoms (dashed line; *n* = 37). Six total prey groups were predicted for both species, with a Chao1 95% Upper Boundary Conf. Interval of 7.58 (TNG) and 7.14 (MAD). Curves were generated using EstimateS (Colwell 2013).
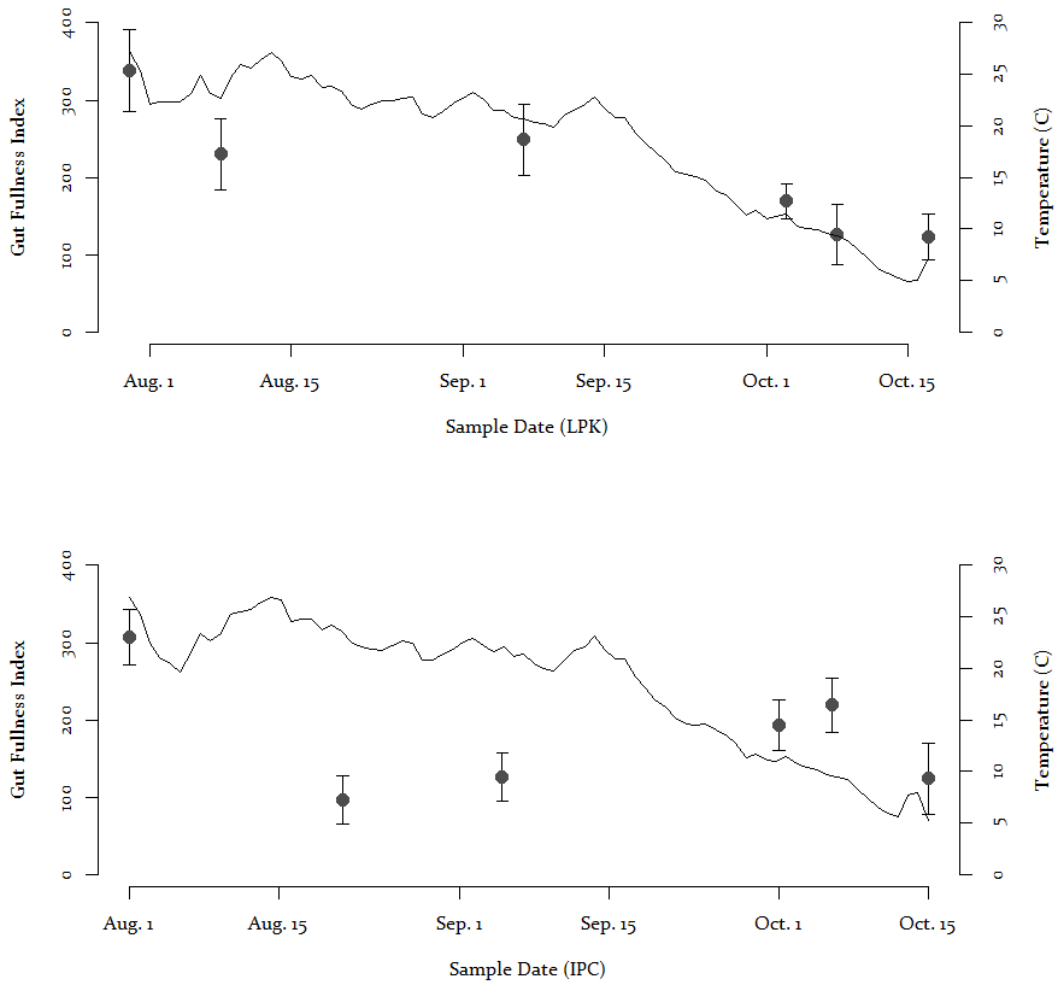
Figure 6. Mean gut fullness (± Standard Error) for tubenose goby at each sample replicate per site (Lower Pokegama = top; Indian Point Campground = bottom). Water temperature (ºC) was measured hourly over the course of the season via HOBO logger at each site; daily average was calculated and is shown via the gray line on each graph. Data collected during 2018 in the St. Louis River estuary, Duluth MN.
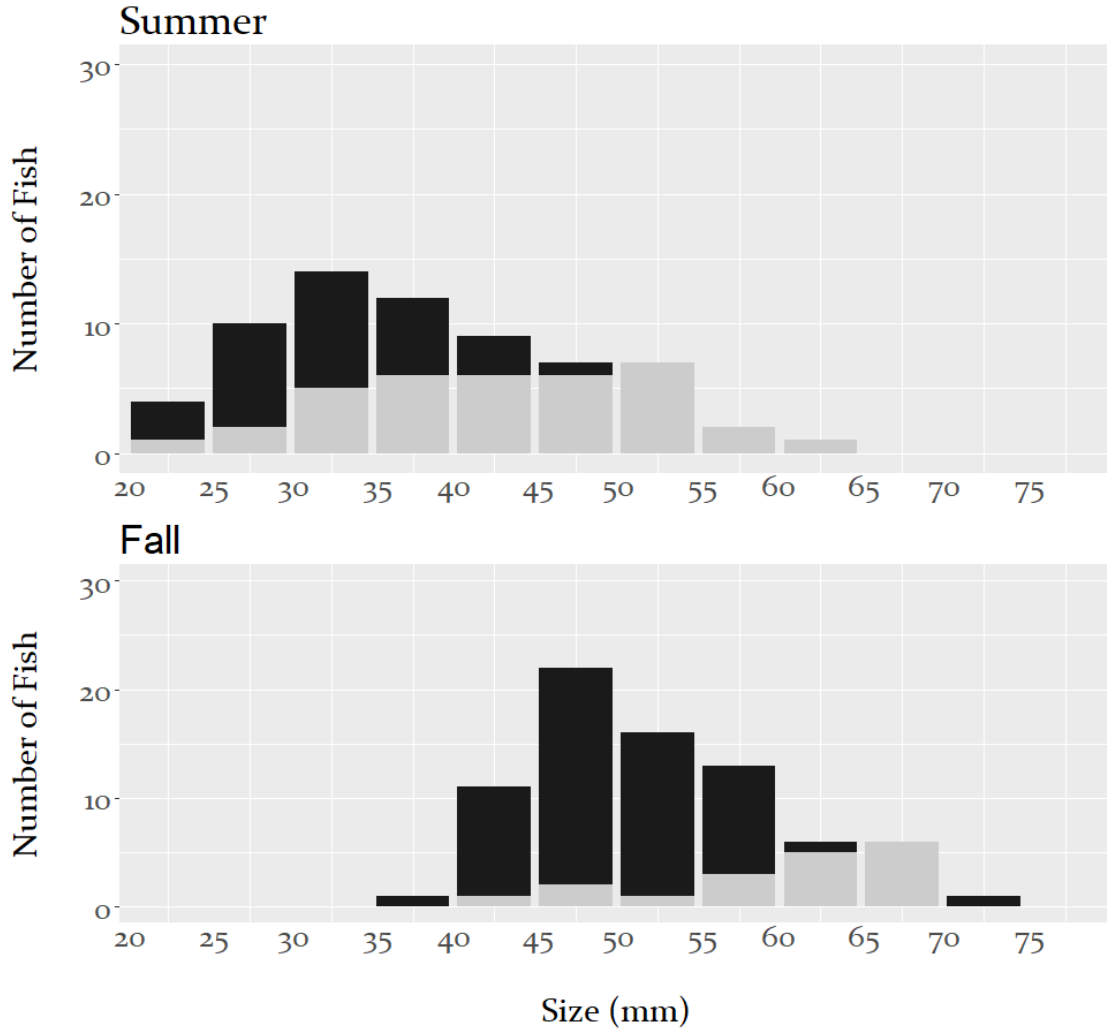
44

Figure 7. Size frequency of tubenose gobies captured in summer (top; $n = 66$) and fall (bottom; $n = 76$). Only fish that were sexed (>20 mm) were used for analysis (142 of 144 attempted were successfully identified). Females = black, males = gray.
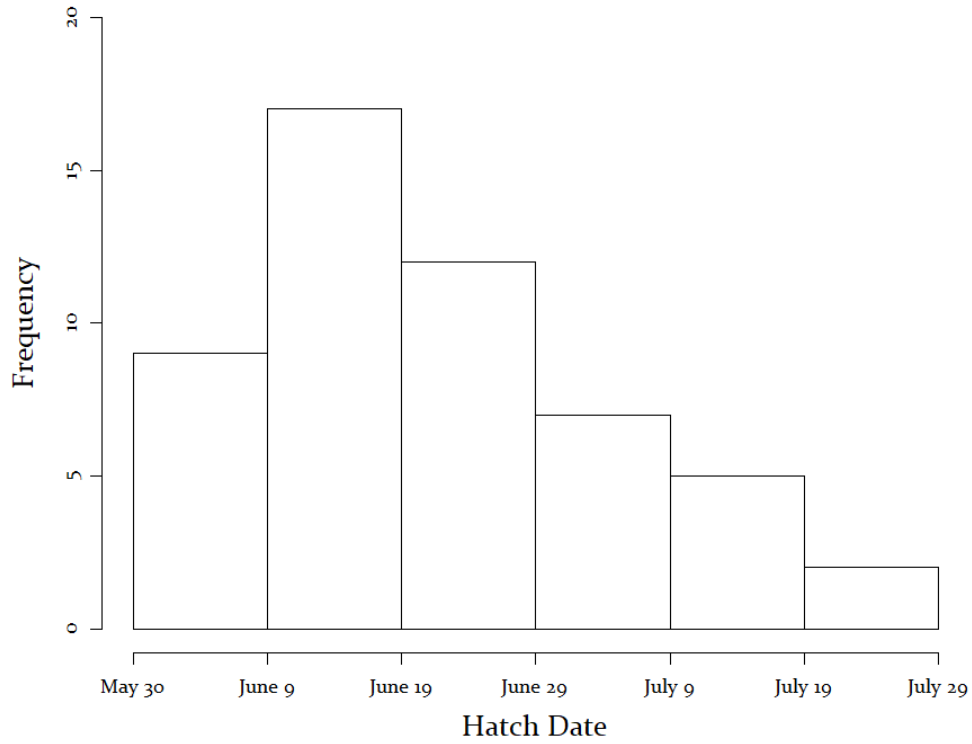
Figure 8. Hatch dates for a subset of tubenose goby (*n* = 52) that underwent age analysis. Tubenose goby were selected that spanned the sampled size range and aged via daily increments; age was then subtracted from capture date to calculate hatch date. Median hatch date was June 19 and ranged from June 4-July 24.
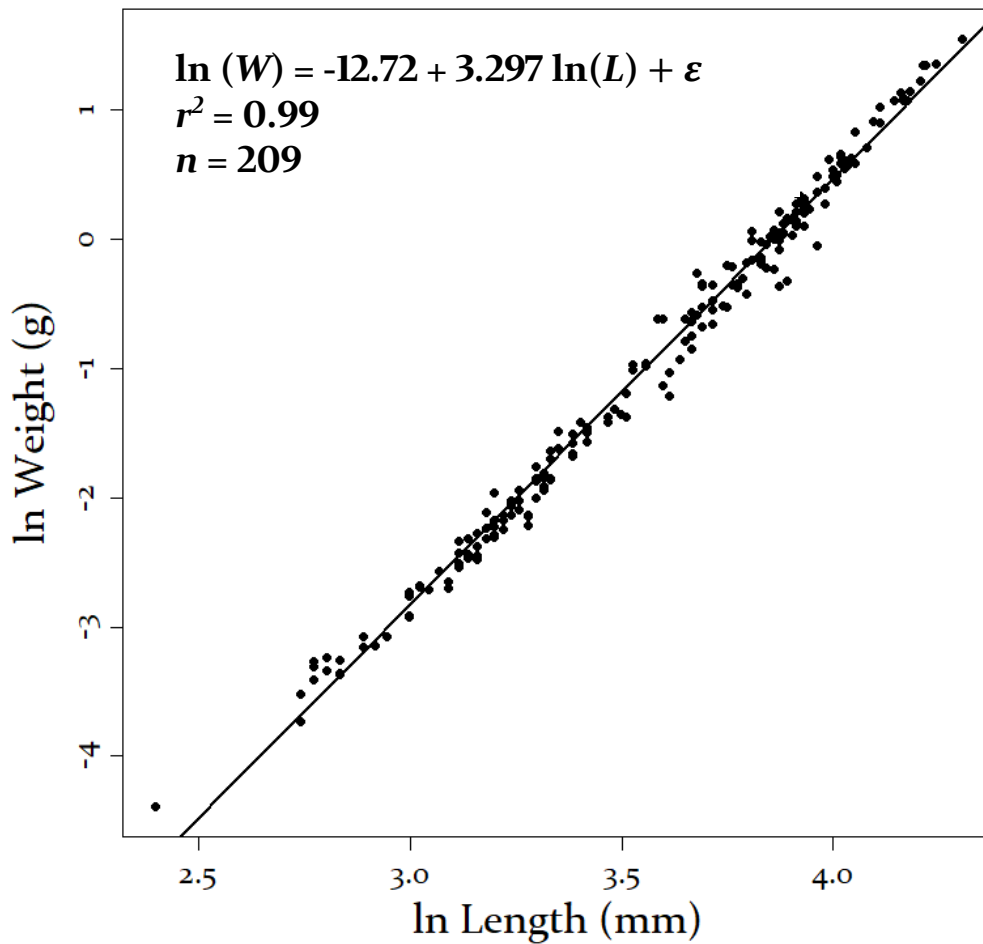
Figure 9. The relationship between the natural log of weight (g) and the natural log of total length (mm) for tubenose goby ($n = 209$; adjusted $r^2 = 0.99$). Allometric growth is apparent from $b > 3$; exponent parameter $b = 3.297 \pm 0.02$ SE, with a 95% conf. interval of 3.25-3.34. Lengths (TL) and wet weights were calculated as fresh, and fish were collected via beach seine from the St. Louis River estuary during July-October 2018.
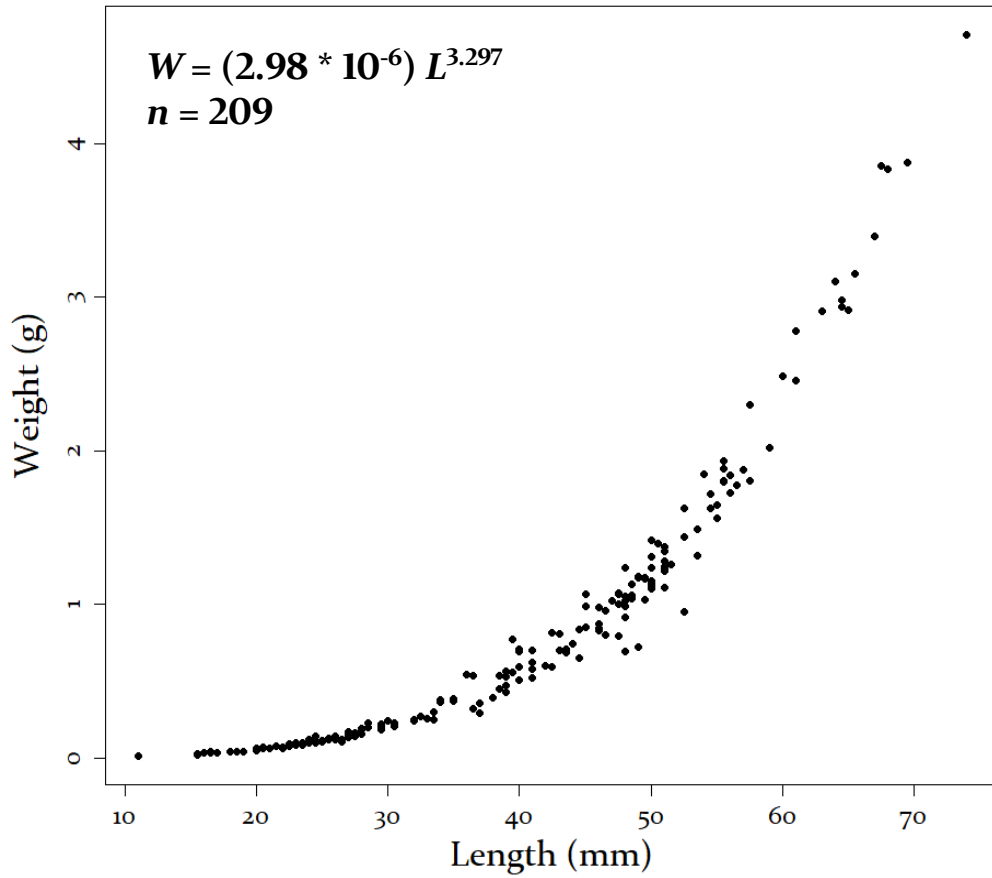
$$W = (2.98 * 10^{-6})\, L^{3.297}$$
$$n = 209$$

Figure 10. Weight-length relationship for tubenose goby ($n = 209$) collected via beach seine from the St. Louis River estuary in July-October 2018. Length (TL; mm) and wet weight (g) are shown as fresh.
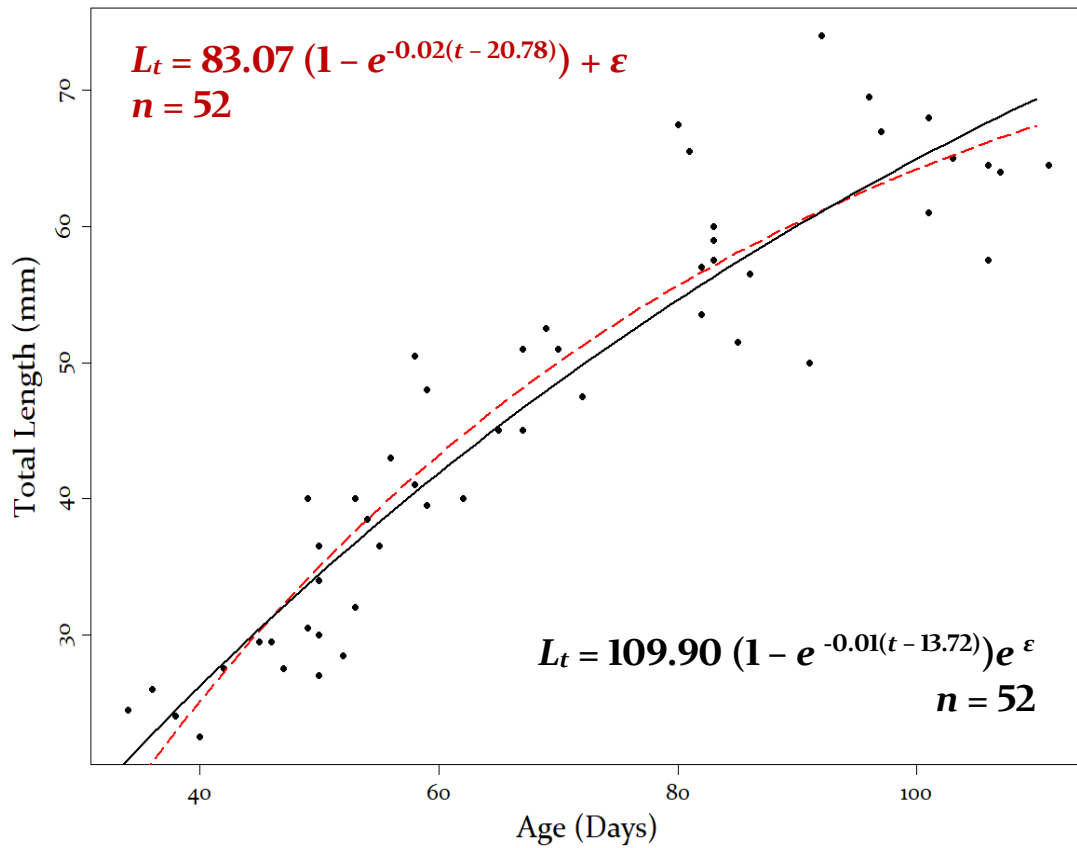
Figure 11. Von Bertalanffy Growth Function for age-0 tubenose goby. Models incorporate additive and multiplicative error assumptions; variance in total length increases as fish age increases, causing data heteroscedasticity. Natural log transformation of total length was successful in equalizing variance, and multiplicative error structure (AIC = -72.28) is preferred over additive structure (AIC = 323.66). Dashed line = additive error & solid line = multiplicative error; equations included on figure. Parameters were fit using "FSA" and "nlstools" packages in R (Ogle et al. 2018).
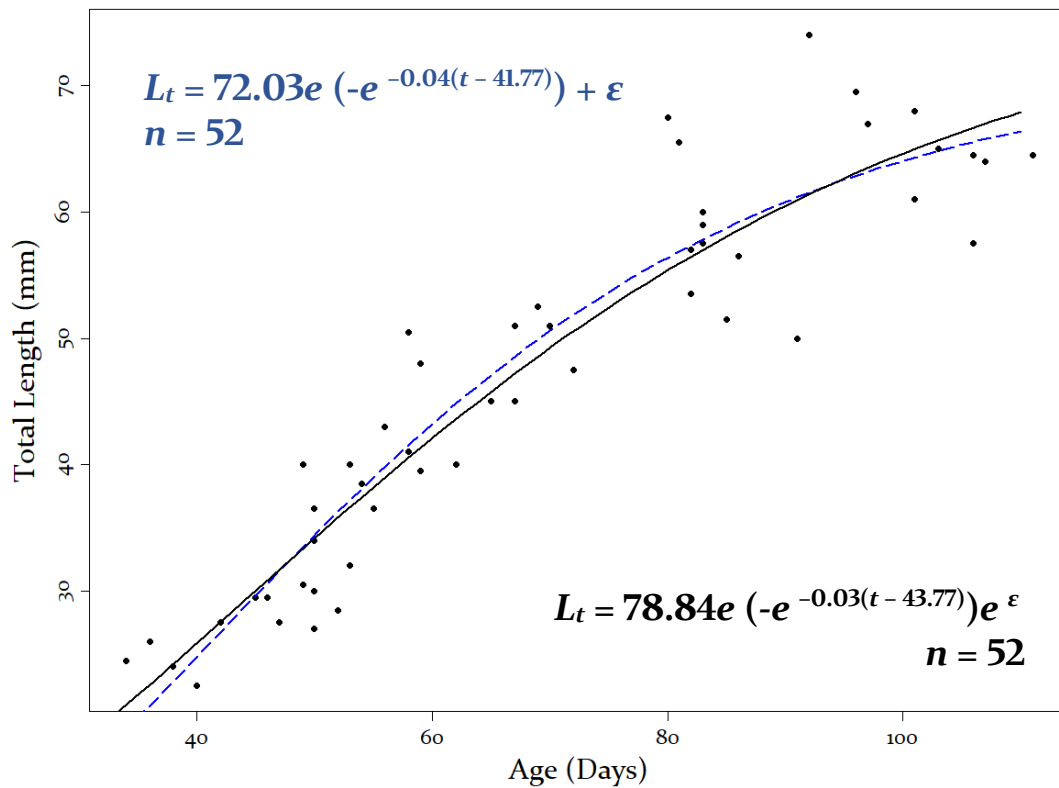
Figure 12. Gompertz Growth Function for age-0 tubenose goby. Models incorporate additive and multiplicative error assumptions; variance in total length increases as fish age increases, causing data heteroscedasticity. Natural log transformation of total length was successful in equalizing variance, and multiplicative error structure (AIC = -75.83) is preferred over additive structure (AIC = 320.37). Dashed line = additive error & black solid line = multiplicative error; equations included on figure. Parameters were fit using "FSA" and "nlstools" packages in R (Ogle et al. 2018).
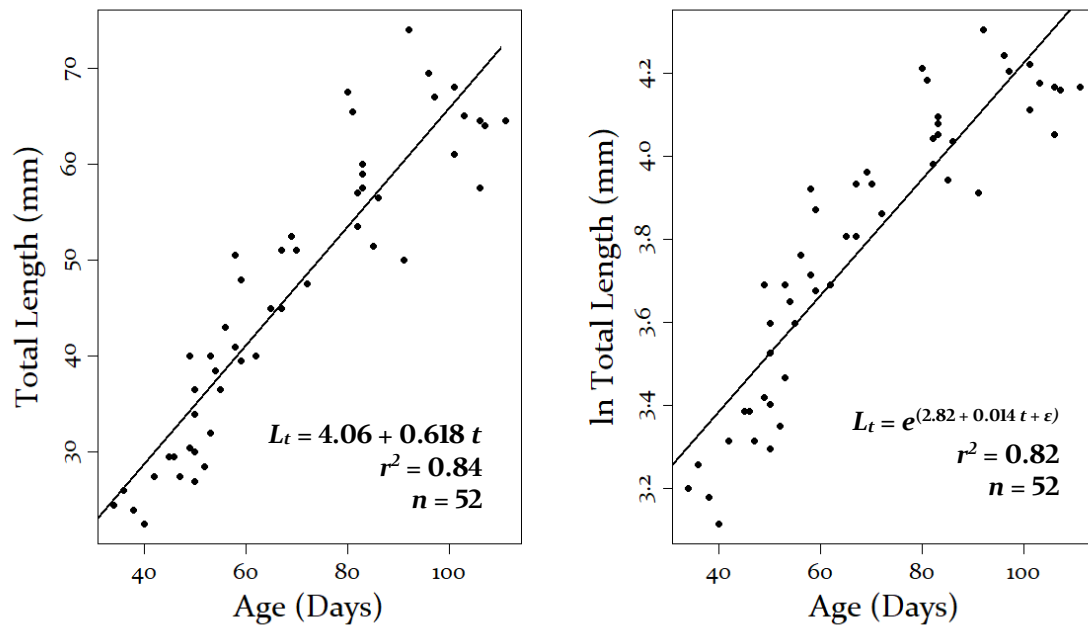
Figure 13. Linear models for length-age (left) and for natural log-transformed length-age (right) data. Equations are shown on figures; the slope of the linear length-age regression (0.62 mm/day) represents the overall daily growth rate for age-0 tubenose goby. Although AIC selected the model of transformed data (AIC = -49.75) over the untransformed model (AIC = 334.43), the r^2 values are similar.

$$L_t = 27.22 + 0.36\,t$$
$$r^2 = 0.41$$
$$n = 26$$
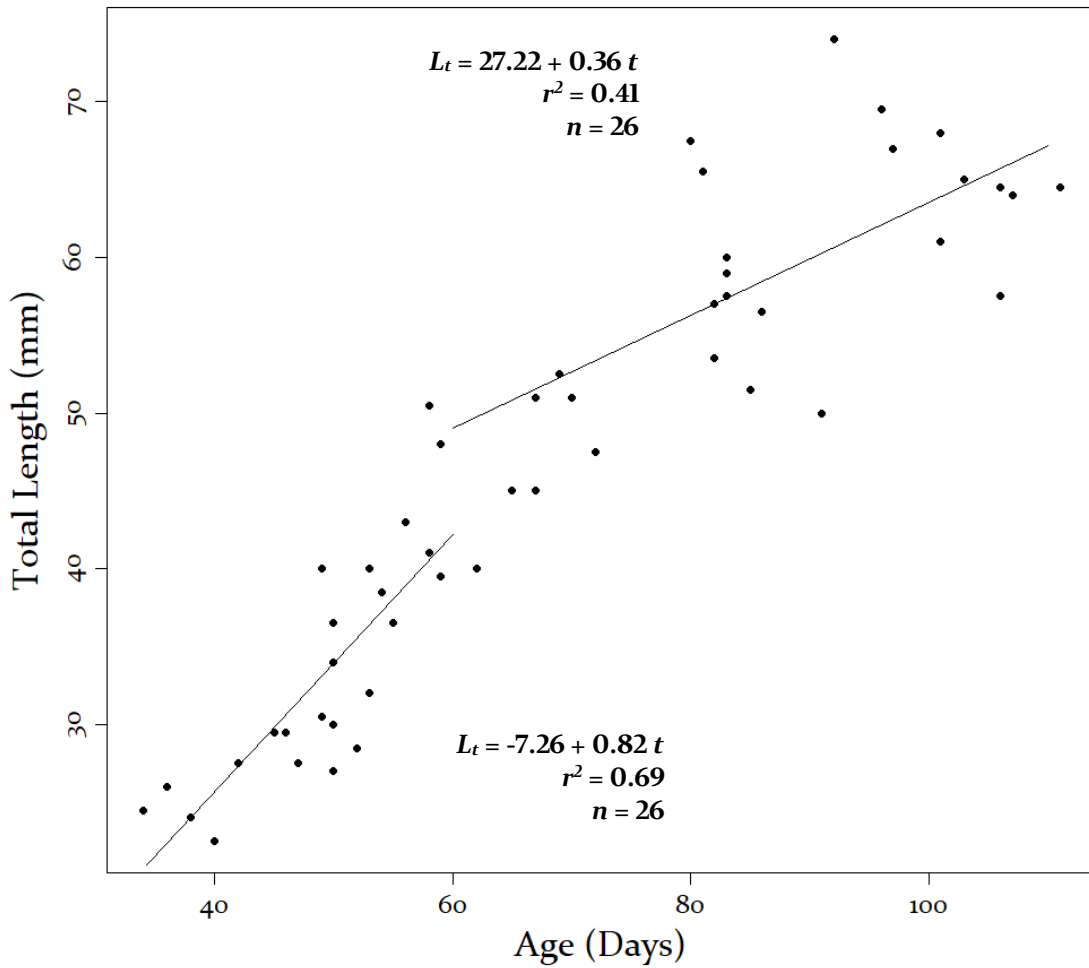
$$L_t = -7.26 + 0.82\,t$$
$$r^2 = 0.69$$
$$n = 26$$

Figure 14. Linear regression for growth of the youngest 26 tubenose goby sampled (34-65 days) vs. the oldest 26 tubenose goby sampled (67-111 days). Different slopes for regressions show a higher growth rate among younger fish (0.82 mm/day) than older fish (0.36 mm/day), consistent with a seasonal growth asymptote.
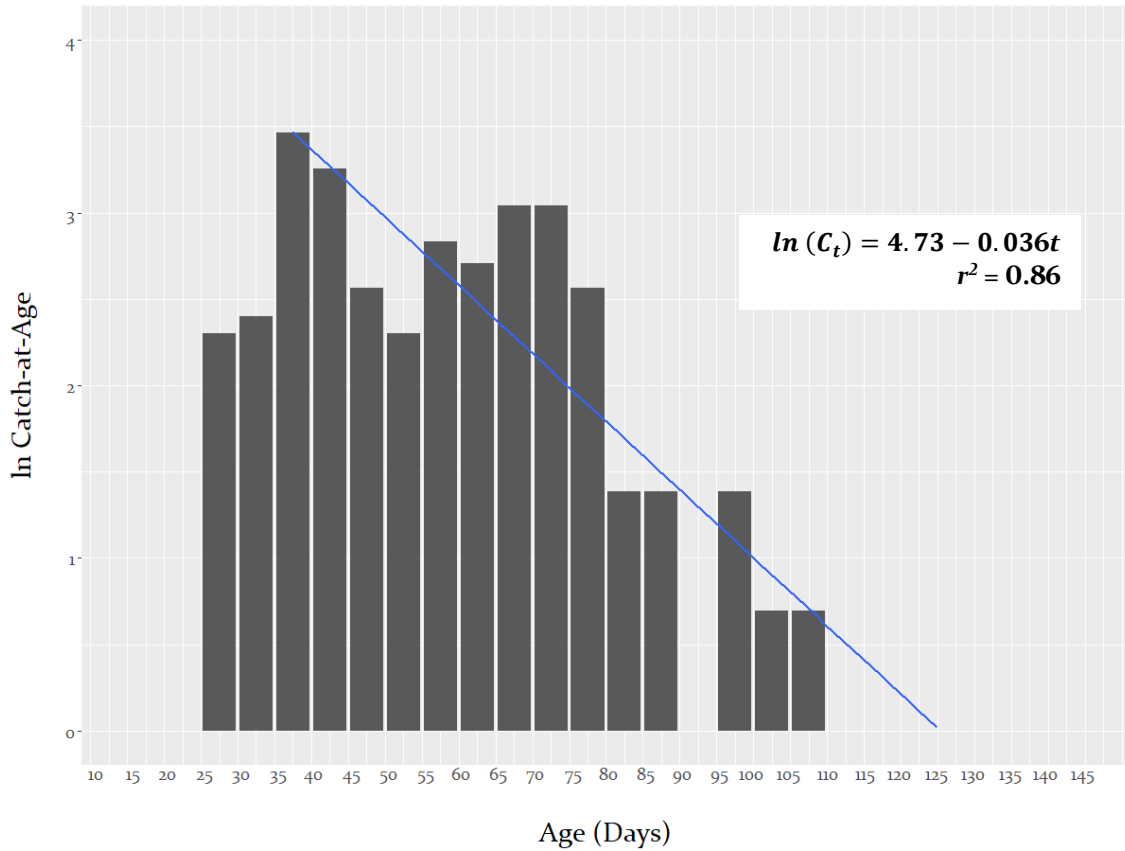
Figure 15. Longitudinal catch curve showing age bins and natural log of catch-at-age for tubenose goby. Age was estimated for each individual ($n = 209$) from length using the Gompertz growth function. Linear regression was used to estimate instantaneous mortality, which is represented by the slope ($Z = 0.036$) and can be used to estimate daily mortality rate ($D = 0.0350$) and approximate annual mortality ($M = 0.9999$). Fish were sampled July-October 2018 at two sites in the St. Louis River estuary.

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