

## ORIGINAL ARTICLE

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# Assessing the success of conservation efforts for a North American topminnow at risk of extinction from spatially variable mosquitofish invasions

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## Abstract

1. Invasive species can cause population declines and extinctions of native species through several mechanisms including predation and competition. One such species, western mosquitofish (*Gambusia affinis*), is an invasive, aggressive predator and competitor of numerous freshwater species around the world that has caused precipitous declines of native species.
2. The Barrens topminnow (*Fundulus julisia*) is a spring-endemic fish of the southeastern U.S.A., and the western mosquitofish limits its recruitment via predation of smaller individuals but there is also potential for competition (i.e. interference competition) between the species in laboratory trials. All of these negative interactions probably contribute to the Barrens topminnow's precipitous decline since the 1980s and the listing of the species as endangered by the U.S. Fish and Wildlife Service in late 2019. Ongoing conservation measures for Barrens topminnow include long-term monitoring of populations and reintroductions through stocking of hatchery-reared individuals into numerous sites.
3. Here, we investigate trends in Barrens topminnow and western mosquitofish occupancy and abundance over an 8-year period (three or four generations for Barrens topminnow) and assess the effectiveness of a stocking programme. We also included habitat covariates including distance to the nearest downstream confluence, stream order, and habitat size.
4. Our findings revealed significant range-wide Barrens topminnow declines in both occupancy and abundance were occurring over the 8-year period. These declines were associated with the presence of western mosquitofish. Overall, the stocking programme of hatchery-reared individuals did not significantly enhance or restore populations of Barrens topminnow but had a negative effect on topminnow abundance when western mosquitofish were present.
5. The lack of a positive effect of stocking hatchery-reared individuals on Barrens topminnow populations and the long-term population declines of Barrens topminnow suggest that propagation and stocking alone are not effective at creating self-sustaining populations of the Barrens topminnow. The presence of western mosquitofish does not currently appear to be compatible with long-term

persistence of topminnow populations. Moving forward, conservation strategies will be needed to create and maintain mosquitofish-free habitats, which may include eradication of mosquitofish, construction of barriers, or other actions.

#### KEYWORDS

barrens topminnow, conservation, endangered species, invasive species, reintroduction

## 1 | INTRODUCTION

Biological invasions have profound effects on biodiversity (Bellard et al., 2016; Blackburn et al., 2019; Simberloff, 2014; Simberloff et al., 2013) and represent one of the greatest threats to native species (Doherty et al., 2016; Wilcove et al., 1998). Invasions are largely responsible for biotic homogenisation seen among geographical regions (Clavero et al., 2009; McClure et al., 2018; Rahel, 2000). Not surprisingly, introduced species have contributed to the relatively high extinction rates for freshwater fauna in North America (Ricciardi & Rasmussen, 1999).

Western mosquitofish have been successfully introduced across the globe (Pyke, 2008), and these introductions have been implicated in the extirpation of native species (Courtenay & Meffe, 1989; Meffe, 1985; Schoenherr, 1981). As an introduced species, western mosquitofish are known to be aggressive predators and superior competitors of native species (Courtenay & Meffe, 1989; Laha & Mattingly, 2007; Pyke, 2008; Sutton et al., 2013). In general, invasive predators and pathogens typically have a much larger negative impact on native species than do those that interact via competition (Gurevitch & Padilla, 2004; Mooney & Cleland, 2001; Sax et al., 2002), and extinction of native fauna driven by invasive species is usually disproportionally attributed to predation rather than competition (Davis, 2003). One such fish species negatively impacted by the competitive and predatory interactions with western mosquitofish is the Barrens topminnow (*Fundulus julisia*). Barrens topminnow are endemic to springs and spring-fed streams of the Barrens Plateau in middle Tennessee (Figure 1), and their distribution and population sizes have contracted largely due to the introduction and spread of western mosquitofish over the past several decades (Bettoli, 2015; Laha & Mattingly, 2007; Rakes, 1989).

Interactions between Barrens topminnow and western mosquitofish are size-structured, where western mosquitofish are predators of small size classes [12–16 mm total length (TL) young-of-year and 20–30 mm TL juveniles]. However, when topminnow and mosquitofish are of similar size, Laha and Mattingly (2007) documented significant aggression towards and fin damage in Barrens topminnow potentially suggestive of interference competition, even though their particular study design did not allow them to test for competition effects directly. These interactions between the two species are the putative mechanisms facilitating the precipitous declines of Barrens topminnow. As a result, most formerly occupied sites have been extirpated leaving only four natural populations (i.e. non-stocked sites containing an extant natural [historical] population) on

the Barrens Plateau by the early 2000s (Laha & Mattingly, 2007) and only three natural populations by 2015 (Bettoli, 2015). The Barrens topminnow is now considered one the most imperilled fish species in the southeastern U.S.A. due to its restricted distribution, precipitous declines and additional threats from drought and habitat degradation within the region (Bettoli, 2015). The species was recently listed as endangered by the U.S. Fish and Wildlife Service (United States Fish & Wildlife Service, 2019).

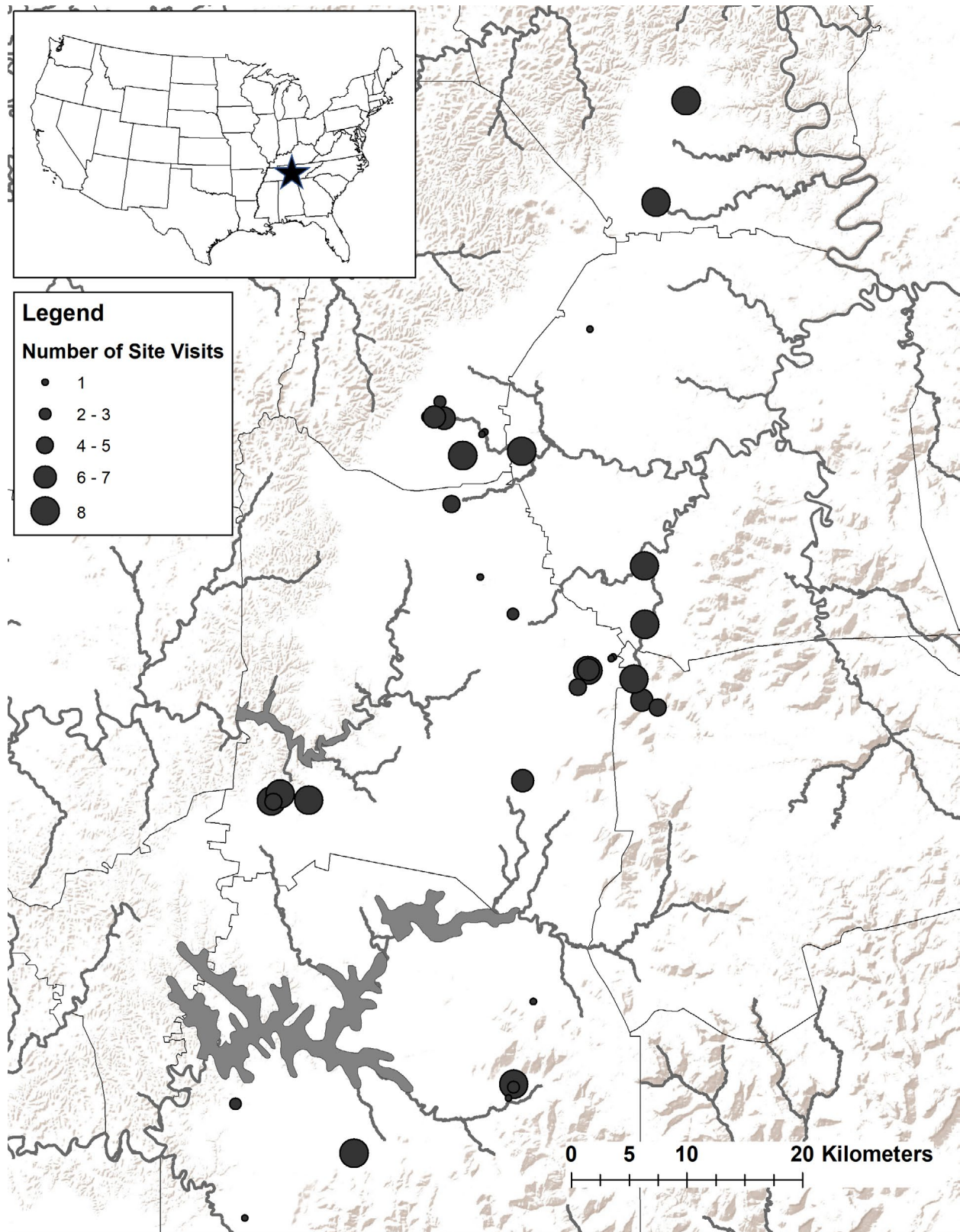
In response to the precipitous declines, an extensive reintroduction programme using stocking (henceforth stocking) for the Barrens topminnow was initiated in 2001 (Goldsworthy & Bettoli, 2006). To date, over 46,000 hatchery-bred Barrens topminnow have been repatriated into the wild through occasional stocking in the 1980s and 1990s, and annual stocking at multiple sites from 2001 to 2015 (USFWS, unpublished data). Short-term monitoring (i.e. 2003–2004) of these repatriated populations reported most cohorts of stocked Barrens topminnow experienced greater than 84% annual mortality; however, these mortality estimates were not related to western mosquitofish density at specific locations (Goldsworthy & Bettoli, 2006). Recruitment of Barrens topminnow over this 2-year period was only detected at locations without western mosquitofish (Goldsworthy & Bettoli, 2006), which supports ex situ experiments by Laha and Mattingly (2007) that western mosquitofish are significant predators of young-of-year Barrens topminnow.

Here, we investigate current population status of the critically endangered Barrens topminnow at natural and stocked sites over an 8-year period (i.e. 2008–2015). Specifically, we evaluate if: (1) occupancy or abundance of Barrens topminnow have changed through time and by site characteristics; (2) co-occurring western mosquitofish and time since invasion are associated with patterns of occupancy or abundance of Barrens topminnow; and (3) the management action of stocking is associated with patterns of abundance of Barrens topminnow.

## 2 | METHODS

### 2.1 | Sampling protocol

Sampling occurred among 38 different sites, although only 17–35 sites were surveyed in a given year. All sites occurred in the Barrens region of middle Tennessee west of the Cumberland Plateau (Figure 1). Site selection was based upon historical records and past and current reintroduction and introduction sites



**FIGURE 1** We sampled 38 sites (circles) from middle Tennessee for Barrens topminnow (*Fundulus julisia*) from 2008 to 2015. Within the main map, our sample sites were overlaid upon topography, and dark grey lines represent large drainages (fourth order and above) within our study area. The various sizes of the circles represent the number of years each site was sampled, where the smallest circle represents 1 year of sampling and the largest circle represents 8 years of sampling. The inset map is of the U.S.A., and star represents the general location of our study

selected by the U.S. Fish and Wildlife Service. Each site was surveyed between one and eight times (mean = 4.8 times, median = 5.5 times) during the 8-year study period (2008–2015). We classified the 38 sites using the following terminology: *non-stocked* sites ( $n = 8$ ) contained either an extant natural (historical) population of topminnow or an introduced population of topminnow into which supplemental stocking of hatchery-reared individuals did not occur during the study period; *stocked* sites ( $n = 25$ ) contained introduced (non-historical) or reintroduced (historical but extirpated) topminnow populations into which hatchery-bred individuals were released during the study period; and *exploratory* sites ( $n = 5$ ) were localities sampled within the topminnow's distributional range that had no historical records of topminnow or released individuals. These latter five sites were sampled once in search of new native populations and were excluded from our analyses. Sampling frequency of the sites was not standardised. Sites with more visits included *non-stocked* and *stocked* sites with known populations of Barrens topminnow, whereas sites with lower frequencies of visits were extirpated *non-stocked*, *stocked*, and *exploratory* sites. Many of these low frequency-visited sites were sampled pre-2008, and we sampled them post-2008 to verify past data. The 8-year study period was selected because of the standardised dataset (i.e. standardised protocols for sampling; see below) available during this time.

A crew of 3–11 persons used seines to sample each site. Sites were ranked as *small* or *large* to standardise sampling effort and were characterised by both the length of the spring and spring run (if present) and the type of pond/pool associated with the spring head. Small sites ranged in length from 23 to 150 m ( $\bar{x} = 80.3$  m), with all sites  $\geq 100$  m either lacking a well-defined spring pool at the spring head or were spring-influenced streams. Large sites varied from 90 to 700 m in length ( $\bar{x} = 263.5$  m). Small sites were sampled a minimum of 6 seine efforts and a maximum of 12. Large sites were sampled a minimum of 10 seine efforts and a maximum of 20. Each seine effort consisted of a c. 6 m haul. Per the protocol adopted by USFWS biologists, we were required to cease sampling at the minimum number of seine efforts once 80 Barrens topminnow were captured to minimise stress. Barrens topminnow were measured to the nearest mm total length and released at the point of capture. Barrens topminnow abundance was determined by direct counts (referred to as captures henceforth), whereas mosquitofish abundance was determined by either direct counts or volumetric estimations based on a linear model.

## 2.2 | Analyses

Because of variable sampling efforts annually, and because sites were only sampled once annually, modelling options available to estimate dynamic occupancy and abundance were limited. We used package unmarked in program R to estimate initial occupancy, detection, colonisation, and extinction probabilities for both Barrens topminnow and western mosquitofish using all sampling data (Fiske & Chandler, 2011; R Development Core Team, 2018). An a priori model was developed

for Barrens topminnow that modelled initial occupancy as a response to habitat covariates, which included distance to the nearest downstream confluence, the order of that stream, the habitat size (large or small), and whether or not the site had received stocked individuals during the study period. We used ArcMap v. 10.6.1 to estimate distance from our sites to the nearest downstream confluence and to determine stream order. We hypothesised that more distant springs located on lower-order streams would be least likely to be invaded by western mosquitofish. Detection and colonisation probabilities were modelled as constant because of consistent methodology and only one known natural Barrens topminnow colonisation event. Extinction probabilities were modelled with the status of western mosquitofish at the end of the study period. In the absence of sampling, we assumed that the site retained the western mosquitofish presence-or-absence state of the last sampling occasion, which is supported by observations from regularly surveyed sites occupied by western mosquitofish. Western mosquitofish were modelled with constant initial occupancy, detection, and extinction rates. Colonisation rates were modelled as a function of the same habitat covariates as the Barrens topminnow—distance to and order of receiving stream and size of the spring.

Even though we were unable to evaluate abundance while accounting for incomplete detection, we used linear mixed models to evaluate capture patterns while accounting for variation among sites as a random effect (i.e. 1|Site). We used Poisson generalised linear mixed models to evaluate trends in Barrens topminnow and western mosquitofish captures because captures were non-normally distributed. As fixed factors, we first investigated temporal trends and characteristics of site location (e.g. distance and order of the nearest stream) on both Barrens topminnow and western mosquitofish abundance:

$$\text{Captures} \sim \text{Year} + \text{Distance} + \text{Stream order} + (1|\text{Site})$$

where Captures represent the number of individuals captured per sampling per site, Year is the sampling year, Distance is the distance from the sites to the nearest downstream confluence, and Stream order is stream order of the nearest stream to the site.

Secondly, we investigated the interaction between the Barrens topminnow and western mosquitofish using Poisson mixed models using Barrens topminnow captures as our response variable and including a random effect of site. We evaluated if abundance of Barrens topminnow was negatively associated with the occupancy or abundance of western mosquitofish. If either was significantly associated with Barrens topminnow abundance, we evaluated their effect when presented via an interaction with the length of time the two species have interacted at a site (i.e. years since the introduction of western mosquitofish:

$$\begin{aligned} \text{Captures} \sim & \text{Mosquitofishoccupancy} + \text{Mosquitofishcaptures} \\ & + \text{Yearsinvasion} + \text{Mosquitofishoccupancy} * \text{Yearsinvasion} \\ & + \text{Mosquitofishcaptures} * \text{Yearsinvasion} + (1|\text{Site}) \end{aligned}$$

where, Captures represent the number of Barrens topminnow captured per sampling per site, Mosquitofish occupancy represents if mosquitofish were present or absent at a site, Mosquitofish captures



effect represent the number of mosquitofish captured per sampling per site, and Years invasion represents the number of years since mosquitofish were first detected at a site.

Finally, we evaluated if the Barrens topminnow population status (non-stocked or stocked) or the number of stocked fish in the previous year increased the captures of Barrens topminnow using a Poisson mixed model including site as a random effect. In this analysis, we also accounted for the presence or absence of western mosquitofish given the likelihood that they also may affect Barrens topminnow captures:

$$\text{Captures} \sim \text{Stocking status} * \text{Mosquitofish occupancy} + (1 | \text{Site})$$

where, Captures represent the number of Barrens topminnow captured per sampling per site, Stocking status represents if a site received hatchery-reared individuals or not, and Mosquitofish occupancy represents if mosquitofish were present or absent at a site.

All models were implemented and evaluated in R using package lme4 (Bates et al., 2015). For the occupancy models, importance for each parameter was determined by confidence intervals overlapping with zero. In all other models, significance was determined using an analysis of deviance in the car package (i.e. type II likelihood ratio tests) and  $\alpha = 0.05$  (Fox & Weisberg, 2011).

### 3 | RESULTS

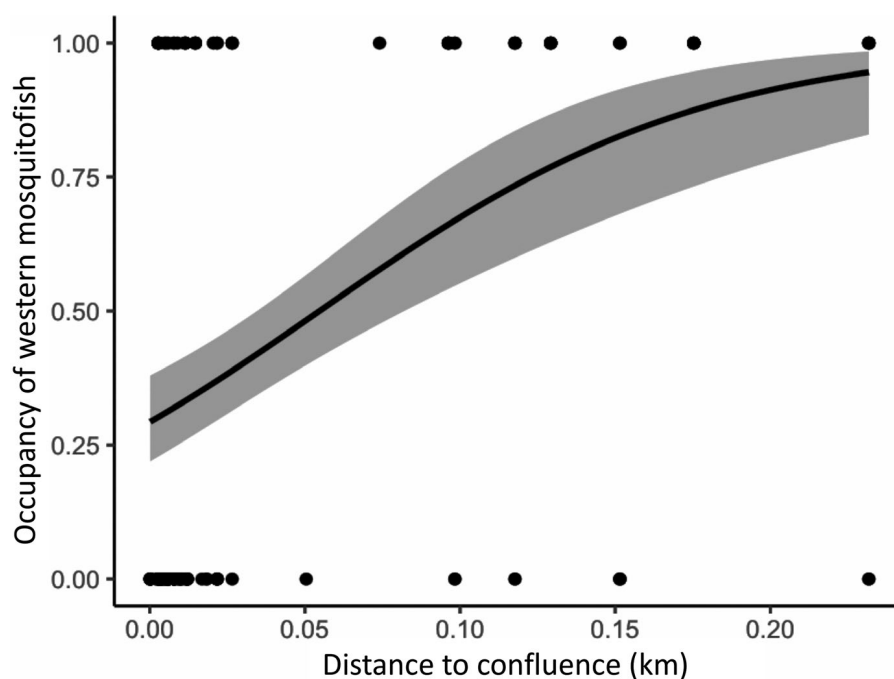
#### 3.1 | Occupancy and abundance

Initial regional site occupancy of Barrens topminnow was 0.855 (0.569–0.964; 95% confidence interval [CI]). By the end of the surveys, we observed 8 sites change from occupied to unoccupied (27.6% of sites surveyed more than once). None of the habitat

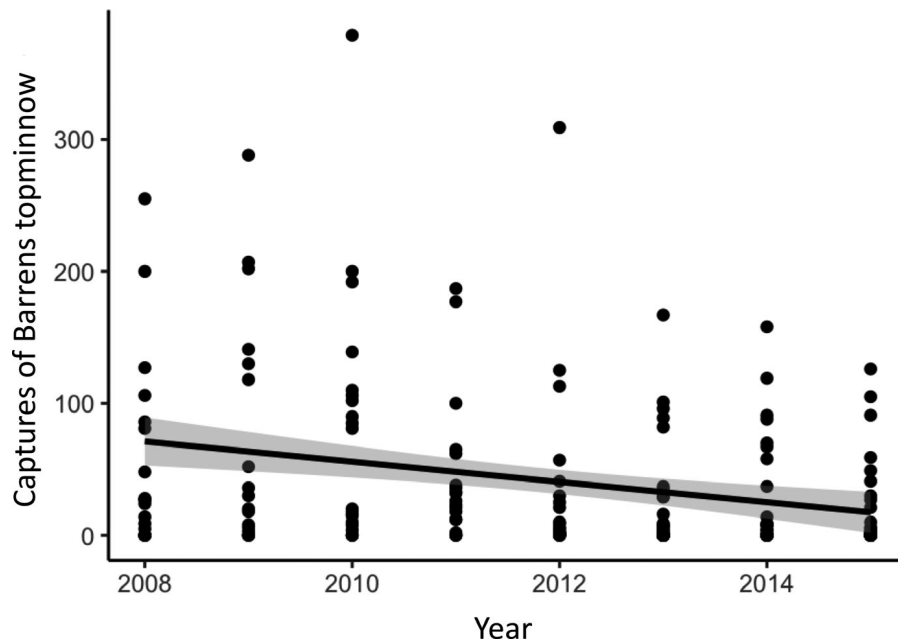
covariates had an influence on initial Barrens topminnow occupancy (distance to receiving stream,  $\beta = -0.117 \pm 11.06$ ; order of receiving stream,  $\beta = 2.56 \pm 9.70$ ; habitat size,  $\beta = 0.56 \pm 0.98$ ). Past stocking of Barrens topminnow did not influence occupancy ( $\beta = 0.64 \pm 7.62$ ). Natural colonisation probability of Barrens topminnow was low at 0.039 (0.027–0.058; 95% CI). While  $\beta$  estimates suggested that the presence of western mosquitofish had limited impact on Barrens topminnow extinction rates ( $\beta = 0.219 \pm 0.443$ ), back-transformed estimates indicate a 6.3% increase in extinction probability due to the presence of western mosquitofish at the end of the study period (western mosquitofish absent, 0.289 [0.221–0.369]; western mosquitofish present, 0.352 [0.258–0.459]).

Estimated western mosquitofish initial occupancy was 0.486 (0.371–0.602; 95% CI) with low extinction estimates (0.116 [0.078–0.169]) and high detection probability (0.847 [0.785–0.893]). Western mosquitofish were more likely to invade large springs ( $\beta = 0.618 \pm 0.802$ ) that were further from a receiving stream ( $\beta = 9.353 \pm 7.314$ ; Figure 2). Receiving stream order had no effect on mosquitofish colonisation probability ( $\beta = -0.156 \pm 0.380$ ).

Mean captures of Barrens topminnow have declined between 2008 and 2015 ( $\chi^2 = 1,163.02$ ,  $df = 1$ ,  $p < 0.001$ ; Figure 3), and western mosquitofish captures have increased during the same time period ( $\chi^2 = 497.23$ ,  $df = 1$ ,  $p < 0.001$ ). Barrens topminnow captures were positively associated with lower-order streams ( $\chi^2 = 3.86$ ,  $df = 1$ ,  $p = 0.049$ ) and unassociated with distance from the nearest stream ( $\chi^2 = 1.34$ ,  $df = 1$ ,  $p = 0.248$ ). Western mosquitofish captures were not associated with stream order ( $\chi^2 = 0.437$ ,  $df = 1$ ,  $p = 0.509$ ) but were positively associated with distance from the nearest stream ( $\chi^2 = 6.67$ ,  $df = 1$ ,  $p = 0.010$ ), which might suggest human-assisted rather than natural dispersal.



**FIGURE 2** Western mosquitofish (*Gambusia affinis*) colonisation probability increased with distance (km) from the nearest stream confluence resulting in a positive relationship between distance to the nearest downstream confluence and western mosquitofish occupancy probability



**FIGURE 3** Barrens topminnow (*Fundulus julisia*) captures (i.e. number of individuals) per site visits (black dots) by year. The line was fitted with a linear smoother and the error band represents the 95% confidence intervals

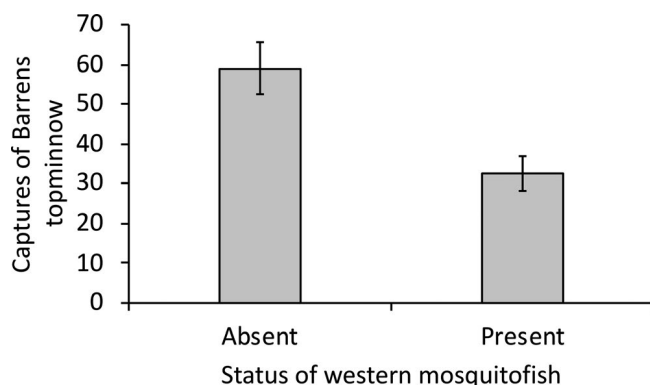
### 3.2 | Influence of western mosquitofish and stocking

Barrens topminnow captures were negatively impacted by the presence of western mosquitofish ( $\chi^2 = 6.31$ ,  $df = 1$ ,  $p = 0.012$ ; Figure 4), the abundance of western mosquitofish ( $\chi^2 = 371.51$ ,  $df = 1$ ,  $p < 0.001$ ), and the years since the first detection of western mosquitofish ( $\chi^2 = 56.71$ ,  $df = 1$ ,  $p < 0.001$ ; Figure 5). Negative impacts of the occupancy or abundance of western mosquitofish on Barrens topminnow captures did not significantly interact with time since their introduction ( $\chi^2 = 2.86$ ,  $df = 1$ ,  $p = 0.091$ ;  $\chi^2 = 3.54$ ,  $df = 1$ ,  $p = 0.060$ , respectively). This analysis suggests that it will take 11.5 years (almost 4 generations) after introduction of western mosquitofish for Barrens topminnow to be extirpated from the site. Stocking had no overall effect on the captures of Barrens topminnow ( $\chi^2 = 0.34$ ,  $df = 1$ ,  $p = 0.562$ ) and had a negative effect on captures when western mosquitofish were present ( $\chi^2 = 210.15$ ,  $df = 1$ ,  $p < 0.001$ ; Figure 6). A similar relationship was observed for the

effect of the number of individuals stocked into a site. The number of Barrens topminnow stocked into a site was positively associated with captures of Barrens topminnow but probably interacted with the presence of western mosquitofish ( $\chi^2 = 3.27$ ,  $df = 1$ ,  $p = 0.071$ ). In this interaction, increasing the number of introduced Barrens topminnow when western mosquitofish were present had a neutral effect on captures, whereas it had marginal positive effects on future captures when western mosquitofish were absent. In sites with western mosquitofish, five Barrens topminnow would need to be released in order to increase captures the following year by one.

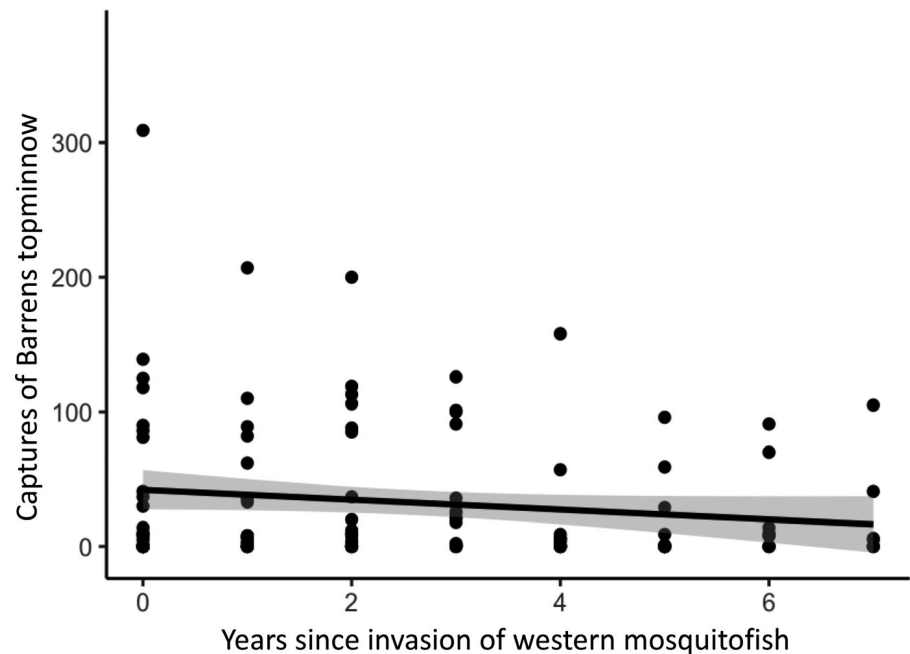
## 4 | DISCUSSION

To understand the impacts of a biological invasion, conservation managers need to identify the mechanism(s) by which native and invasive species interact, but also need long-term (i.e. time series) studies to identify population trends. A long-term study is critical in identifying population trends for threatened and endangered species for several reasons (Hughes et al., 2017; Lindenmayer et al., 2012). One is that data collection over multiple generations dampens inter-annual population variability and elucidates population-level responses to the environment (McCain et al., 2016), or in the Barrens topminnow's case, elucidates population-level responses to invasive species. Here, we report several critical implications for conservation and management efforts of the Barrens topminnow: (1) population declines (i.e. occupancy and abundance) occurred across the distribution over an 8-year period, which is roughly 4 generations (i.e. maximum longevity 3 years) for this species (Rakes, 1989); (2) these declines were associated with the presence of western mosquitofish; and (3) the stocking programme of hatchery-reared individuals had little influence on landscape-scale occupancy or abundance. For example, we found no overall significant relationship between stocking

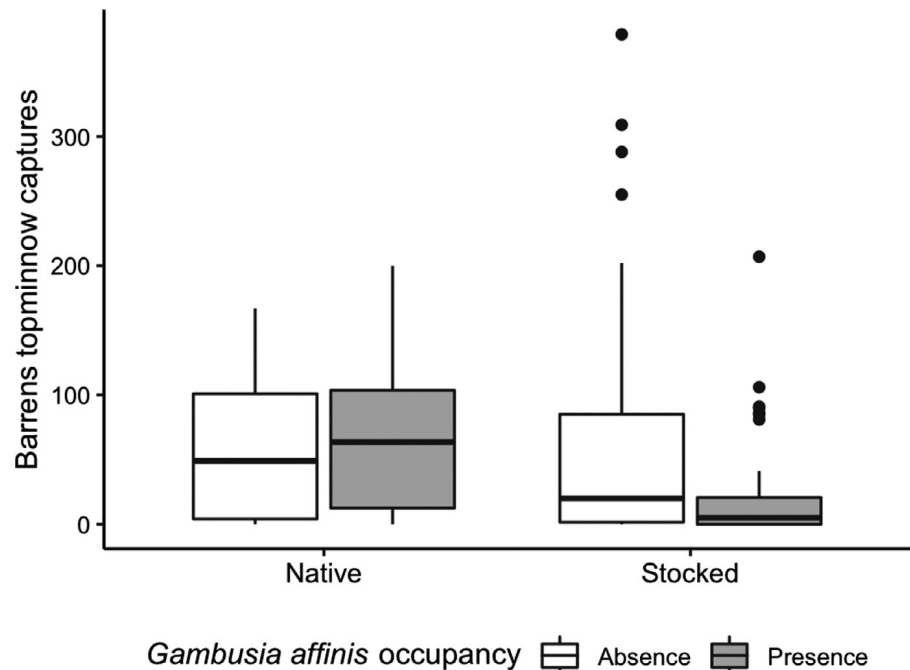


**FIGURE 4** Mean and standard error of captures (i.e. number of individuals per site) of the Barrens topminnow (*Fundulus julisia*) by the status of western mosquitofish (*Gambusia affinis*). Status was defined as presence or absence of mosquitofish

**FIGURE 5** Barrens topminnow (*Fundulus julisia*) captures (i.e. number of individuals per site) by time since site invasion (i.e. number of years) by western mosquitofish (*Gambusia affinis*). The line was fitted with a linear smoother and the error band represents the 95% confidence intervals



**FIGURE 6** A box-whisker plot displaying median (i.e. middle line in box), first and third quartile (i.e. top and bottom line of box), whiskers (95% confidence intervals), and outliers (open circles) for overall captures (i.e. number of individual per site) of Barrens topminnow (*Fundulus julisia*) by stocking status and occupancy of western mosquitofish (*Gambusia affinis*) of sites. Native sites were sites that received no hatchery-reared individuals, whereas stocked received hatchery-reared individuals



of hatchery-reared individuals (i.e. artificial recruitment) on the captures of Barrens topminnow, and stocking had a negative effect on the captures of Barrens topminnow in the presence of western mosquitofish. This lack of a significant response of Barrens topminnow abundance to stocking may be evidence of hatchery-reared individuals being less fit than their counterparts. It may also be due to habitat destruction and alteration from agricultural practices (personal observation, B. Kuhajda) accompanied by predation and competition pressures from western mosquitofish, which is likely to be driving population declines and the eventual extinction of the species.

Our study provides additional evidence that the presence and abundance of western mosquitofish are associated with the decline

of Barrens topminnow across their distributional range. These results are not unexpected since mosquitofish (collectively, *G. affinis* and the eastern mosquitofish, *Gambusia holbrooki*) are implicated in numerous declines of native fish species around the world (Courtenay & Meffe, 1989; Meffe, 1985; Pyke, 2008), in particular of native topminnow (Meffe, 1985; Sutton et al., 2013), including an ex situ Barrens topminnow study (Laha & Mattingly, 2007).

Stocking for reintroduction is an important conservation technique that has been utilised over 260 times with varying degrees of success in freshwater fishes around the world (Cochran-Biederman et al., 2015). The stocking of hatchery-reared Barrens topminnow individuals began extensively in 2001 and continued through

2014, with limited stocking continuing to occur thereafter. In the early to mid-2000s, short-term studies assessing the success of the stocking reported high mortality and little natural reproduction (Goldsworthy & Bettoli, 2006; Johnson, 2004), both of which are considered two important variables in gauging success of a stocking programme (Cochran-Biederman et al., 2015). However, another variable important in predicting stocking success—duration of stocking or repetitive stocking events through time—cannot be assessed via short-term studies (Cochran-Biederman et al., 2015). Our results using longer-term data provide evidence that stocking of hatchery-reared Barrens topminnow has not significantly influenced occupancy or abundance over an 8-year period, in part due to the presence of western mosquitofish at many of the reintroduced locations but also potentially due to reduced fitness of hatchery-reared individuals. Often hatchery-reared individuals have lower fitness (Araki et al., 2008; Jonsson et al., 2003) due to lack of social learning (Brown & Laland, 2001) and predator avoidance behaviours (Álvarez & Nicieza, 2003; Malavasi et al., 2004), and being weaker competitors (Orpwood et al., 2004) than their wild counterparts. Given our Barrens topminnow findings, freshwater managers should carefully consider decisions related to stocking of hatchery-reared individuals. In many cases, stocking of hatchery-reared individuals might be best reserved for locations where biological invaders are absent and unlikely to colonise. Stocking for population maintenance in the presence of a biological invader could expend valuable conservation resources and could even be associated with population declines as observed in our study.

Extinctions caused by invasive species are quite rare in the literature with the exception of island systems (Bellard et al., 2016). However, springs are analogous to aquatic islands in a terrestrial setting, and spring-endemic species mirror island endemics by sharing several characteristics. These include a patchy distribution, higher levels of genetic structuring and lower levels of genetic diversity compared to mainland (in this case, stream) species due to limited movement (gene flow), and increased vulnerability to stochastic events (Fluker et al., 2010). Endemic freshwater fishes are found in these aquatic islands across the world, and *Gambusia* species are a common invasive threat in these unique aquatic ecosystems—e.g. Iran (Esmaeili et al., 2007); Australia (Fensham & Fairfax, 2003) and (Kerezszy & Fensham, 2013); Turkey (Keskin, 2016); and U.S.A. (Mills et al., 2004). Barrens topminnow possess these *island species* attributes, having a patchy distribution, two genetically distinct management units within the same river drainage, reduced levels of genetic variation compared to stream-dwelling topminnow, and disproportionately affected by drought (Bettoli, 2015; Hurt et al., 2017; Rakes, 1989). Thus, the introduction of the invasive western mosquitofish into Barrens topminnow's spring habitat has the real potential to cause its extinction.

In conclusion, Barrens topminnow populations have declined over an 8-year period moving the species closer to extinction. Our analyses failed to reveal a positive impact of a multi-year stocking programme on Barrens topminnow occupancy and abundance partially due to the presence of western mosquitofish. In other words,

the stocking programme is probably prolonging the inevitable—population extirpation and potentially extinction. We recommend that more active measures (e.g. exclusion or eradication of western mosquitofish) are needed to prevent the extinction of the Barrens topminnow, and current conservation efforts—stocking—are only creating conservation-dependent populations without addressing the primary cause of the rapid declines. Ultimately, the best predictor of stocking failure is not adequately “addressing the initial cause of decline” (Cochran-Biederman et al., 2015) and, in the case of the Barrens topminnow, we recommend shifting some resources away from the stocking programme to focus on conserving springs through management actions more directly addressing the initial cause of decline. One possible action is the construction of barriers or selectively permeable fish-passage structures to prevent upstream colonisation by western mosquitofish at the remaining sites without western mosquitofish. Another active management practice is eradication of western mosquitofish. This practice was successfully performed at a small site with Barrens topminnow, Vervilla Spring (United States Fish & Wildlife Service, 2019), but is extremely difficult and unlikely to be an effective strategy in large spring habitats because of site characteristics (e.g. vegetation density) and sheer numbers and reproductive potential of western mosquitofish. Finally, an important strategy to consider is managing human actions through outreach and education efforts, especially deterring human-introduced establishment of additional mosquitofish populations. The recent federal listing of the Barrens topminnow under the U.S. Endangered Species Act may provide greater access to resources for conservation planning and recovery actions to prevent extinction of this native North American fish despite its current ecological challenges. However, unless active measures are taken, the Barrens topminnow unfortunately represents a species on a rapid downhill trajectory toward extinction.

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## CONFLICT OF INTEREST

The authors declare no conflict of interest.



## DATA AVAILABILITY STATEMENT

All data available from the corresponding author upon reasonable request.

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## REFERENCES

- Álvarez, D., & Nicieza, A. G. (2003). Predator avoidance behaviour in wild and hatchery-reared brown trout: The role of experience and domestication. *Journal of Fish Biology*, 63(6), 1565–1577. <https://doi.org/10.1111/j.1095-8649.2003.00267.x>
- Araki, H., Berejikian, B. A., Ford, M. J., & Blouin, M. S. (2008). Fitness of hatchery-reared salmonids in the wild. *Evolutionary Applications*, 1(2), 342–355. <https://doi.org/10.1111/j.1752-4571.2008.00026.x>
- Bates, D., Mächler, M., Bolker, B., & Walker, S. (2015). Fitting Linear mixed-effects models using lme4. *Journal of Statistical Software*, 67(1), 1–48. <https://doi.org/10.18637/jss.v067.i01>
- Bellard, C., Cassey, P., & Blackburn, T. M. (2016). Alien species as a driver of recent extinctions. *Biology Letters*, 12(2), 20150623. <https://doi.org/10.1098/rsbl.2015.0623>
- Bettoli, P. W. (2015). The last stand of the Barrens topminnow. *Fisheries*, 40(3), 102–103. <https://doi.org/10.1080/03632415.2015.1006324>
- Blackburn, T. M., Bellard, C., & Ricciardi, A. (2019). Alien versus native species as drivers of recent extinctions. *Frontiers in Ecology and the Environment*, 17(4), 203–207. <https://doi.org/10.1002/fee.2020>
- Brown, C., & Laland, K. (2001). Social learning and life skills training for hatchery reared fish. *Journal of Fish Biology*, 59(3), 471–493. <https://doi.org/10.1111/j.1095-8649.2001.tb02354.x>
- Clavero, M., Brotons, L., Pons, P., & Sol, D. (2009). Prominent role of invasive species in avian biodiversity loss. *Biological Conservation*, 142(10), 2043–2049. <https://doi.org/10.1016/j.biocon.2009.03.034>
- Cochran-Biederman, J. L., Wyman, K. E., French, W. E., & Loppnow, G. L. (2015). Identifying correlates of success and failure of native freshwater fish reintroductions. *Conservation Biology*, 29(1), 175–186. <https://doi.org/10.1111/cobi.12374>
- Courtenay, W. R., & Meffe, G. K. (1989). Small fishes in strange places: A review of introduced poeciliids. In G. K. Meffe & F. F. Snelson (Eds.), *Ecology and evolution of livebearing fishes (Poeciliidae)* (pp. 319–331). Prentice Hall.
- Davis, M. A. (2003). Biotic globalization: Does competition from introduced species threaten biodiversity? *BioScience*, 53(5), 481.
- Doherty, T. S., Glen, A. S., Nimmo, D. G., Ritchie, E. G., & Dickman, C. R. (2016). Invasive predators and global biodiversity loss. *Proceedings of the National Academy of Sciences of the United States of America*, 113(40), 11261–11265. <https://doi.org/10.1073/pnas.1602480113>
- Esmaili, H., Teimory, A., & Khosravi, A. (2007). A note on the biodiversity of Ghadamghah spring-stream system in Fars province, south-west Iran. *Iranian Journal of Animal Biosystematics*, 3(1), 15–23.
- Fensham, R. J., & Fairfax, R. J. (2003). Spring wetlands of the Great Artesian Basin, Queensland, Australia. *Wetlands Ecology and Management*, 11, 343–362. <https://doi.org/10.1023/B:WETL.0000005532.95598.e4>
- Fiske, I., & Chandler, R. (2011). unmarked: An R Package for fitting hierarchical models of wildlife occurrence and abundance. *Journal of Statistical Software*, 43(10), 1–23. <https://doi.org/10.18637/jss.v043.i10>
- Fluker, B. L., Kuhajda, B. R., Lang, N. J., & Harris, P. M. (2010). Low genetic diversity and small long-term population sizes in the spring endemic watercress darter, *Etheostoma nuchale*. *Conservation Genetics*, 11(6), 2267–2279. <https://doi.org/10.1007/s10592-010-0111-y>
- Fox, J., & Weisberg, S. (2011). *An R companion to applied regression* (2nd ed.). Sage Publications.
- Goldsworthy, C. A., & Bettoli, P. W. (2006). Growth, body condition, reproduction and survival of stocked Barrens topminnow, *Fundulus julisia* (Fundulidae). *The American Midland Naturalist*, 156(2), 331–343. [https://doi.org/10.1674/0003-0031\(2006\)156\[331:gbcra\]2.0.co;2](https://doi.org/10.1674/0003-0031(2006)156[331:gbcra]2.0.co;2)
- Gurevitch, J., & Padilla, D. K. (2004). Are invasive species a major cause of extinctions? *Trends in Ecology & Evolution*, 19(9), 470–474. <https://doi.org/10.1016/j.tree.2004.07.005>
- Hughes, B. B., Beas-Luna, R., Barner, A. K., Brewitt, K., Brumbaugh, D. R., Cerny-Chipman, E. B., ... Carr, M. H. (2017). Long-term studies contribute disproportionately to ecology and policy. *BioScience*, 67(3), 271–281. <https://doi.org/10.1093/biosci/biw185>
- Hurt, C., Kuhajda, B., Harman, A., Ellis, N., & Nalan, M. (2017). Genetic diversity and population structure in the Barrens Topminnow (*Fundulus julisia*): Implications for conservation and management of a critically endangered species. *Conservation Genetics*, 18(6), 1347–1358. <https://doi.org/10.1007/s10592-017-0984-0>
- Johnson, A. B. (2004). *Monitoring introductions of the barrens topminnow*. Masters, Tennessee Technological University, Cookeville.
- Jonsson, N., Jonsson, B., & Hansen, L. P. (2003). The marine survival and growth of wild and hatchery-reared Atlantic salmon. *Journal of Applied Ecology*, 40(5), 900–911. <https://doi.org/10.1046/j.1365-2664.2003.00851.x>
- Kerecsy, A., & Fensham, R. (2013). Conservation of the endangered red-finned blue-eye, *Scaturiginichthys vermeilipinnis*, and control of alien eastern gambusia, *Gambusia holbrooki*, in a spring wetland complex. *Marine and Freshwater Research*, 64(9), 851. <https://doi.org/10.1071/mf12236>
- Keskin, E. (2016). Molecular evidence for the predation of Critically Endangered endemic *Aphanius transgrediens* from the stomach contents of world wide invasive *Gambusia affinis*. *Mitochondrial DNA*, 27(2), 1210–1215. <https://doi.org/10.3109/19401736.2014.945526>
- Laha, M., & Mattingly, H. T. (2007). Ex situ evaluation of impacts of invasive mosquitofish on the imperiled Barrens topminnow. *Environmental Biology of Fishes*, 78(1), 1–11. <https://doi.org/10.1007/s10641-006-9040-5>
- Lindenmayer, D. B., Likens, G. E., Andersen, A., Bowman, D., Bull, C. M., Burns, E., ... Wardle, G. M. (2012). Value of long-term ecological studies. *Austral Ecology*, 37(7), 745–757. <https://doi.org/10.1111/j.1442-9993.2011.02351.x>
- Malavasi, S., Georgalas, V., Lugli, M., Torricelli, P., & Mainardi, D. (2004). Differences in the pattern of antipredator behaviour between hatchery-reared and wild European sea bass juveniles. *Journal of Fish Biology*, 65(s1), 143–155. <https://doi.org/10.1111/j.0022-1112.2004.00545.x>
- McCain, C., Szewczyk, T., & Bracy Knight, K. (2016). Population variability complicates the accurate detection of climate change responses. *Global Change Biology*, 22(6), 2081–2093. <https://doi.org/10.1111/gcb.13211>
- McClure, M. L., Burdett, C. L., Farnsworth, M. L., Sweeney, S. J., & Miller, R. S. (2018). A globally-distributed alien invasive species poses risks to United States imperiled species. *Scientific Reports*, 8(1), 5331. <https://doi.org/10.1038/s41598-018-23657-z>
- Meffe, G. K. (1985). Predation and species replacement in American southwestern fishes: A case study. *The Southwestern Naturalist*, 30(2), 173. <https://doi.org/10.2307/3670732>
- Mills, M. D., Rader, R. B., & Belk, M. C. (2004). Complex interactions between native and invasive fish: The simultaneous effects of multiple negative interactions. *Oecologia*, 141(4), 713–721. <https://doi.org/10.1007/s00442-004-1695-z>
- Mooney, H. A., & Cleland, E. E. (2001). The evolutionary impact of invasive species. *Proceedings of the National Academy of Sciences of the United States of America*, 98(10), 5446–5451. <https://doi.org/10.1073/pnas.091093398>

- Orpwood, J. E., Griffiths, S. W., & Armstrong, J. D. (2004). Effect of density on competition between wild and hatchery-reared Atlantic salmon for shelter in winter. *Journal of Fish Biology*, 65(s1), 201–209. <https://doi.org/10.1111/j.0022-1112.2004.00530.x>
- Pyke, G. H. (2008). Plague minnow or mosquito fish? A review of the biology and impacts of introduced *Gambusia* species. *Annual Review of Ecology, Evolution, and Systematics*, 39(1), 171–191. <https://doi.org/10.1146/annurev.ecolsys.39.110707.173451>
- R Development Core Team (2018). *R: A language and environment for statistical computing*. R Foundation for Statistical Computing. Retrieved from <http://www.R-project.org>
- Rahel, F. J. (2000). Homogenization of fish faunas across the United States. *Science*, 288(5467), 854–856. <https://doi.org/10.1126/science.288.5467.854>
- Rakes, P. L. (1989). *Life history and ecology of the Barrens Topminnow, Fundulus Julisia Williams and Ethier (Pisces, Fundulidae)*. Master of Science Masters, University of Tennessee, Knoxville, TN.
- Ricciardi, A., & Rasmussen, J. B. (1999). Extinction rates of North American freshwater fauna. *Conservation Biology*, 13(5), 1220–1222. <https://doi.org/10.1046/j.1523-1739.1999.98380.x>
- Sax, D. F., Gaines, S. D., & Brown, J. H. (2002). Species invasions exceed extinctions on islands worldwide: A comparative study of plants and birds. *American Naturalist*, 160(6), 766–783. <https://doi.org/10.1086/343877>
- Schoenherr, A. A. (1981). The role of competition in the replacement of native fishes by introduced species. In R. J. Naiman & D. L. Soltz (Eds.), *Fishes in the North American Deserts* (pp. 173–203). John Wiley and Sons.
- Simberloff, D. (2014). Biological invasions: What's worth fighting and what can be won? *Ecological Engineering*, 65, 112–121. <https://doi.org/10.1016/j.ecoleng.2013.08.004>
- Simberloff, D., Martin, J.-L., Genovesi, P., Maris, V., Wardle, D. A., Aronson, J., ... Vilà, M. (2013). Impacts of biological invasions: What's what and the way forward. *Trends in Ecology & Evolution*, 28(1), 58–66. <https://doi.org/10.1016/j.tree.2012.07.013>
- Sutton, T. M., Zeiber, R. A., & Fisher, B. E. (2013). Agonistic behavioral interactions between introduced western mosquitofish and native topminnow. *Journal of Freshwater Ecology*, 28(1), 1–16. <https://doi.org/10.1080/02705060.2012.688492>
- United States Fish and Wildlife Service (2019). *Endangered and threatened wildlife and plants; endangered species status of the Barrens topminnow*. Federal Register. Retrieved from <https://www.govinfo.gov/content/pkg/FR-2019-10-21/html/2019-22857.htm>
- Wilcove, D. S., Rothstein, D., Dubow, J., Phillips, A., & Losos, E. (1998). Quantifying threats to imperiled species in the United States. *BioScience*, 48(8), 607–615. <https://doi.org/10.2307/1313420>

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