Factors Limiting Reintroduced Plains Topminnow, *Fundulus sciadicus*, Populations in Central Great Plains Streams

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ABSTRACT The plains topminnow (*Fundulus sciadicus*) is an endemic Great Plains stream fish that has experienced declines in geographic range and local abundance. Due to these declines, the species has been considered for federal protection and designated with conservation status in states throughout its historic range. The reasons for declines are likely similar to hypothesized factors for other endemic stream fish declines in the Great Plains. To investigate potential limiting factors a suite of 17 historic sites with reintroduced plains topminnow populations across Nebraska were evaluated for current populations and if plains topminnow were absent, additional fish were introduced. These sites were sampled for plains topminnow persistence with fall backpack shocking in 2014-2016. A suite of 10 abiotic and biotic variables were selected *a priori*, based on previous research and guidance from fisheries personnel with working knowledge of the species, to evaluate potential factors that regulate populations of plains topminnow following reintroductions. Variables were combined to develop models based on plains topminnow life history characteristics, trophic interactions, and habitat requirements. Competing models were compared and variables were prioritized using an information theoretic approach. Limited backwater pool habitat and high predator fish abundances have the greatest relative importance in limiting reintroduced plains topminnow populations. Future management efforts to reintroduce plains topminnow should prioritize locations with these available habitats and communities and habitat renovation efforts should focus on these identified parameters.

KEY WORDS: Great Plains, limiting factors, native fish, reintroduced, plains topminnow

The native freshwater stream fishes of North America are declining (Minckley and Douglas 1991, Saunders et al. 2002). Approximately 70% of freshwater fishes throughout North America are at risk of continued declines in both local abundances and distribution (Ricciardi and Rasmussen 1999, Fischer and Paukert 2008*a*, Smith et al. 2014). Multitudes of abiotic and biotic alterations have been postulated to negatively influence native fish populations and assemblage diversity across the US (Pierce et al. 2001, Rahel 2002, Fischer and Paukert 2008*b*). However, the identification of important threats to imperiled species is limited, and often hinders the establishment of effective conservation measures (Campbell et al. 2002).

Increased legal protection of imperiled fishes in North America has resulted in efforts to conserve, not only entire species, but also individual populations (Minckley 1995). Conservation strategies to protect populations of imperiled species have included minimum flow requirements, habitat preservation and reserves, habitat enhancements or restoration, repatriation, and predator fish removal (Marsh et al. 2005; Mueller 2005). The recovery of imperiled species commonly employs stocking strategies such as augmentations, translocations, and reintroduction in attempts to sustain or reestablish historic populations (Sheller et al. 2006, Schumann et al. 2017). However, the majority of reestablishment efforts fail to establish subsequent year-classes due to the lack of considerations for potential limiting factors (Minckley 1995). Assessing stocking and reestablishment feasibility prior to implementation would likely result in greater success (Dunham 2011). Identifying the biotic and habitat features that influence abundance after reintroduction can help to maximize capital investments and the probability of species reestablishment.

Plains topminnow (*Fundulus sciadicus*) is a Great Plains stream fish, which has experienced declines in range-wide distribution as well as measurable reductions in local abundance (Haas 2005, Fischer and Paukert 2008*a*, Pasbrig et al. 2012). Nebraska comprises over 60% of the species distribution, and currently lists plains topminnow as a Tier 1 at risk species (Schneider et al. 2011). Theoretically, plains topminnow should be resilient to changes that minimize their distribution. Plains topminnow are robust, and durable backwater specialists that tolerate a wide range of abiotic conditions (Rahel and Thel 2004). Plains topminnow demonstrate a large home range that can allow reestablishment of desiccated stream reaches (Schumann et al. 2015*b*) and seek calm, shallow, warm waters with prolific aquatic vegetation (Rahel and Thel 2004). The presence of stream crossing structures has been identified to create deeper pool habitat which favor predator fish such as green sunfish (*Lepomis cyanellus*) and largemouth bass (*Micropterus salmoides*), and potentially limit the ability to move upstream (Dodds et al. 2004). While plains topminnow are generalized feeders they do demonstrate a selective preference for gastropods (Thiessen et al. 2018), which are commonly associated with heavily vegetated aquatic habitats (Ross and Ultsch 1980), suggesting alterations in substrate composition and shifts in flow regimes that limit submerged vegetation may be important to plains topminnow persistence (Schumann et al. 2017).

A variety of conservation efforts for this species have been undertaken in Nebraska including the development of a cultivation pond (Schumann et al. 2012) and subsequent species reintroduction efforts (Schumann et al. 2017). Supplementing plains topminnow populations through stocking increases local abundance, maintains genetic diversity, and temporarily preserves the ecosystem's community value (Reading et al. 2002, Marsh et al. 2005). However, stocking efforts do not address the factors prompting population declines and local extirpation. The data needed to identify specific abiotic and biotic factors limiting population persistence after reintroductions are lacking.

Identifying potential limiting factors can aid in attempts to establish and manage populations by prioritizing optimal conservation efforts. The environmental and biotic variables that influence plains topminnow populations have been postulated based on factors associated with the reduction of other endemic stream fishes (Dauwalter and Rahel 2008; Smith et al. 2014), topminnow morphologic characteristics (Rahel and Thel 2004), interactions with competitors and predators (Schumann et al. 2015*a*, Schumann et al. 2016), and observed behavior of wild individuals (Bestgen 2014). Great Plains native fish populations are at risk of declines due to alterations to physical habitat and invasion of introduced species caused by changes in water and land use practices, illegal introductions, and fish stocking programs (Fischer and Paukert 2008*b*, Smith et al. 2014). The changing landscape of Great Plains streams has resulted in reduced sinuosity, which is essential for the formation of preferred backwater pool habitat (Beschta and Platts 1986). Similarly, water impoundments, changes in water use practices, stream fragmentation, and hydro-morphologic stream alterations may have substantial impacts on native prairie fish assemblages (Wanner et al. 2011, Pasbrig et al. 2012, Smith et al. 2014). Biotic pressures have been found to control other fish species with predator control (Lundgren et al. 2014, Munter et al. 2019), as well as prey availability (Kaemingk et al. 2014). Introductions of sport fish and invasions of introduced western mosquitofish (*Gambusia affinis*) may also be decreasing plains topminnow populations by predating on both juveniles and adults (Schumann et al. 2015a). Compounding the challenge of identifying appropriate limiting factors is the reality that each of these proposed factors may work separately or in concert to decrease plains topminnow abundance.

Evaluating factors limiting species success prior to fish reintroductions is rarely done (Minckley 1995, Seddon et al. 2007, George et al. 2009). Because wild plains topminnow populations are considered at risk and the species occurs naturally in low abundances, this study utilizes experimentally reintroduced populations paralleled with adaptive stocking strategies to identify factors that influenced the abundance of plains topminnow at extirpated historic occurrence sites. Our objectives were to: (1) identify factors that influenced the success of reintroduced plains topminnow populations at 17 Nebraska stream sites, and (2) examine model weight averages to direct future management feasibility models.

STUDY AREA

Study sites were a continuation of Schumann et al. (2017), where 17 plains topminnow reintroduction locations (Figure 1) consisted of 14 separate streams or rivers so that all ecoregions in Nebraska were represented (Dauwalter and Rahel 2008). These sites historically contained plains topminnow but were currently considered relict populations since this species had not been sampled there for a minimum of 10 years. The length of each study site was 40X the mean wetted stream width, with a minimum 150 m and a maximum 300 m. Study sites received stockings of plains topminnow in 2010 (Schumann et al. 2017). Species presence was assessed in 2014 and sites where plains topminnow were not encountered received an additional stocking of 1,012 fish per habitat hectare (2,500 per acre) in 2014. A habitat hectare was defined by Schumann et al. (2017) as the wetted area with stream flows ≤ 0.407 m/s, which constituted pool, backwater and marginal bank areas. In total, nine sites received stockings and eight sites received no additional stockings.

METHODS

Fish assemblage

Fish community sampling utilized single-pass backpack electro-shocking with a Smithroot LR-24 backpack

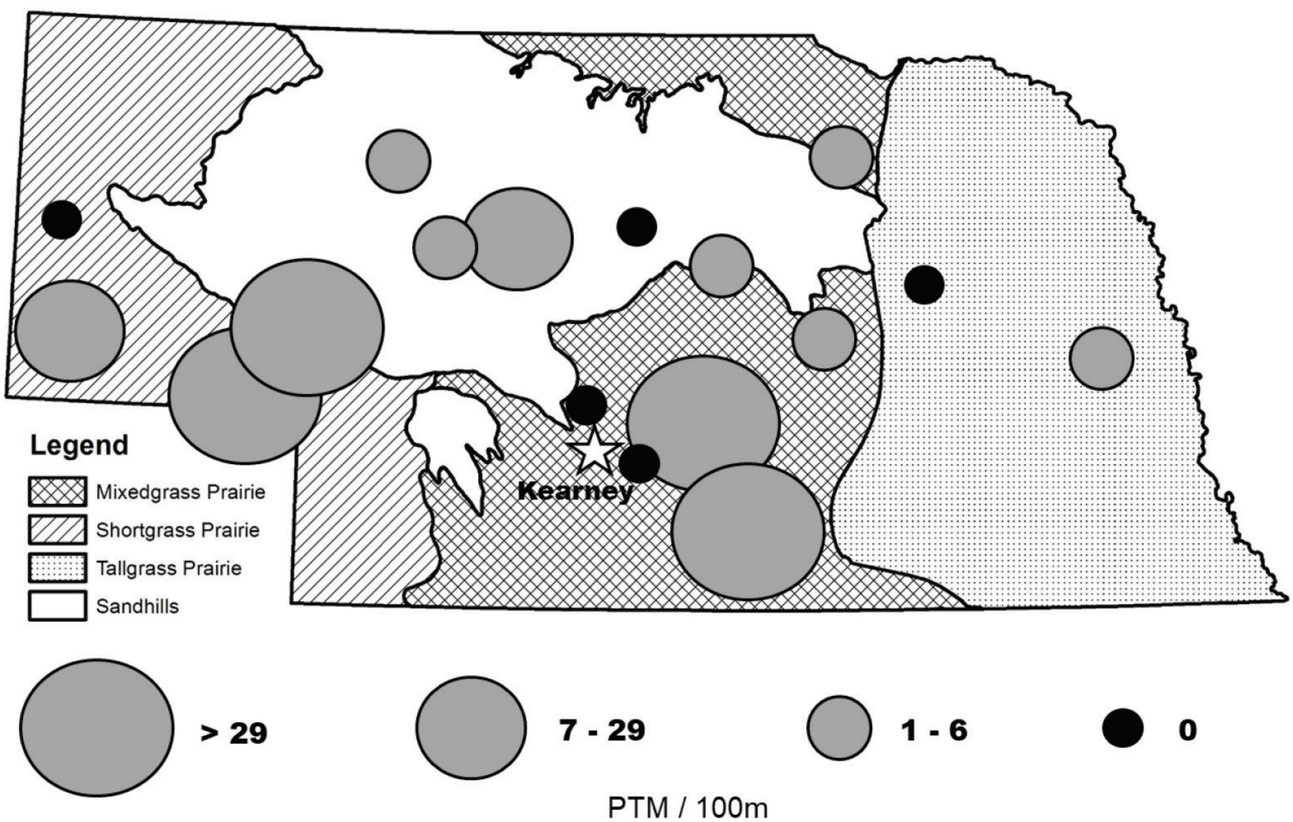

Figure 1. Plains topminnow (PTM) reintroduction sites across Nebraska ecoregions and individual site catch per unit effort (CPUE; number/100 m) from backpack electrofishing efforts post reintroduction efforts.

shocker, at optimized outputs for each site (Bertrand et al. 2006). Sampling sites were consistent with the previously established locations (Schumann et al. 2017). Fish collected were held in a bucket containing a portable aerator and water from the sample location. All captured fish were identified and enumerated before being released back into the stream. Sampling was conducted in 2015 between August and October as this timeframe was previously identified as having the highest seasonal capture efficiency of plains topminnow (Pasbrig et al. 2012). Relative abundance was indexed as catch per unit effort (fish/100 m of shocking) for all collected species.

Abiotic sampling

Abiotic data were collected in 2015 following the EPA Wadeable Streams and Rivers Rapid Biomass Standardized Sampling Protocol (Barbour et al. 1999), which included stream width and stream depth. Physical habitat sampling protocol followed EPA standards set by Kaufmann et al. (1999) and included slope, flow, temperature, and thalweg. Bank slopes and stream depths (m) were measured at five random locations within each stream reach. Bank slopes (degree angle) were measured from the current waters-edge at the time of visit. Total dissolved solids (TDS; mg/L) and water temperature (temp; °C) were measured prior to other data collection at the furthest downstream point of each study transect, using the HANNA combo HI98129 meter. Available backwater pool (BWP) habitat was determined based on stream flow regimes, where velocities ≤ 0.407 m/s were considered habitable by plains topminnow, as this is the average swimming velocity for the species (Prenosil et al. 2016). Hydrologic habitats encountered included trench pools, runs, lateral scour pools, backwater pools, dam pools, glides, and riffles. The transition between stream flows and aquatic habitat velocity were identified using a single reading with an OTT MF pro handheld flow meter at 60% of stream depth. Riffles were identified based on their range of flow; then counted and measured to the nearest cm^2 for the entire transect length of each study site to determine the available hydrologic habitat. Dominant substrate coarseness was visually estimated by the percentage composition of silt (<0.5mm), sand (0.5-2mm), fine gravel (2-16mm), coarse gravel (16-64mm), and cobble (64-240mm) at each study reach. Sinuosity was quantified as the ratio of thalweg length compared to straight line length in the described study site.

Variable selection and model development

We selected 10 variables thought to potentially limit plains topminnow from the published literature or in conjunction with Nebraska Game and Parks Commission fisheries staff with working knowledge of regional freshwater systems (Table 1). Variables included were characterized as either physicochemical, geomorphic, hydrologic, biotic, or physical habitat and were collected in sampling efforts conducted in August – October 2015. These included available macrohabitats (i.e., backwater pool, flow regime) predator fish relative abundance (pred), total dissolved solids (TDS), water temperature (temp), average stream depth (streamdepth), estimated dominant substrate, average bank slope, estimated percent of submerged vegetation (stream veg.), sinuosity (Sinu), and species richness (total count of species presence). Multiple linear regression models were used to quantify the relationship between each model and plains topminnow relative abundance using R-Studio version 0.99.491 (RStudio 2015). The relationship of selected variables with plains topminnow relative abundance was considered to construct 15 competing models using the 10 biotic and abiotic variables, based on the working understanding of life history characteristics and ecosystem requirements of this species (Table 2).

Fish species were divided into two categories: (1) predator (piscivorous) and (2) non-predator based on life history. Predatory fish that were represented by the presence of a single individual at multiple sites consisted of channel catfish (*Ictalurus punctatus*), western mosquitofish, creek chub (*Semotilus atromaculatus*), green sunfish, and largemouth bass. Recent studies suggest negative plains topminnow population impacts result from *Gambusia* spp. aggressive harassment towards adult and predation on juveniles (Haas 2005, Schumann et al. 2016) and that minimal diet overlap was observed (Thiessen et al. 2018). Therefore, western mosquitofish were included as a predator for model development. Non-predator fish that were represented by the presence of a single individual at multiple sites included gizzard shad (*Dorosoma cepedianum*), common carp (*Cyprinus carpio*), fathead minnow (*Pimephales promelas*), brassy minnow (*Hybognathus hankinsoni*), emerald shiner (*Notropis atherinoides*), white sucker (*Catostomus commersonii*), plains killifish (*Fundulus zebrinus*), sand shiner (*Notropis stramineus*), bigmouth shiner (*Notropis dorsalis*), red shiner (*Cyprinella lutrensis*), longnose dace (*Rhinichthys cataractae*), orangethroat darter (*Etheostoma spectabile*), and brookside stickleback (*Culaea inconstans*).

Available habitat was defined by collected flow readings based on the published threshold for maintained swimming

Table 1. Variable codes and description included in AICc model development for candidate model analysis, with value range (min-max), mean value, and standard error for each variable to predict relative abundance of reintroduced plains topminnow populations at 17 reintroduction sites in Nebraska. The PTM code was the response variable in the models. Backwater pools (BWP) was defined as the percent wetted area with stream flows ≤ 0.407 m/s.

Code	Description	min-max	mean	SE
PTM	Plains topminnow /100m	0-243.6	27.1	15.2
pred	Predator fish /100m	0.7-243.6	60.6	20.9
speciesrich	Total species/100m	5.0-19.0	9.9	0.9
TDS	Total dissolved solids (PPM)	80.0-630.0	257.8	42.8
sinu	Sinuosity (thalwag)	10-16.6	12.2	0.5
temp	Avg. stream temperature (C°)	10.9-23.7	16.3	0.9
streamdepth	Stream depth (m)	0.18-3.16	0.6	0.2
bankslope	Avg. degree of bank angle	0.16-3.16	1.4	0.2
stream.veg	In-stream vegetation (%)	0-100	23.1	10
substrate	Dominant substrate (mm)	0.25-12	2.4	0.7
BWP	Available backwater pool habitat/100m (%)	0.42-100	22.8	8.3

Table 2. AIC_c candidate models and rank for best fit models predicting relative abundance of reintroduced plains topminnow populations in Nebraska, as determined by the Akaike's information criterion for small sample size AIC_c rankings. Δ_i is the change in AIC_c values between models and wi is the Akaike's weight. Individual model code parameters are located in the methods section.

Model	R^2	AIC_c	Δ_i	w_i
pred+temp+BWP+TDS	0.62	135.11	0.00	0.57
pred+sinu+BWP	0.49	138.41	3.30	0.11
pred+temp+sinu+BWP+stream.veg	0.59	138.70	3.59	0.09
bankslope+streamdepth+BWP	0.47	139.02	3.91	0.08
sinu+temp	0.39	139.33	4.22	0.07
temp+streamdepth+substrate+BWP+speciesrich	0.54	140.33	5.22	0.04
pred+speciesrich	0.26	142.65	7.54	0.01
substrate+bankslope	0.24	143.04	7.93	0.01
TDS+streamsdepth+substrate	0.28	144.21	9.10	0.01
sinu+bankslope+substrate	0.24	145.03	9.92	0.00
streamveg+speciesrich	0.06	146.56	11.45	0.00
TDS+speciesrich	0.02	147.29	12.18	0.00
TDS+bankslope+streamveg	0.13	147.31	12.20	0.00
sinu+speciesrich	0.00	147.70	12.58	0.00
sinu+streamdepth+streamveg.	0.07	148.42	13.31	0.00

speed of this species (Prenosil et al. 2016). Estimated dominant substrate was included as Schumann et al. (2015*b*) found this to be a predictor of plains topminnow presence at site locations. Total dissolved solids (TDS) was included as plains topminnow have been associated with clear headwater streams with low TDS (Rahel and Thel 2004). Average stream depth was included because plains topminnow have been associated with shallow backwater habitats, as deeper pools have the potential for holding predator fish (Rahel and Thel 2004, Schumann et al. 2015*b*). Plains topminnow rely on instream vegetation for egg deposition and gastropod feeding (Rahel and Thel 2004, Thiessen et al. 2018), therefore estimated percent of instream vegetation was included as an explanatory variable. Species richness was included due to it being a common predictor for endemic fish presence at stream sites (Poff et al. 1997).

A total of 15 competing models were developed by the assembled review team to reflect combinations of conditions that have previously been associated with Plains topminnow CPUE (Table 2). We used Akaike's Information Criterion for small sample sizes (i.e., AICc) to rank the competing models (Burnham and Anderson 2002). Model averaging was used across all candidate models with associated parameter estimate standard error by calculating,

$$\tilde{\bar{\beta}} = \sum_{i=1}^{R} w_i \hat{\beta}_i$$

$$\widehat{var}\left(\tilde{\bar{\beta}}\right) = \sum w_i \ [\widehat{var}\ \left(\tilde{\bar{\beta}}\right) + \left(\beta_i - \tilde{\bar{\beta}}\right)^2]$$

where, $\tilde{\bar{\beta}}$ is the parameter estimate, w_i is the perspective model weight, and $\hat{\beta}_i$ is the regression estimate for model i (Burnham and Anderson 2002). We estimated the relative importance of each individual predictor variable by summing the weights of all models containing each variable (Σw_i; Burnham and Anderson 2002, 2004). Models with zero weights were omitted (Burnham and Anderson 2002, 2004). Predictor variables with the largest total weight were considered to have the greatest relative importance for explaining the dependent variable, topminnow abundance (Burnham and Anderson 2004). Ranking factors in terms of relative importance using this approach rather than making inferences from best model fit alone reduces variable selection bias and increases precision, which can be useful when multiple candidate models exhibit support of the dependent variable (Burnham and Anderson 2002, Burnham and Anderson 2004).

RESULTS

Plains topminnow relative abundance ranged from 0.0 – 243.6/100 m at the 17 sample sites (Figure 1). Abiotic conditions were variable as an eight-fold difference was noted between sites for total dissolved solids readings and a two-fold difference in recorded water temperature (Table 1). Available backwater pool habitat ranged from <1-100%, but other habitat variables like sinuosity were more consistent across sites (Table 1).

The top performing model included predator CPUE, stream temperature, backwater pool availability, and total dissolved solids (Table 2). Backwater pool availability appeared in five of the top six models, while predator CPUE was in the top three models (Table 2). Sinuosity was not included in the top model but did appear in three of the top five models (Table 2). Variable weight summation determined limited backwater pool availability (Σw_i = 0.89), increased predator fish abundance (Σw_i = 0.78), and colder stream temperatures (Σw_i = 0.77) to be the three variables with the greatest relative importance limiting plains topminnow relative abundances (Table 3). Model averaging estimates suggest low plains topminnow CPUE was best predicted by relatively high predator fish CPUE and total dissolved solids; while high plains topminnow CPUE was best predicted by increased backwater pool availability and stream temperatures (Table 3).

DISCUSSION

The anthropogenic degradation of Great Plains streams has been observed over the last century (Dodds et al. 2004) and has impacted native fishes such as the plains topminnow.

The factors suggested by this study to be limiting plains topminnow relative abundance are commonly associated with degraded prairie streams, while factors suggested to increase relative abundance are descriptive features in minimally disturbed Great Plains streams (Falke and Gido 2006, Fischer and Paukert 2008*a*). This study determined that relative abundance of reintroduced plains topminnow populations decreased with increased predator fish abundances, turbidity, and bank slope. Increased plains

Table 3. Final model averaging estimates for variables influencing reintroduced Plains topminnow abundance at 17 release sites in Nebraska, with standard error (SE), and AIC relative importance (Σw_i).

Predictor variables	Parameter estimate	SE	Σw_i
Backwater pools	0.52	0.64	0.89
Predator fish	-0.04	0.19	0.78
Stream temperature	1.63	0.38	0.77
Turbidity	-0.01	0.04	0.57
Sinuosity	-0.23	0.52	0.28
Average stream depth	-0.84	0.58	0.13
% Submerged vegetation	-0.02	0.03	0.10
Average bank slope	0.01	0.09	0.10
Dominant substrate	-0.51	0.90	0.06
Species richness	-0.05	0.26	0.06

topminnow relative abundance was higher when sites had increased backwater pool habitat, water temperatures, stream sinuosity, and submerged vegetation. Large scale alterations of Great Plains waterways have decreased shallow backwater stream habitat availability, which has shifted fish assemblages favoring lentic sport fish, introduced generalists, and decreased native fish populations (Smith et al. 2014). Collectively, this study suggests minimally disturbed stream sections may provide increased potential for higher abundances of reestablished plains topminnow populations, while the factors associated with degraded stream systems potentially limit the size of reintroduced populations. A lack in effort to recover the plains topminnow will inevitably increase considerations for Federal protection designation. However, recovery efforts have been initiated in Nebraska by reintroducing and supplementing historic locations and river drainages (Koupal et al. 2015, Schumann et al. 2017). These efforts are key to stabilizing plains topminnow populations, but also represent an avenue for better understanding what factors influence the persistence of these populations, which was the focus of this work.

MANAGEMENT IMPLICATIONS

The results of the current study suggest limited backwater pool availability, relative predator fish abundance, and stream temperature at reintroduction sites influence plains topminnow abundance post stocking. Because of our findings we suggest conservation efforts to recover plains topminnow populations should focus on these parameters by looking to maintain the natural integrity of Great Plains streams with consideration of variables like stream sinuosity. Our results also indicate abiotic conditions such as geomorphology, hydrology, and physical habitat loss limit reintroduced plains topminnow populations. Future reintroduction efforts of plains topminnow should be completed at historically inhabited sites where ample warm, backwater habitat persists with low turbidity and low predator abundance. Although the findings of this assessment resulted from reintroduced populations, the short life span of this species means that the specimens collected had not been cultured and consequently represent naturally recruited populations. Therefore, we believe the defined limitations identified in this study also persist for wild populations.

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