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Research Article

Identifying drivers of pumpkinseed invasiveness using population models

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Abstract

Pumpkinseed (*Lepomis gibbosus*) is listed among the most invasive fish species and has been demonstrated to have adverse impact on native species when present in high numbers. However, not all introductions result in high density populations. There are several possible underlying mechanisms behind this variation in population density (e.g. water quality, availability of nesting substratum and biotic interactions), but their relative importance remains poorly known. With this in mind, we studied vital rates (i.e. growth, maturation and reproduction) of pumpkinseed in 19 isolated standing waters of different pumpkinseed density. The fish were collected in early summer to determine their density, sex, age, growth and reproductive effort as well as environmental variables (i.e. availability of nesting substratum, acidity, nutrient concentrations, fish assemblage structure). To construct a population projection model with which to assess the relative importance of each vital rate for the growth of the populations, a stable population structure was assumed. Most environmental variables that affected vital rates (e.g. pH effect on individual growth) had little effect on population growth, or the associations were spurious (e.g. the negative effect of nesting substratum availability on gonad production). The environmental effects were dictated by a strong density dependent feedback of pumpkinseed density on the growth of age 2 fish, and gonad size and maturation state. This finding has important repercussions for management of pumpkinseed invasions: if only part of the population is removed or if complete eradication is followed by a re-introduction, then the population will rapidly recover to its former size. It was not possible to identify environmental drivers of pumpkinseed survival because the data had to be pooled across populations in order to estimate survival rates. However, a negative correlation was found between pumpkinseed and predator density, indicating that predator-induced mortality may be key in determining pumpkinseed invasiveness. Although the measure still needs thorough evaluation, introduction of native piscivores, especially northern pike (*Esox lucius*), may be a suitable way to prevent pumpkinseed from becoming the dominant species and reduce damage to local biodiversity.

Key words: Centrarchids, life history, non-native species, growth, reproduction, maturation, Integral Projection Model

Introduction

Non-native species can potentially exert devastating effects on native species (Clavero and Garcia-Berthou 2005; Light and Marchetti 2007) and this often depends on their abundance (Yokomizo et al. 2009). For example, in low abundances the non-native species can coexist with, and even be beneficial to native species. The pumpkinseed *Lepomis gibbosus* (Linnaeus, 1758), a North American centrarchid (Cooke and Phillipp 2009), is listed amongst the top ten non-native fishes with adverse ecological impact (Casal 2006), though the data supporting this is limited. Since the late 19th century, pumpkinseed have been introduced in various locations, but mainly in

Europe, where the species has been classified as invasive in many countries, including the Netherlands, but mainly those in the south (Cucherousset et al. 2009). Adult males excavate shallow nests on bottom substrata, primarily alluvial, where females are induced to deposit eggs. The mean age at maturity of female European pumpkinseeds is 1.7 yr (Copp and Fox 2007), and life span rarely reaches ten years (Copp et al. 2004). Primarily a species of still waters, pumpkinseed also occur in the lentic sections of streams and rivers. An opportunistic predator, the pumpkinseed feeds on all available invertebrate prey and small fishes. Pumpkinseed have been reported to be the cause of decreased water quality (Angeler et al. 2002), zooplankton biomass (Angeler et al. 2002) as

well as declines in native fish species (Prenda-Marin et al. 2003) which in water courses are likely to result from its adverse interactions with native fishes (Almeida et al. 2014). All pumpkinseed impact studies emanate from Iberia except one on pond macroinvertebrates in the Netherlands (Van Kleef et al. 2008). However, when present in low densities the ecological effects of introduced pumpkinseed on native species may be negligible or not discernable (Copp et al. 2010).

Invasiveness (defined here as high abundance) in pumpkinseed populations has been attributed to their ability to adapt morphologically and in life history traits to a variety of environmental conditions (Tomoček et al. 2007), such as different water velocities (Robinson et al. 2000), habitat structural complexity (Robinson et al. 1996) and available food resources (Robinson et al. 1993; Gillespie and Fox 2003). Pumpkinseed invasiveness in Europe has also been suggested to be induced by warmer climate, due to an increase in growth rate and a decrease in age at maturity with latitude (Copp and Fox 2007; Cucherousset et al. 2009). However, pumpkinseed abundance can vary considerably on a small geographical scale (Van Kleef et al. 2008) suggesting that environmental conditions other than climate are also influencing pumpkinseed invasiveness and that the species' ability to adapt to its environment has its limits. For example, Klaar et al. (2004) demonstrated that pumpkinseed abundance is low in streams of southern England. Densities of the species also appear to be influenced by environmental management practices (Van Kleef et al. 2008). High-impact practices such as dredging of water bodies and digging new ponds were often found to be associated with localized explosions in pumpkinseed densities. However, the driving environmental conditions behind pumpkinseed invasiveness outside of its native range are still poorly understood. This knowledge is urgently needed as it may offer new management options for controlling pumpkinseed numbers in waters where conventional eradication efforts fail.

By correlating environmental variables with pumpkinseed densities or proxies of pumpkinseed fecundity, it is difficult to identify causal mechanisms. Such an approach may even result in failure to identify key factors. To be able to compare the effects of different environmental variables on various vital rates (i.e. growth, reproduction and survival probabilities that emerge from studying a group of individuals), a proper measure of fitness is needed, rather than a proxy of fitness.

Population projection models allow for the hierarchical inclusion of the effects of environmental variables and produce population growth rates as useful fitness measures (Bakker et al. 2009; Jongejans et al. 2010). Typically these matrix projection models require the imposition of often arbitrary size boundaries, but newer versions (integral projection models, IPMs) no longer require this and therefore are better suited for species, e.g. pumpkinseed, that do not have discrete size classes (Ellner and Rees 2006; Metcalf et al. 2013).

The present study aims to identify environmental drivers of pumpkinseed invasiveness with three specific objectives: (i) test for correlations between the variability in vital rates and a set of potentially influential environmental variables; (ii) next an IPM was used to describe how age- and size-specific vital rates influence population growth; and finally (iii) the sensitivity of the model to the associated environmental variables was determined to identify key mechanisms for controlling pumpkinseed numbers.

Materials and methods

Sample collection

The study was performed in 19 isolated, standing waters in the Netherlands and Belgium in which pumpkinseed has established self-sustaining populations. These ponds and shallow lakes were selected because previous studies (Van Kleef et al. 2008) have shown them to contain different densities of pumpkinseed. To minimize the chance of including recently established and still developing populations locations were included only where pumpkinseed had been recorded at least four years earlier.

The fish population of the water bodies was censused in spring (May/June) and autumn (September) 2009 using a Deka 3000 portable electrofishing unit. In each water body a transect was sampled parallel to the shore at a depth of approximately 1 m. Thus the pond was encircled or in the case of the larger lakes a length of approximately 300 m was sampled. Actual transect length was determined using GPS and aerial photographs. Mean transect length was 260 m (min 25 m, max 550 m). Total length (TL) of native piscivorous fish was determined in cm. In spring all pumpkinseed were collected and stored in a slurry of ice water after sedation and euthanasia in a solution of water and 0.1% eugenol. After transportation to the laboratory, pumpkinseed were stored at -20°C for subsequent laboratory processing.

Environmental factors collected at each pond were chosen because they have been shown to structure fish assemblages and therefore would be likely to affect pumpkinseed vital rates. These population-level factors were: pumpkinseed density, predator density and availability of suitable nesting sites (Danylchuk and Fox 1996). Other factors that are highly variable and may influence eco-system function in moorland pools, where most pumpkinseed in Belgium and The Netherlands have established populations, are nutrients (Robillard and Fox 2006) and pH (Leuven and Oyen 1987).

At each pond a surface water sample was taken in an iodated polyethylene bottle. Water pH was measured within 24 h of collection. After filtering (Whatman GF/C filter) and adding 1 mg of citric acid per 25 ml of water, samples were stored at -20ºC until further analysis. The following concentrations were determined colorimetrically; NO_3^- (as per Kamphake et al. 1967) and NH4+ (as per Grasshoff and Johanssen 1972), using a Bran and Luebbe, TRAACS 800+, and ortho-PO_4^{3-} as per Henriksen (1965), using a Technicon AA II system. Concentrations of nitrate and ammonium were summed to calculate total nitrogen concentration.

In these moorland pools, >90% of pumpkinseed nests are located near the bank, where water depth is < 40 cm and 95% of the nests have < 6 cm of organic layer covering the substratum, which is mainly sand (unpubl. data H. van Kleef). In July 2009 a census of bank suitability for pumpkinseed reproduction was undertaken along transects perpendicular to the pond shore line with four transects taken in small water bodies (0.1 ha) and seven in the largest (6 ha). Each transect was 30 m long and at 1-m intervals depth and thickness of the organic sludge layer were determined. At each location, bank suitability was calculated as the percentage surface area with water depth < 40 cm and sludge depth < 6 cm.

Pumpkinseed density was calculated as the number of pumpkinseed caught per 100 m transect. The fresh weight of captured piscivorous species was estimated from published length-weight relationships (Klein Breteler and de Laak 2003) using recorded total length (TL) values. Recorded piscivorous species were European perch *Perca fluviatilis* Linnaeus, 1758, northern pike *Esox lucius* Linnaeus, 1758 and black bullhead *Ameiurus melas* Rafinesque-Schmaltz, 1819. For each study site total predator density was calculated as the estimated weight of piscivorous fish species per 100 m transect and the mean of the censuses in

spring and autumn. Avian and mammalian predators were not considered in the study as they are either extremely scarce or absent in the study area and their impact on pumpkinseed populations is expected to be nil.

Population characteristics

Collected pumpkinseed were processed as per Fox (1994) to determine population characteristics, which included measurements of TL (nearest mm) as well as wet body weight (FW) and gonad weight (GW) to 0.1 g and 0.01 g accuracy, respectively. Each fish was dissected in order to determine sex and maturation status. A minimum of ten scales was collected in the anterior part of the fish, ventrally of the lateral line. Age of each pumpkinseed was determined as described by Steinmetz and Müller (1991) using a Consolidated Micrographics model 9785 microfiche reader. Age determinations were checked by a second person on a subset of specimens. The linear relationship between scale radius and TL was used to back-calculate size at age. For all populations combined a fixed intercept of 18.3 mm was used (Fox and Crivelli 2001).

To allow comparison of the studied populations with other European populations we calculated mean pseudo-gonadosomatic index (PGSI), mean age at maturity (AaM) and mean TL at maturity (LaM). PGSI was calculated for mature females as the fresh gonad weight expressed as percentage of total body weight. AaM of females was calculated following Fox (1994):

$$\alpha = \sum_{x=0}^{w}(x)\,[f(x) - f(x-1)]$$

where α is the mean age at maturity, x is the age in years, f(x) is the proportion of fish mature at age x, and w is the maximum age in the sample. A modified version of this formula, using 10 mm TL intervals in place of age-classes (Trippel and Harvey 1987), was used to calculate mean TL at maturity (LaM) according to Fox and Crivelli (2001).

Statistical analysis

An Integral Projection Model (IPM; Easterling et al. 2000; Ellner and Rees 2006) of the population dynamics of pumpkinseed was constructed. IPMs are population transition models with a continuous state variable (in our case body size) and a discrete time step (in our case one year). The moment of population census and therefore the start of the

annual time step in our IPM was spring. More precisely, the state variables in our IPM were age (1, 2, 3, and 4-year olds) and the TL of the fish as continuous size variable within each of the four age classes. Our IPM was thus defined by a set of age-dependent continuous functions of individual size (i.e. 'vital rate' functions in the IPM literature; Metcalf et al. 2013), as summarized below. To use the analytical toolbox of matrix algebra we had to parameterize a large matrix model with the vital rate functions in our IPM (as is common practice when analysing IPMs). To discretize the continuous size axes, we subdivided each age-dependent size axis in 50 size classes of equally width from 1 and 15 cm, resulting in a 200×200 transition matrix.

Since we wanted to see how environmental variables affected the vital rates and hence the pumpkinseed population dynamics, we chose to analyse each vital rate across all populations. Thus rather than parameterizing an IPM for each of the 19 populations, we constructed one general IPM based on all data. In most of the vital rate analyses (described below) we analysed data on individual fish (rather than using population means) and accounted for the nested character of the data by including location as a random factor.

Since the populations were monitored only once (in spring 2009) and the captured fish were removed, the IPM could not be parameterized with mark-recapture analyses. Instead, the parameterization of the survival and growth rates were based on back-calculated growth of the captured pumpkinseeds (embedded in the annual growth 'rings' of their scales) and on the assumption that the age and size structure of the populations was stable. This assumption, unlikely to be true in most cases due to temporally varying environmental conditions, was necessary because the only population data with which to estimate survival rates were the observed age-class distributions. The estimated survival rates were therefore rough estimates, but nonetheless necessary for the exploratory population modelling.

For the juvenile (1+) fish we tested whether their TL in spring 2009 was a function of explanatory environmental variables, i.e. pumpkinseed density, predator density, bank suitability, phosphate, total N and pH, with location as a random factor - using the 'lme' function of the 'nlme' package in R (R Core Team 2013; Pinheiro et al. 2013). The maximum likelihood method was used to find the most parsimonious model of offspring TL from a set of models that included the full model with all explanatory variables and a range of other models with fewer variables. The environmental variables were normalized by dividing the deviate from the mean by the standard deviating prior to analysis, with the strongly left-skewed variables (pumpkinseed density, predator density, phosphate concentration, and total N) being ln-transformed prior to normalisation. Correlations between the environmental variables were weak, with the strongest (-0.6) and only significant correlation being between pumpkinseed density and predator densities and these did not affect this or subsequent models (i.e. variance inflation factors were < 3 in all cases).

To estimate annual growth, we back-calculated fish TL at 12 months prior to their capture date in spring 2009. Annual growth rings in pumpkinseed scales are formed in early spring, so TL was regressed against total scale length in spring 2009, and then fitted models in which the total scale length was taken as the length from the scale origin to the ring of the preceding growth year (2008–2009). Using these relationships and the observed lengths of the last spring-to-spring scale increments, we estimated the previous (spring 2008) TL of each caught fish. For each age class separately, fish TL in spring 2009 was then modelled as a function of their estimated TL a year before, of previous TL squared, and of the explanatory environmental variables (again with 'lme', maximum likelihood, and location as random factor).

For each age class, we analysed the presence of female gonads in individual fish as a function of current fish TL and of the explanatory environmental variables. This method combined two probabilities: whether a fish was female and whether a female fish produced gonads. We modelled these binomial data of female gonad presence with the mixed-effects model function 'lmer' of the 'lme4' R-package (Bates et al. 2013), with location as random factor.

For each age class and for the subset of females with gonads, gonad production was modelled using the ln-transformed wet GW as a function of their current TL and of the environmental variables. We assumed a normal error distribution and fitted mixed-effect models ('lme') with location as random factor.

Next, to estimate offspring number from GW for each location, we calculated the ratio of the number of offspring (1+ fish) and the total female wet GW along a transect. This ratio thus provided an estimate of the mean number of

offspring per g of wet GW. The between-locations variation in this offspring-gonad ratio was then linearly regressed ('lm') against the explanatory environmental variables. Please note that, in the absence of data from multiple years, we related total GW found in spring 2009 to the number of recruits, also in spring 2009. This means that we had to assume population age and size to be stable and that we could estimate total GW in spring 2008 from that of 2009.

Since the populations were monitored only once, survival of individual fish was estimated using the relative frequency of fish in each age-class to estimate the regression parameters of a model in which annual survival is related to fish TL. Again, stable population size and age-structure were assumed. These analyses were carried out separately for 1, 2 and 3-year old fish, with data from all 19 locations combined. We used the R-function 'optim' to find those binomial regression parameters of the survival function that optimized its fit. To assess the fit of a survival model to the observed fish TL distribution and number of one-year older fish (given the number and distribution of fish in a particular age class), the 'optim' function was set to search for those survival model parameters which minimized the sum of three statistics. These three statistics were the relative differen-ces (between model expectation and observation) in next-year's population size, next-year's mean TL of the survivors, and the standard deviation of that mean. Individual growth of the survivors was modelled in this optimization analysis using the growth models (based on annual scale growth) introduced above. This optimization converged for the survival functions of 1- and 3-yr olds, but not for 2-yr olds, because (summed over all populations) we caught more 3-yr old fish than 2-yr old fish. Because mean survival was biased by this approach, these survival functions were rescaled to realistic values. This rescaling was done by adjusting the entire size-dependent survival function with a certain factor. This adjustment factor was calculated by dividing a) the survival estimate of a simple (size-independent) mean model by b) the above model's prediction for the survival rate for individuals of median size. Overall mean survival was estimated by the slope of an exponential regression fitted to the number of caught fish per age class. We estimated the survival of 2-yr olds by calculating the mean for survival estimates of 1- and 3-yr olds for each size bin.

With the vital rate functions we constructed the general IPM (outlined above) using the R package *IPMpack* (Metcalf et al. 2013). As a robustness test we also repeated the vital rate analyses and IPM construction with 19 subsets of the raw data: from each subset we removed the data from a different population. With this robustness test we checked whether or not data from a single population strongly affected our findings and conclusions. Using the environmental variables mean values we calculated the projected population growth rate (λ, i.e. the dominant eigenvalue) and the associated stable stage distribution and elasticity matrix (De Kroon et al. 2000). We also calculated vital rate elasticity values by numerically multiplying vital rates one at a time by a factor 1.001 and evaluating the relative increase in λ by this relative increase in a vital rate. Elasticity values quantify the relative sensitivity of λ to small proportional changes to the model parameters. The advantage of elasticity values is that they can be interpreted as relative contributions to population growth, and that they can be directly compared between survival, growth and reproduction rates. Finally, we studied the effect of each environmental variable by calculating λ over the range of observed values of that variable (see e.g. Dahlgren and Ehrlén 2009; Hegland et al. 2010).

Results

Pumpkinseed density in the studied water bodies was highly variable (Table S1). This was also the case for predator densities, which were absent from many locations but could also reach densities of 1088 g·100 m^{-1} sampling transect. The most abundant predator was northern pike, which was recorded at nine sites representing 93% of total predator biomass (Table S1). The second most abundant predator was Eurasian perch (five sites), followed by black bullhead (two sites). Surface area of the water bodies varied from less than a hectare to multiple hectares. Maximum depth was less variable as most water bodies < 1.5 m deep. Most study sites had low concentrations of phosphate (ortho-PO$_4$ < 0.5 µmol·L^{-1}). Nitrogen on the other hand could reach relatively high concentrations (> 40 µmol·L^{-1}). Pond pH ranged from acid (pH 4.6) to highly alkaline (pH 9.7), with most sites being slightly acid to neutral. Bank suitability on average was 39% (8–100%), providing plenty of reproduction sites for pumpkinseeds on most locations.

Table 1. Mean TL at age and the number (given in parenthesis) of pumpkinseed caught at each age in ponds in the Netherlands and Belgium (*), with the mean pseudo-gonadosomatic index (PGSI) in % for mature females as well as mean age at maturity (AaM) in years and mean TL at maturity (LaM) in mm of female pumpkinseed.

Site	Mean TL at age (N)					PGSI	AaM	LaM
	1	2	3	4	5			
Dommeldal pond 1	44.7 (35)	76.6 (4)	105.1 (15)			7.0	2.0	69.3
Dommeldal pond 2	34.9 (0)	71.5 (18)	98.7 (10)			3.1	2.0	70.0
Grootmeer	36.7 (77)	80.2 (11)				6.7	1.8	75.0
Grote Klotteraard*	45.4 (5)	86.3 (3)	109.6 (16)	130.0 (1)		10.0	1.6	90.0
Kranenbroekerpoel	33.0 (344)	58.5 (0)	87.5 (2)			3.1		
Kranenbroekerven	35.2 (263)	65.5 (2)	81.5 (100)	97.0 (1)		4.9	1.8	70.7
Woldersven	38.7 (51)	75.9 (14)	96.4 (14)	105.8 (20)		7.8	2.0	65.0
Meeuwven	35.5 (63)	53.6 (42)	69.8 (18)			2.1	2.8	65.7
Gitstappermolen	35.1 (7)	56.7 (23)	77.1 (19)			6.4	2.0	60.0
Rietven	32.0 (566)	53.5 (13)	64.7 (21)	71.2 (5)		2.8	2.2	60.0
Schaapsloopven	41.5 (5)	76.3 (2)	118.3 (4)	138.0 (1)		7.4		
Schoapedobbe	38.1 (11)	79.1 (47)	119.0 (0)	140.0 (0)	150.0 (1)	8.4	1.8	68.0
Uden pond 1	42.9 (87)	88.3 (17)				10.1	2.0	85.0
Uden pond 2	34.0 (56)	59.0 (5)	73.4 (15)	86.0 (24)		8.4	2.5	65.0
Uden pond 3	35.0 (1)							
Uden pond 4	34.0 (22)	45.5 (155)	58.6 (145)			1.2	1.6	51.7
Uden pond 5	29.9 (0)	55.7 (0)	89.1 (10)			2.8		
Uden pond 6	36.1 (16)	87.3 (0)	101.7 (40)			10.1		
Zwart water*	38.3 (1)	74.9 (7)	101.0 (34)	124.0 (2)		8.4	2.0	75.0

Growth, reproduction and maturity varied strongly between the studied pumpkinseed populations (Table 1). Mean TL at age 1 was as low as 30 mm in Uden pond 4 and as high as 46 mm in Grote Klotteraard. Mean TL at age 2 (i.e. when most fish become mature, Copp and Fox 2007), was 69 mm and varied between 46 mm and 87 mm. The oldest and largest fish were caught in the moorland pool Schaopedobbe. Reproductive investment of mature female pumpkinseed varied between 1.2 and 10.1% of the total body weight. Age and TL at maturity could not be calculated for five populations when mature females were not equally distributed among age classes. Fastest maturation was recorded in the Grote Klotteraard and Uden pond 4, with mean age at maturity of 1.6 years. Slowest maturation of mean age at maturity of 2.8 was recorded in the Meeuwven. Mean TL at maturity of female pumpkinseed was 70 mm (min = 52 mm, max = 90 mm).

Vital rate functions

Size (i.e. TL) of first-year fish (Table 2) was not related to any of the studied explanatory variables and was therefore described as a normal distribution with a mean of 37 mm (SD = 5.6 mm). The growth functions, which modelled the following year's size for age 1, 2 and 3 fish, all contained this year's size: fish that were large were also above-mean a year later. The growth of age 2 fish was further explained by pH (a positive effect) and pumpkinseed density (a larger, negative effect; Table 2).

The probability that females were mature was a significantly positive function of their size at age 1 and 2 (although age 2 fish also had a significantly negative 2nd order size term), but a significantly negative function in 3-yr olds, and no relationship with size in the oldest size class (Table 2). Pumpkinseed density increased the female reproduction probability of age 1 fish, but significantly reduced that of age 3 fish. Larger reproductive females of any age produced heavier gonads (Table 2). Pumpkinseed density reduced gonad weight in age 2 and 4 fish, whereas predator density had a positive effect in 2-yr olds, and bank suitability a smaller negative effect, also in 2-yr olds. Over all populations, 10 young (age 1) fish in spring were produced per gram of female gonad the year before.

Mean annual survival was estimated as 36%. The resulting survival function of age 1 fish showed a negative relationship with TL, ranging from 47% for the smallest 1-yr olds (22.7 mm) to 12% for the largest (62.7 mm). The survival function of age 3 fish, however, was estimated to

Table 2. Vital rate models for the Integral Projection Model (IPM). The continuous state variable 'size' represents the TL of the fish. Explanatory variables are: pumpkinseed density and predator density (ln-transformed and normalized); bank suitability and pH (normalized only). Behind regression parameter and residuals (σ) upto two characteristics are given between curly brackets: standard error (SE) and the λ-elasticity value (e; calculated with all IPM-covariates set to zero).

Vital rate	Function
Offspring size distribution	size = 37.14{SE=1.02;e=-0.124}, σ = 5.57{e=-0.005}, n = 569
Next year's size of surviving age 1 fish	sizeNext = 35.83{SE=5.39;e=0.391} +0.79{SE=0.10;e=0.321} size, σ = 7.84{e=0.033}, n = 214
Next year's size of surviving age 2 fish	sizeNext = 34.01{SE=3.30;e=-0.041} +0.84{SE=0.04;e=-0.074} size -9.41{SE=1.69} pumpkinseedDensity +4.49{SE=1.54} pH, σ = 5.29{e=-0.004}, n = 260
Next year's size of surviving age 3 fish	sizeNext = 8.43{SE=2.48;e=0.009} +1.02{SE=0.03;e=0.083} size, σ = 3.10{e=-0.001}, n = 50
Reproduction probability of age 1 fish	logit(probRepr) = -14.08{SE=2.52;e=-0.002} +0.19{SE=0.05;e=0.001} size + 2.73{SE=0.84} pumpkinseedDensity, n = 569
Reproduction probability of age 2 fish	logit(probRepr) = -6.51{SE=2.24;e=-0.636} +0.18{SE=0.07;e=1.202} size -0.0011{SE=0.0005;e=-0.541} size^2, n = 214
Reproduction probability of age 3 fish	logit(probRepr) = 7.89{SE=1.58;e=0.303} -0.097{SE=0.018;e=-0.323} size -1.33{SE=0.48} pumpkinseedDensity, n = 260
Reproduction probability of age 4 fish	logit(probRepr) = -0.66{SE=0.30;e=-0.037}, n = 50
Gonad production by reproductive age 1 females	ln(gonadWeight) = -7.97{SE=0.65;e=-0.001} +0.096{SE=0.015;e=0.001} size, n = 16
Gonad production by reproductive age 2 females	ln(gonadWeight) = -4.47{SE=0.46;e=-1.023} +0.051{SE=0.006;e=0.805} size -0.74{SE=0.14} pumpkinseedDensity +0.38{SE=0.12} predatorDensity -0.20{SE=0.07} bankSuitability, n = 89
Gonad production by reproductive age 3 females	ln(gonadWeight) = -2.21{SE=0.70;e=-0.150} +0.021{SE=0.008;e=0.120} size, n = 102
Gonad production by reproductive age 4 females	ln(gonadWeight) = -8.87{SE=3.42;e=-0.747} +0.19{SE=0.07;e=1.568} size -0.00088{SE=0.00039;e=-0.731} size^2 -1.58{SE=0.45} pumpkinseedDensity, n = 17
Number of offspring per g gonad	nOffspringPerGonadWeight = 10.0{e=0.382}, n = 19
Survival probability of age 1 fish	survival = 0.68{e=0.418} (exp(2.09{e=0.062} -0.060{e=-0.154} size) / (1 +exp(2.09{e=0.062} -0.060{e=-0.154} size))
Survival probability of age 3 fish	survival = 2.12{e=0.200} (exp(-1.59{e=0.000} +0.00020{e=0.000} size) / (1 +exp(-1.59{e=0.000} +0.00020{e=0.000} size))

be virtually size-independent, ranging from 36.2% for 48.8 mm small fish to 36.7% for 128.8 mm large fish. Estimated survival was based on the data of all 19 locations combined, preventing estimation of the effect of environmental variables. Correlations between these factors and pumpkinseed density revealed a significant negative correlation between pumpkinseed and predator densities (r = -0.60, P = 0.0073), indicating a negative influence on survivorship. The robustness tests, in which we excluded data from single populations, showed that none of the populations had a strong effect on our analyses: no matter which population we excluded from the analyses, positive regression parameters always stayed positive, while negative parameters remained negative.

Population-level effects of environmental variables

The starting point in the IPM for determining the effects of environmental variables on projected population growth rate (λ) was the mean scenario
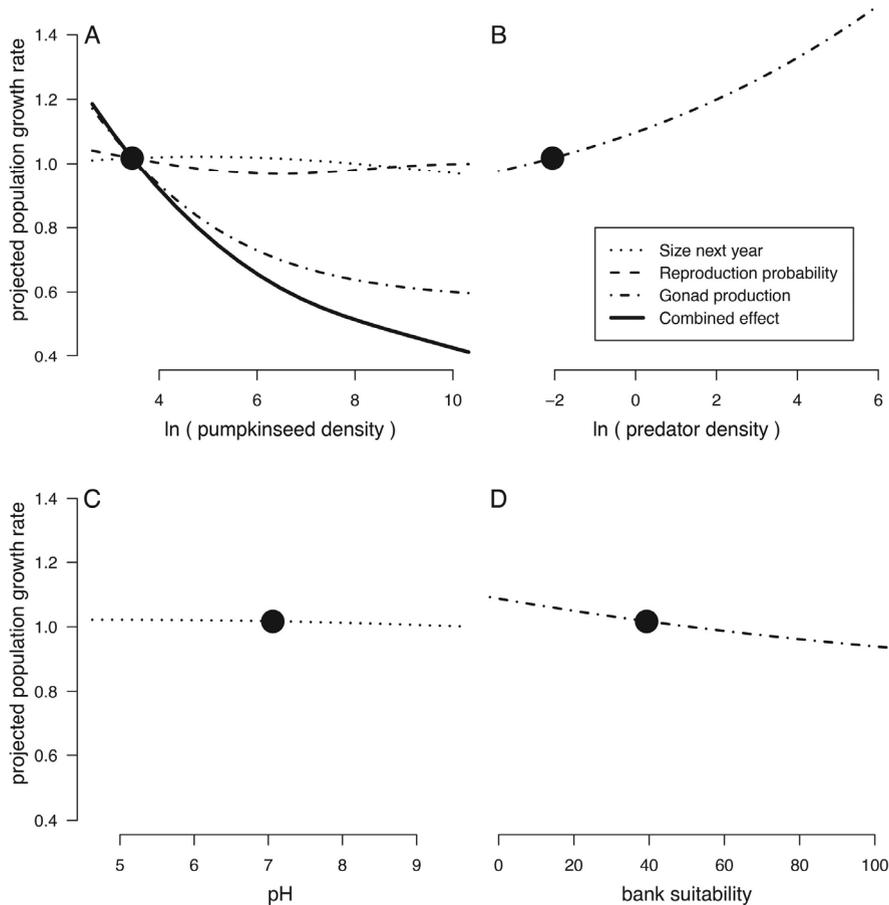
Figure 1. Population model (IPM) projections of pumpkinseed population growth as a function of pumpkinseed density (A), predator density (B), pH (C) and bank suitability for nesting (D). Effects on population growth through either effects on next year's size of the fish, on reproduction probability and on their gonad production are shown separately (interrupted lines). As pumpkinseed density significantly affected multiple vital rates, the combined effect in panel A (thick line) is also shown. The large dots indicate the location of the mean values for the environmental factors across populations, corresponding with a projected population growth rate of 1.016.

in which all environmental variables were set to zero. This resulted in $\lambda = 1.016$, i.e. a modestly growing population, with survival and growth contributing 68% and reproduction 32% (based on λ-elasticity sums over the survival-growth and reproduction kernels of the IPM). The numerically-calculated vital rate elasticity values were -0.123 for the offspring size distribution. The population model projected a stable age distribution of 70% for age 1, 23% for age 2, 5% for age 3 and 2% for age 4 fish.

The only significant effect of pH was on the growth of age 2 fish (Table 2). However, when λ was calculated over the range of observed pH values (4.6 to 9.7) while keeping other environmental variables constant, population-level effects

were negligible (Figure 1C). Bank suitability negatively correlated with gonad weight of reproductive age 2 females, which did have an effect on λ: 8% bank suitability resulted in $\lambda=1.07$, whereas 100% bank suitability resulted in 0.94 (Figure 1D). However, this correlation is likely spurious as females spend most of their time away from the spawning grounds. Predator density, on the other hand had a positive effect on the gonadic weight of reproductive 2-yr olds - from $\lambda=0.98$ when no predators were present to $\lambda=1.48$ for the observed maximum observed predator abundance of 12.4 kg $\cdot$ 100 m^{-1} (Figure 1B).

The largest impact, however, was related to pumpkinseed density, which had a significant influence on five vital rate functions (Table 2;

322

Figure 1A), i.e. next year's size of age 2 fish, reproduction probability of age 1 and age 3 fish, gonad production of age 2 and age 3 fish. The greatest negative effect was on the gonadic weight of age 2 females, Although there was a slight buffering effect through the positive effect of pumpkinseed density on the reproduction probability of age 1 fish (Figure 1A), the joint effect of pumpkinseed density was very negative: from $\lambda=1.18$ when pumpkinseed density was at its observed minimum (1 fish · 100 m^{-1}) to $\lambda=0.41$ at maximum pumpkinseed density (1288 · 100 m^{-1}).

Discussion

Vital statistics, such as growth, maturation and reproduction, determine the population growth trajectory of species. For pumpkinseed, the relationship between juvenile growth and age at maturity has been proposed as predictor of the species' invasion potential (Copp and Fox 2007; Cucherousset et al. 2009). Following this hypothesis, nearly all of the populations in our study were transitional between invasive and non-invasive populations. However, the potentially high invasiveness of the Dutch and Belgian populations is not unequivocally supported by the highly variable pumpkinseed population sizes that were found in this study. Apparently, there were environmental factors that limited pumpkinseed abundance in a number of the studied water bodies.

We were able to construct a population model of Dutch and Belgian pumpkinseed using a one-time population census along with determination of age, sex, fecundity and growth of the captured fish. According to our model, different environmental factors correlated with pumpkinseed vital rates and influenced projected population growth. Most interactions between vital rates and environmental variables either had little effect on population growth, such as effect of pH on the growth of age 2 fish, or they were likely spurious, as observed for the negative effect of reproduction site availability. Correlations of pH and bank suitability were overshadowed by strong density-dependent feedback of pumpkinseed abundance on the growth of age 2 fish, gonad development and gonad size. Predator density had a positive effect on gonad development. It is unlikely that this is a direct effect. More likely, predators are able to reduce pumpkinseed numbers, thereby increasing resource availability of the remaining pumpkinseed and increasing gonad production.

High population densities have been associated with slow growth for European pumpkinseed (Fox and Crivelli 2001; Copp et al. 2002), with resource competition being a plausible mechanism for the observed density dependence. The species exhibits an ontogenetic diet shift, with larger prey becoming more important as the fish grow larger (García-Berthou and Moreno-Amich 2000; Van Kleef et al. 2008). However, when pumpkinseed become dominant, the abundance of potential prey strongly decreases (Van Kleef et al. 2008). Eventually, dissimilarity in diet between fish of different sizes disappears (Copp et al. 2002), probably resulting in increased intraspecific competition. Indeed, resource competition among pumpkinseed has been demonstrated to occur in its native range when the species is abundant (Hanson and Leggett 1986), resulting in weight loss (Hanson and Leggett 1985).

The observed density dependent feedback on reproduction will have repercussions for the management of invasive pumpkinseed populations. Incomplete eradication efforts will be followed by a rapid recovery due to an increase in reproduction and subsequent population growth rate (Figure 1A). Identical density-dependent effects have been recorded for commercial fishing (Rochet 1998), where growth and maturity increased following harvesting part of the population. Even when eradication is complete, it is possible that some pumpkinseed populations will quickly recover as repeated introductions have been shown to be highly likely (Van Kleef et al. 2008). Therefore, identifying external factors that regulate pumpkinseed abundance still has a high priority. Unfortunately, for our population model we could not identify clear determinants of pumpkinseed population growth other than the density of the population itself. This conclusion does not correspond to field observations over multiple years, where populations with apparently continuous low densities were found (Van Kleef et al. 2008).

One of the limitations of our approach in constructing a population model based on a one-time census was that data from all populations had to be pooled despite the survival functions being size- and age-dependant. Pooling of the data was necessary because fish numbers rarely declined continuously with age within the data from single populations. A continuously-declining (and stable) age-structure is a requirement when estimating survival from a one-time census (Huijbers et al. 2013). As a consequence of data pooling we could not determine whether survival depended on pumpkinseed or predator densities

or on other environmental factors. However, the observed negative correlation between pumpkinseed density and predator density may indicate a possible role of predators lowering survival rates in pumpkinseed. This is even more likely because the only effect of predator density that we found in the analyses of the other vital rates was in the opposite direction: gonad production (and population growth) increasing with predator density (Figure 1B).

In our estimation of survival rates we assumed a stable age distribution. However, many of the studied populations had skewed population structures with fish of some older age classes being more abundant than younger ones (Table 1). Such large differences from the projected stable stage distribution of our overall population model suggests that age- and size-dependent survival varied between years and populations. Mechanisms that can cause such fluctuations in age/size-dependent survival are variability in dietary overlap of age-classes (Copp et al. 2002) or cannibalism (Guti et al. 1991; Godinho et al. 1997; Copp et al. 2002). It is likely that these mechanisms are especially important when pumpkinseed densities are high, because then resources are likely to be depleted and the density of juvenile pumpkinseed high.

The negative correlation between pumpkinseed and predator densities (potentially indicating lowered survival rates caused by predators), and the effects of predator and pumpkinseed densities on gonad production, both point to potential pumpkinseed control by these predators. Of the two piscivorous species frequently recorded, northern pike was the more abundant both in number and in biomass. Northern pike begins preying on fish between 4 to 8 cm TL (Paat 1988), whereas perch switches from invertebrates to fish between 18 and 40 cm TL (Thorpe 1977). Northern pike is predator of pumpkinseed both in its native range and Europe (Gutti et al. 1991), making it most suitable of native piscivorous fishes for biological control of pumpkinseed in Europe. These results have already persuaded managers of several pumpkinseed-infested water bodies to plan introduction of northern pike as a biological control agent. However, the diet of pike is not restricted to pumpkinseed (Beyerle and Williams 1968; Mauck and Coble 1971). Introducing pike may be a suitable option in most Dutch and Belgian pumpkinseed habitats, where other fish species are absent. However, this should be cautioned as in parts of Europe where there are no native piscivorous fishes, e.g. Iberia,

the introduction of pike or other top-predators has proved to be detrimental to native fish assemblages (Elvira 1995).

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Supplementary material

The following supplementary material is available for this article:

Table S1. Environmental characteristics of the study sites.

This material is available as part of online article from:
http://www.aquaticinvasions.net/2014/Supplements/AI_2014_vanKleef_Jongejans_Supplement.xls