VARIATION IN LIFE-HISTORY TRAITS BETWEEN A NEWLY ESTABLISHED AND LONG-ESTABLISHED POPULATION OF NON-NATIVE PUMPKINSEED, *LEPOMIS GIBBOSUS* (ACTINOPTERYGII: PERCIFORMES: CENTRARCHIDAE)

Markéta KONEČNÁ, Michal JANÁČ, Kevin ROCHE*, and Pavel JURAJDA

Institute of Vertebrate Biology, Academy of Sciences of the Czech Republic, Brno, Czech Republic

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Background. The life-history traits of non-native species are believed to change in a predictable manner in relation to time since introduction, with populations in the early stages of establishment predicted to invest more energy into reproduction than long-established populations, mainly due to lower intraspecific competition for resources. In Europe, the range of non-native pumpkinseed, *Lepomis gibbosus* (Linnaeus, 1758), continues to increase. Despite this, the majority of hitherto conducted studies have investigated long-established populations only, very few focusing on newly established populations (<10 years old) or comparisons of life-history traits of new and older populations.
Material and methods. In this study, we compared the fecundity, condition, and population structure of a new and a long-established pumpkinseed population (two- and 14-years after introduction, respectively) in two small Central European lakes in order to identify any differences in life-history traits.
Results. We confirmed that the established population displayed lower fecundity and poorer overall condition than the new population. Unexpectedly, there was no significant difference in size-at-maturity.
Conclusion. While this study confirmed that life-history traits of introduced species appear to change with time since introduction, it also emphasises the possible effect of other factors such as temperature, size of body of water, and relative predator pressure in shaping such traits.

Keywords: freshwater fish, alien species, introduced species, date of introduction, size-at-maturity, fecundity

INTRODUCTION

The majority of successful introductions of non-native species pass through three stages: introduction—when the species arrives at a new environment, establishment—in which the species successfully reproduces and rapidly increases in number, and assimilation—when the non-native species integrates into the native ecosystem (Feiner et al. 2012). During each of these stages, life-history strategies employed by the species may vary along an *r*–*K* continuum (see Fox et al. 2007). Traits typical for the establishment stage (defined by a low density of conspecifics and low competition for resources) include fast individual growth (for juveniles especially), early maturation, and high investment in reproduction. During the assimilation stage, investment into reproduction decreases and growth is limited by higher competition for food resources (Fox et al. 2007).

Successful fish invaders are mostly characterised by having suitable traits for establishment in a new environment, such as: a broad diet spectrum, wide environmental tolerance, and parental care (Garcia-Berthou 2007) or by

the ability to shift from *r*-strategy life-history traits, which are suitable for the years immediately after introduction, to *k*-strategy traits, more suitable for established populations (Feiner et al. 2012). The pumpkinseed, *Lepomis gibbosus* (Linnaeus, 1758), is a freshwater fish known for its recent successful establishment and rapid spread in many non-native areas (Fox and Copp 2014). A native of North America, the species was first introduced into European waters: Spain (Rooke and Fox 2014), France and Germany (Przybylski and Zięba 2011) during the late 19th century, since when it has spread to at least 28 European countries (Copp and Fox 2007). The species' high invasive potential is supported, at least in part, by its ability to survive and successfully establish itself in both lentic and lotic waters under a range of environmental conditions, including low or high water temperatures or hypoxic conditions (Fox and Copp 2014). As a result, the pumpkinseed is presently one of the most-frequently studied invasive fish in Europe. The majority of these studies have focused on differences in life-history traits between the various European populations (e.g., the influence of latitude and thermal regime;

* Correspondence: Dr Kevin Roche, Ústav biologie obratlovců, Akademie věd České republiky, Květná 8, 603 65 Brno, Czech Republic, e-mail: (KR) *kevin.roche@hotmail.co.uk*, (MK) *marketakon@seznam.cz*, (MJ) *janac@ivb.cz*, (PJ) *jurajda@brno.cas.cz*.

Tomeček et al. 2007) and shifts in life-history traits between native and non-native ranges (e.g., Fox and Copp 2014).

Despite distribution of pumpkinseed continuing to increase in Europe, the majority of studies have investigated long-established populations only, with very few focusing on characteristics of newly established populations (<10 years; Oliva-Paterna et al. 2005, Diripasko et al. 2007) or comparisons of life-history traits of new and older populations (Fobert et al. 2013).

In this study, we compared two pumpkinseed populations in the Czech Republic, one established in 1997 (long-established population) and the other in 2011 (new population). Based on life-history theory (Sakai et al. 2001, Bøhn et al. 2004), we hypothesised that newer pumpkinseed populations would mature younger and display increased fecundity compared with long-established populations. Further, low population density would decrease competition for resources and lead to higher body condition per fish (Carol et al. 2009). As increased energy depletion following reproduction may cause increased mortality and/or a shorter life span, however, we further predicted that newer and older populations would differ in population structure, body condition, and reproductive characteristics. We discuss the results in relation to other European pumpkinseed populations.

The goals of this work were to evaluate selected life-history traits of two pumpkinseed populations, one long-established and the other established relatively recently, and to assess such traits change with time since introduction.

MATERIAL AND METHODS

Study sites. Pumpkinseeds (*Lepomis gibbosus*) for this study were sampled from two lakes in southeast Moravia, Czech Republic: Annin (49°25′20.366″N, 17°17′49.275″E; 119 ha) and Rohlik (48°38′57.541″N, 16°55′38.807″E; 0.8 ha). Annin Lake was created in the 1950s through flooding of a gravel extraction pit. The lake is oligotrophic and has a maximum depth of 16 m. The littoral zone ranges from 0.1 m to 1.5 m and supports many macrophytes, making it suitable for pumpkinseed of all ages, along with juveniles of other fish species. The fish fauna is dominated by roach, *Rutilus rutilus* (Linnaeus, 1758), and rudd, *Scardinius erythrophthalmus* (Linnaeus, 1758), with perch, *Perca fluviatilis* Linnaeus, 1758, and pike, *Esox lucius* Linnaeus, 1758, as predators. Pumpkinseed was first introduced into Annin Lake from an unknown source (probably the River Morava or one of its tributaries) during extreme flooding in 1997. Since then, it has established itself as one of the few successfully reproducing populations in the Czech Republic.

Rohlik Lake was formed through flooding of a gravel borrow pit in 1983. It has a mean depth of 2 m and the bottom is sand–gravel with a thin layer of organic mud. Despite being shallow, the water levels never dropped below 1 m, even during the driest periods (Janáč et al. 2010). While aquatic vegetation is almost absent, numerous submerged branches and tree stumps provide sufficient

shelter for fish. Rohlik's fish fauna is dominated by bitterling, *Rhodeus amarus* (Bloch, 1782); common bream, *Abramis brama* (Linnaeus, 1758), and Prussian carp, *Carassius gibelio* (Bloch, 1782); with pike, *Esox lucius*, and wels catfish, *Silurus glanis* Linnaeus, 1758, as predators. Pumpkinseed was first registered there in 2011, following extensive local flooding (P. Jurajda, personal observation). The Dyje River is just 700 m away, suggesting this was the source of the new population.

Though both lakes are situated at the same latitude, differences in depth and surface area could have influenced water temperatures. As temperature range is an important factor for comparing populations across Europe, we monitored the annual temperature range using HOBO 64K data loggers (Onset Computer Corporation, MA, USA) placed at 0.8 m depth at each locality between April and October 2013. Though the data loggers were unavailable for monitoring temperatures at Annin Lake in 2011 (when fish were sampled), we were able to download data for this site from a Czech on-line diving website[*] for comparison (see Fig. 1).

Fish collection. Pumpkinseed were sampled from Annin Lake at the end of May 2011, and from Rohlik Lake at the end of May 2013 (in each case, prior to the predicted start of the spawning season) using a five-metre beach seine and electrofishing equipment (Lena, Bednář Olomouc, Czech Republic). All fish caught were euthanized with an overdose of clove oil, placed in crushed ice, and transported to the laboratory for further analysis.

Ethical issues. The presently reported study has been carried out in strict accordance with the Czech Law No. 246/1992 about "Animal welfare".

Assessment of size, age, sex, and sexual maturity. In the laboratory, all fish were measured to the nearest 0.01 mm of total length (TL) and standard length (SL) using digital callipers and weighed to the nearest 0.01 g of total weight (W_T). The age of each fish was determined by counting the annuli on scales. Scales were removed from the left side of the body, between the lateral line and dorsal fin, cleaned and placed between two glass slides, and allowed to dry.
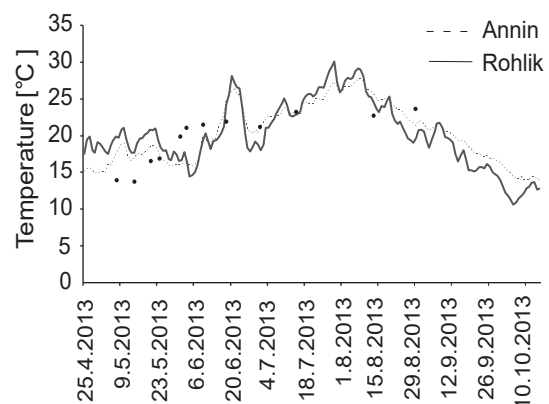
Fig. 1. Water temperature regime for Annin (dashed line) and Rohlik (solid line) lakes in 2013; Individual points indicate separate temperature measurements for Annin in 2011

The scales were examined by two independent observers using a microfiche projector. The fish were then dissected to remove the gonads and gastrointestinal tract to determine the eviscerated weight (W_{ev}) and the gonad weight (W_G). Sex and maturity of each fish was determined during dissection based on the presence and type of gonads. Fish with no-, indistinguishable-, or very small and clearly immature gonads (according to colour in males; colour and absence of mature or maturing/vitellogenic oocytes in females) were considered as immature juveniles. The proportion of each sex (juveniles excluded) was used to calculate the population sex ratio.

Assessment of body condition and fecundity. Changes in body condition were expressed using Clark's condition coefficient (K_C) (Clark 1928), expressed as

$$K_C = 100\,000\,W_{ev}\cdot TL^{-3}$$

where W_{ev} represents eviscerated fish weight and TL is total length. Gonad development in females was determined by the gonadosomatic index (GSI) (West 1990), expressed as

$$GSI = 100\,W_G\cdot W_T^{-1}$$

where W_G represents weight of gonads and W_T is total weight. Fecundity was assessed through a detailed study of the female gonads. Each ovary was removed, opened, and spread thinly on a viewing slide. An image of each opened ovary was obtained using a uEye-1540C digital camera coupled with an Olympus SZX7 binocular microscope (20×, 25×, and 32×). The image was then examined using LUCIA 5 image analysis software (Laboratory Imaging Ltd.) to obtain the number and precise measurements of the oocyte size. Individual oocyte developmental stage was characterised according to Burns (1976), and Santos et al. (2012) as previtellogenic (primary growth), cortical alveoli, vitellogenic, or mature. For each female, absolute fecundity was assessed as the total number of oocytes in the ovaries minus previtellogenic oocytes.

Data analysis. The effect of fish size, site and their interaction on female fecundity, and female GSI was revealed using analysis of covariance (ANCOVA). All data complied with the assumptions of ANCOVA (normality, homoscedasticity) and the validity of the model was confirmed through residual analysis (Shapiro test at α level of 0.05 for normality of residuals; visual check for absence of trend in residuals vs. fitted values). Any significant difference in temperature range between the two sites was assessed using the paired Wilcoxon test as data did not comply with the assumptions of parametric tests (normality). The chi-square test was used to evaluate any deviation in the sex ratio from 1 : 1. Any effect of site on body condition was tested for on juveniles, males, and females separately. As the data did not comply with the assumptions of parametric tests (normality, homoscedasticity), effect of site on body condition was revealed using the non-parametric Mann–Whitney test. The effect of fish size, site, and their interaction on size-at-maturity was revealed using a generalised linear model (GLM; binomial distribution of data; model design corresponding to ANCOVA). For each site, GLM was used to estimate the size at which 10%, 50% (size-at-maturity for comparison with literature), and 90% of fish became mature (SL10, SL50, and SL90, respectively).

RESULTS

At Annin Lake 198 pumpkinseed were caught (81 females, 90 males, and 27 juveniles) with an adult female : male sex ratio of 1 : 1.1. Sixty-four fish were sampled from Rohlik Lake (15 females, 24 males, and 25 juveniles), with a female : male sex ratio of 1 : 1.6. At both sites, there was no significant deviation from a 1 : 1 sex ratio (Annin, $\chi^2 = 0.24$, $P > 0.05$; Rohlik, $\chi^2 = 1.04$, $P > 0.05$). Juveniles represented 13% of all pumpkinseed caught at Annin and 39% at Rohlik. Scale reading indicated four age groups in both populations (Fig. 2), though the proportions of each age group differed at each site. More than 70% of fish sampled at both localities were in their second (1+) or third (2+) year of life. In the newly established population at Rohlik, 1+ fish constituted 20% and 2+ fish—50% of the pumpkinseed caught. In the long-established population of Annin 1+ fish represented just 5% of fish caught and 2+ fish—65% (Fig. 2). At both localities, all 1+ fish were juveniles; however, just 10% of 2+ fish at Annin and 19% of 2+ fish at Rohlik were juvenile, indicating that the majority of fish reached maturity in their third year. Whilst the majority of immature 1+ fish and older individ-
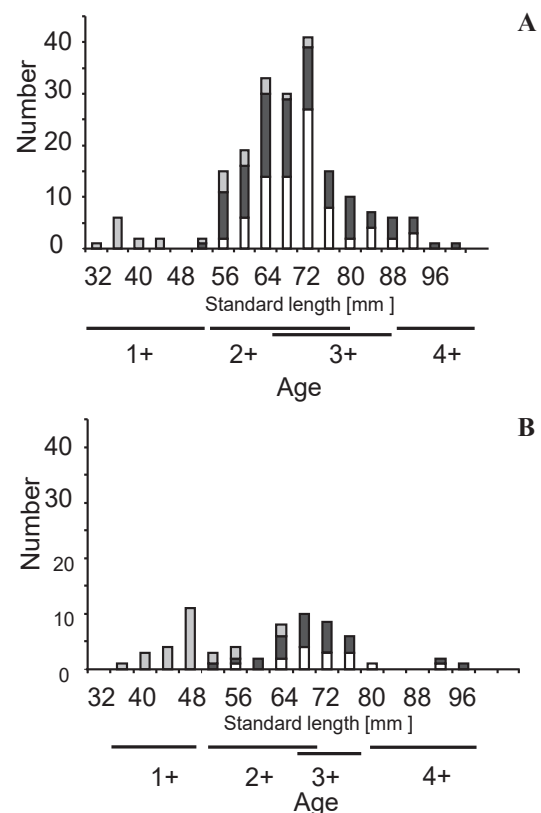
Fig. 2. Standard length frequency distribution of pumpkinseed, *Lepomis gibbosus*, at Annin (**A**) and Rohlik (**B**) lakes (white bars = females; black bars = males, grey bars = juveniles)

uals (4+) could be clearly identified in the length frequency histograms for both localities, there was considerable overlap in 2+ and 3+ fish of 64–80 mm at Annin and for 2+ and 3+ fish of 68–70 mm at Rohlik (Fig. 2).

No significant difference in daily temperature range was observed between the two sites (Wilcoxon paired test, $V = 6883$, $n = 348$, $P = 0.273$; Fig. 1). The GLM model (Fig. 3) showed no significant effect for both site and size–site interaction (effect of site, df = 1 and 255, $P = 0.241$, effect of size–site interaction, df = 1 and 255, $P = 0.175$), thereby indicating no significant difference in size-at-maturity between the two sites. Based on the GLM model, SL_{50} at Annin was slightly lower than that at Rohlik (51.1 mm compared to 55.0 mm; $SL_{10} – SL_{90}$ range = 40.7–61.5 for Annin and 48.3–61.8 for Rohlik; Fig. 3).
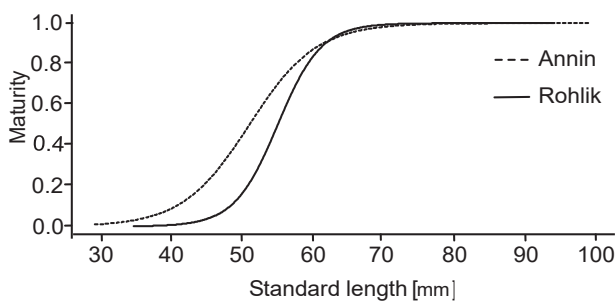
Fig. 3. Generalised linear model predictions of maturity of pumpkinseed, *Lepomis gibbosus*, (i.e., proportion of mature females) as a function of fish length at Annin (dashed line) and Rohlik (solid line) lakes; Model formulae: Annin, $y = 1 \cdot (1 + e^{(10.7866 – 0.2112x)})^{-1}$; Rohlik, $y = 1 \cdot (1 + e^{(17.92078 – 0.32563x)})^{-1}$

There was no significant difference in female GSI between Rohlik and Annin (ANCOVA, effect of site, $F_{1, 92} = 0.45$, $P = 0.506$; Fig. 4). There was, however, a significant difference in the relation between GSI and fish size between the two sites (ANCOVA, effect of site–size interaction, $F_{1, 92} = 13.28$, $P < 0.001$; Fig. 4), with GSI increasing significantly with body length at Rohlik (linear regression, $F_{1, 13} = 18.81$, $P < 0.001$) but not at Annin, where high variability in GSI was observed (linear regression, $F_{1, 79} = 3.23$, $P = 0.076$; Fig. 4).
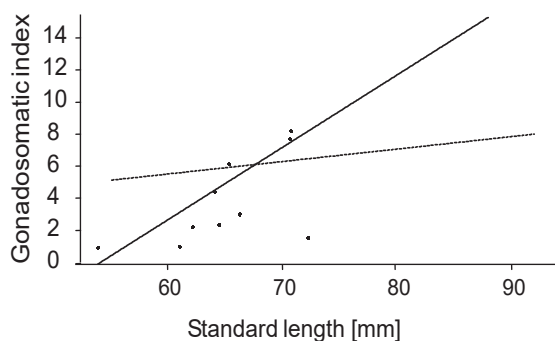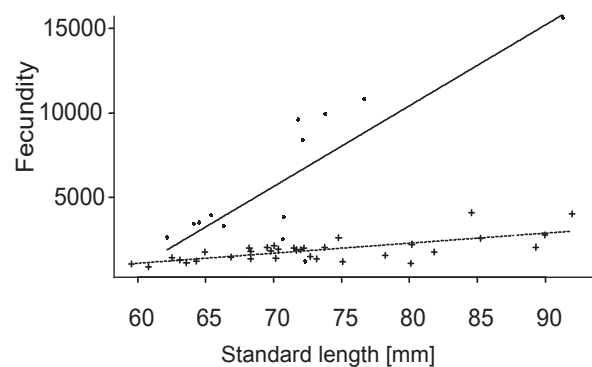

Fig. 4. Female gonadosomatic index (GSI) of pumpkinseed, *Lepomis gibbosus*, as a function of fish length

for Rohlik (full points, solid line) and Annin (crosses, dashed line) lakes; The lines show the curves predicted by linear models. Model formulae: Annin, GSI = $0.82769 + 0.07795 \cdot SL$ (model slope is not significantly different from 0); Rohlik, GSI = $-24.5300 + 0.4527 \cdot SL$ (SL = standard length)

Absolute fecundity for pumpkinseed from Rohlik (62–92 mm) was significantly higher (ANCOVA, effect of site, $F_{1, 43} = 89.23$, $P < 0.001$; Fig. 5) than that for Annin pumpkinseed (58–92 mm), with fecundity ranging from just 907 to 4097 oocytes at Annin and 1235–15 650 oocytes at Rohlik. In addition, fecundity increased significantly more with fish size at Rohlik (ANCOVA, effect of site–size interaction, $F_{1, 43} = 49.17$, $P < 0.001$; Fig. 5).

Body condition of juveniles, males and females was significantly higher at Rohlik than Annin (Mann–Whitney test; juvenile: $W = 10$, $n = 52$, $P < 0.001$; male: $W = 14$, $n = 113$, $P < 0.001$; female: $W = 52$, $n = 96$, $P < 0.001$; Fig. 6), with coefficients approximately 30% higher for all three categories (average condition of male = 1.5 vs. 2.1; female = 1.5 vs. 1.9; juvenile = 1.4 vs. 1.9; Fig. 6).

Fig. 5. Fecundity (Fa) of females of pumpkinseed, *Lepomis gibbosus*, as a function of fish length for Rohlik (full points, solid line) and Annin (crosses, dashed line) lakes; The lines show curves predicted by linear models. Model formulae: Annin, Fa = $-2443.98 + 59.33 \cdot SL$; Rohlik, Fa = $-27761.56 + 477.28 \cdot SL$ (SL = standard length)
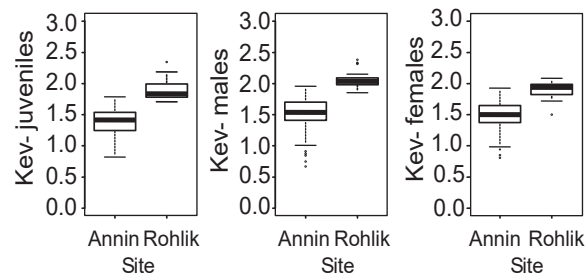
Fig. 6. Body condition of juveniles, males, and females of pumpkinseed, *Lepomis gibbosus*, at Annin and Rohlik lakes; Central bar = median, box = interquartile range, whiskers = non-outlier range (i.e., 1.5 × interquartile range) and points = outliers

DISCUSSION

According to Fox et al. (2007), life-history traits of introduced and successfully established species are predicted to shift along a continuum between *r* and *K* strategies, depending upon the time that has passed since introduction. The extent of this shift can be influenced by a range of biotic and abiotic factors. During the first years following the introduction, fish populations typically display high investment into reproduction, thereby aiding successful establishment in the new environment. This is reflected in a lowered age-at-maturity and higher fecundity (Bøhn et al. 2004). On the other hand, newly colonised areas typically have a low density of conspecifics, resulting in lowered intra-specific competition, faster growth, and improved body condition (Feiner et al. 2012).

In this study, we compared life-history traits and population characteristics of two pumpkinseed populations, one still in the early phase of establishment (Rohlik; sampled two years after its introduction in 2011) and the other an established population introduced in 1997 (Annin; sampled 14 years after introduction). We predicted that our 'new' and 'old' populations would differ in population size structure, body condition and reproductive characteristics.

While we were able to sample 198 fish from the long-established and abundant population in Annin, only 64 fish were obtained from the lower-density population in Rohlik, despite an order-of-magnitude higher sampling effort. We found no evidence, however, that differences observed in life-history traits (or lack of them) were related to the number of fish caught. Furthermore, as the Dyje River is a main tributary of the Morava River, joining close to the Morava's confluence with the Danube, and the Danube and lower (Slovakian) stretch of the Morava is believed to be the source for pumpkinseed in the Dyje (Hanel and Lusk 2005), it is highly likely that the fish from Annin and Rohlik both came from a common source population, i.e., the Danube, effectively ruling out genetics as a cause of differing life-history traits.

Both populations comprised four age groups (1+ through 4+), with the oldest fish at Rohlik probably representing individuals from the founder population. Population structure at the two sites differed only in the proportion of 1+ fish at each site, the relatively weak 1+ class observed at Annin in 2011 probably being caused by exceptional flooding in 2010 (there was no severe flooding prior to sampling at Rohlik). Both populations showed a high degree of overlap between the 2+ and 3+ size classes, almost certainly related to the pumpkinseed's ability to produce multiple clutches each breeding season (Ribeiro and Collares-Pereira 2010).

While pumpkinseed can live for up to eight years and measure up to 162 mm TL (Lake of Banyoles, Spain; Vila-Gispert and Moreno-Amich 2000), they display great variability both in life-span and length-frequency distribution depending on the locality sampled and the prevalent abiotic and biotic conditions (Copp et al. 2004, Fox and Copp 2014). In both age- and length-structure, the Rohlik and Annin populations are comparable with results from other small European bodies of water (e.g., ponds and sand pits in Hungary: Copp et al. 2004; sand pits in Bulgaria: Uzunova et al. 2010). Unlike populations in larger bodies of water in Spain and Greece, which may reach 8+ (Vila-Gispert and Moreno-Amich 2000, Villeneuve et al. 2005), the majority of European populations display a much shorter life-span, with the 4+ maximum observed in our study being typical. This is most likely linked to temperature differences between the study regions, with most (non-Mediterranean) European waters showing high annual variation in temperature, resulting in rapid heating during summer and ice-cover during winter in the small bodies of water typically inhabited by pumpkinseed (as observed at both our study sites). Cold winter weather could lead to higher fish mortality in smaller bodies of water, and especially for smaller fish such as pumpkinseed (Uzunova et al. 2008).

Pumpkinseed show great plasticity in age- and size-at-maturity, both in their native and non-native range. In Europe, fish from southerly populations (e.g., Spain) can reach maturity as early as their second year (1+); however, the majority of populations in the rest of Europe tend to mature in their third year (2+; Przybylski and Zięba 2011). Both our study populations confirm this trend, with most fish maturing at age 2+ and immature individuals representing a very small proportion.

As predicted, pumpkinseed from Rohlik displayed the increased body condition typical for fish populations in the early stage of settlement (Carol et al. 2009), presumably due to lower inter- (absence of perch) and intra-specific competition for food resources at this site. Despite the slight shift toward smaller size-at-maturity evident in the newly established population at Rohlik (55 mm compared to 51 mm at Annin), the difference between two sites was not significant. Size-at-maturity at these two sites was comparable to that at other sites at similar latitudes, e.g., Grand-Lieu Lake in France (Cucherousset et al. 2009) and Tanyard Lake in England (Villeneuve et al. 2005).

Pumpkinseed fecundity in the established population at Annin was comparable with that at several other established populations in Europe such as those of: Lake of Banyoles, Spain (Villa Gispert and Moreno Amich 2000), Lake Kerkini, Greece (Neophitou and Giapis 1994), Mrtva Tisa, Serbia (Maletin et al. 1989), all of which displayed a similar water temperature range during summer to that at Annin (unfortunately, we were unable to find any information on pumpkinseed fecundity at higher or lower temperature ranges). In comparison, female fecundity at Rohlik was extremely high, which appears to confirm our prediction of a higher investment into reproduction at the newly established site. Investment into reproduction, however, is not only measured by fecundity (i.e., the number of possible offspring) but also by GSI. Surprisingly, despite the higher fecundity of fish in the newly established population, we found no significant difference in GSI, probably due to the unexpectedly high GSI of some 2+ fish in the long-established population (Fig. 4) and a relatively low number of females sampled from the new population. GSI is strongly affected by both total num-

ber of oocytes and stage of maturation (i.e., the readiness of females to spawn; Jons and Miranda 1997). Given the lower number of oocytes in 2+ fish from Annin, the higher GSI in these fish could have been caused by an increase in the number of mature oocytes (confirmed by direct observation; in fact 2+ fish from Annin had a higher number of mature oocytes than 4+ fish), suggesting that we caught a group of females just prior to spawning (older/larger females tend to spawn earlier in the season). The unexpectedly high GSI in 2+ Annin females was only observed in eight of 46 individuals; hence, if the scales from these fish had been read incorrectly and the fish were actually older, this could also have introduced bias. On the other hand, scale readings were undertaken independently by two experienced observers, suggesting this was not an issue. In some pumpkinseed populations (e.g., rivers Ardilla and Degebe; Ribeiro and Collares-Pereira 2010) GSI can show considerable variation, both at the beginning of spawning and during the spawning season. During August sampling at Annin, several 4+ females were caught with high GSI and mature oocytes. While this could suggest either a protracted spawning season or a later start and end to the spawning season for larger individuals, we have no definitive evidence for this (and other bias factors) and suggest that further long-term studies of life-history traits over the spawning studies are needed to help shed light on factors affecting GSI at these, and other, sites.

Further development of the two populations remains uncertain. While the long-established population at Annin appears to be stable, the newer population at Rohlik may yet fail due to the relatively small surface area of the body of water (0.8 ha), which could prove limiting for further population growth due to the potential risk of high intraspecific competition and predator pressure. On the other hand, the overall carrying capacity at Rohlik is enhanced by its relatively high nutrient content. More importantly, several other 'new' populations were observed at adjacent sites in autumn 2014 (Jurajda, personal observation), strongly suggesting that regular spring flooding both maintains and increases connectivity between pumpkinseed meta-populations and decreases the overall risk of local extinction, even in small water bodies.

To summarise, pumpkinseed at Rohlik displayed life-history traits typical for a newly established population, i.e., they invested more energy into reproduction (high fecundity) and had a relatively high condition factor, presumably as a low density of conspecifics resulted in lower competition for food. On the other hand, pumpkinseed at Annin displayed a mixture of features typical for populations at an early stage of establishment (i.e., a relatively short life-span and relatively low size-at-maturity) and of more established populations (i.e., lowered body condition and lowered fecundity). The preservation of features typical for newly established populations at Annin was probably caused by the typically central European temperature regime; with low temperatures and ice cover in winter increasing overwinter mortality, resulting in a relatively short lifespan. In addition, these temperature conditions appear to have reduced age-at-maturity in these

Czech populations to 2+ (compared to 4+ in warmer Mediterranean waters), even in newly established populations.

It should be noted that these results were based on just two sites and one year's monitoring and, as such, should be considered a pilot study for future research. In order to confirm our results, and perhaps reveal further factors influencing life history traits in such populations, we suggest more frequent sampling before, during and after the spawning season with more detailed measurement of biotic and abiotic factors at the sites. In this study, we focused on time since establishment; however, the factors contributing to the differences observed in our two populations (e.g., temperature, size of the body of water, and relative predator pressure; see Fox and Copp 2014) may yet prove to be more complex.

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