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Non-native fish dispersal as a contaminant of aquatic plant consignments – a case study from England

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Editor's note:
This is one of five papers prepared by participants of the conference "Freshwater Invasives – Networking for Strategy II". Held in Zagreb, Croatia from the 11th – 14th July 2016, the conference was organized by the University of Zagreb, Faculty of Agriculture, European Inland Fisheries and Aquaculture Advisory Commission (EIFAAC) and the Croatian Biological Society (HBD). The primary objective of the conference was to share new information and provide a forum where international scientists, policy makers and stakeholders could encourage the development of the management and policy in the increasingly important area of biological invasions.

Abstract

The introduction of non-native species as contaminants of aquatic plant consignments is poorly documented. This paper reports on the introduction of pumpkinseed *Lepomis gibbosus*, a North American sunfish, into an angling lake as a contaminant of native aquatic plants during their stocking to enhance the fishery. Growth and life-history data for the *L. gibbosus* specimens captured in the water body provided biological evidence (relatively rapid juvenile growth and early maturation) that supports the assumption that *L. gibbosus* was accidentally introduced as a contaminant of the aquatic plant consignment. This study highlights the importance of adhering to current guidelines on the movement of aquatic plants (e.g. Great Britain's "Be Plant Wise" educational initiative), which aims to prevent unwanted transfer of aquatic organisms.

Key words: Be Plant Wise, unintentional introductions, non-native species vector, alien species

Introduction

The introduction of non-native organisms to inland waters for ornamental purposes (e.g. Mack and Lonsdale 2001), by pet owners (e.g. Copp et al. 2005; Duggan et al. 2006; Rixon et al. 2005), or as contaminants of aquatic animal consignments (e.g. Copp et al. 2007, 2010; Patoka et al. 2016) is becoming increasingly well documented both for imported and within-country consignments (translocations). Recognition in the UK of the risk of introducing invasive plant species along with desirable species is evident in the "Be Plant Wise" educational initiative (www.nonnativespecies.org/beplantwise/), as is the translocation risk of contaminated recreational equipment, e.g. boats and angling gear (e.g. Zięba et al. 2010; Bacela-Spychalska et al. 2013; Anderson et al. 2014), through the "Check-Clean-Dry" initiative (www.nonnativespecies.org/checkcleandry/). Much less well documented are cases of non-native species introductions as contaminants of aquatic plant consignments (e.g. Kipping 2006; Buczyński and Bielak-Bielecki 2012;

Measey et al. 2017). Indeed, a review of non-native species introductions reported that ≈ 90% of aquatic plant consignments were found to arrive contaminated with unintended live organisms (Keller and Lodge 2007), but animal contaminants were invertebrates and no fish species were reported.

However, the introduction of non-native fish and aquatic plants has a long history, which is evinced by the introduction of Canadian pond weed *Elodea canadensis* to Loch Leven (Scotland) when fish tank residues were jettisoned into the lake by "an itinerant hawker of goldfish" (West 1910, p. 172). As a contribution to such rare events, the aim of this short communication is to report on the introduction of the North American sunfish, pumpkinseed *Lepomis gibbosus*, to an angling venue where the species had not been observed during annual fishery surveys prior to the stocking of native aquatic plants for habitat enhancement. Growth and life-history data for the *L. gibbosus* specimens captured in the water body are provided in support of the assumption that this non-native species was accidentally introduced as a contaminant of the aquatic plant consignment, which had been transported in trays of water. The pumpkinseed is of concern because it is one of six species predicted to benefit, and thus become invasive, under the forecasted future warmer climate conditions, both in England (Britton et al. 2010a) and in France (Masson et al. 2015).

Materials and methods

The study site was Langdon Lake, a small water body (area ≈ 0.8 ha) formerly known as Dunton Lake (latitude: 51.564872; longitude: 0.393362) and situated near Basildon (County of Essex, England). Prior to the arrival of *L. gibbosus*, annual electrofishing surveys of Langdon Lake revealed the presence of common carp *Cyprinus carpio* and rudd *Scardinius erythrophthalmus* only. In March 2014, the lake was stocked with native aquatic plants by an independent consultant who transported the plants by automobile from the supplier to the water body in water-filled trays. Subsequent investigation revealed that the source of the aquatic plants was a supplier situated within the current UK range of pumpkinseed and known to have the species on site. On 7 April 2015, specimens of *L. gibbosus* were collected by continuous electrofishing (generator-powered DC unit), which was undertaken as three separate passes around the borders of the water body. Captured pumpkinseed were immediately killed with an overdose of 2-phenoxyethanol, then immersed in a slurry of iced water (a procedure permitted under U.K. Home Office project licence), and transported on ice to the laboratory.

After defrosting, the specimens were measured for total length (TL) to the nearest mm and weighed (Wt) to the nearest 0.1 g, with scale samples taken from the area between the lateral line and dorsal fin from each specimen to assess age, growth and body condition. Gonads were examined to determine sex, with female specimens classified according to egg status: immature = those with unrecognizable or non-yolked eggs; and mature = those with yolked eggs. The scales were aged using a projecting microscope (×48 magnification), with ages for each individual fish determined by through interpretation of scale features representing true annuli. The accuracy of age estimates were improved via a quality control procedure in which 25% of scales were aged independently by a second reader; where there were differences in estimates then these scales were re-aged to determine the source of the ageing discrepancy so that the consistency in ageing precision was improved (Musk et al. 2006).

Data analyses

The logarithmic relationship between TL and body weight was elaborated using simple linear regression. For the analysis of somatic growth rate, to avoid pseudo-replication in analyses resulting from the use of repeated measures from the same fish (i.e. multiple lengths at age whose number varies according to the age of the individual), only one TL at age per individual was determined (Britton et al. 2010b). This length at age was the TL at the last annulus. As the fish were sampled in April, following completion of their growth season the previous year but prior to the commencement of their growth season in the sampling year, then the TL at capture was taken as the TL at the last annulus and thus there was no requirement for back-calculation. The mean TLs at each age (± 95% confidence limits) were then calculated for the population and compared with the mean TLs at age of native North American and introduced European pumpkinseed populations through calculation using the base data from Fox and Copp (2014). Initial comparisons were of the variability (± 95% confidence limits) around the mean TL at age data between Langdon Lake and populations in Europe and North America. As the Langdon Lake pumpkinseed were dominated by fish of age 2 (*cf.* Results), their TLs at age 2 were then tested against those from North America and Europe; as these data violated assumptions for parametric tests (assumptions of normality and homogeneity of variances) then a Mann Whitney U test was used.

Fish condition was assessed using Fulton's condition (plumpness) factor (Mills and Eloranta 1985: K =

Wt × TL^{-3} × 10^{5}) to allow a standard comparison of body condition for the present specimens relative to published values elsewhere. Mean TL and age at maturity of the population was calculated from the percentage of mature individuals in each age-class using the DeMaster (1978) formula as adapted by Fox (1994):

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

where α is the mean age of 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 (10 mm TL intervals in place of age-classes) was used to calculate mean TL at maturity as per Fox and Crivelli (2001). To assess whether a relationship exists between age at maturity and juvenile growth (e.g. Fox 1994), and to compare with published values for other introduced populations in Europe (Masson et al. 2015), the former was regressed against the latter, which was defined as total length at age 2 ($TL_{AGE\ 2}$), i.e. generally the earliest age at which female pumpkinseed mature.

Results and discussion

Of the 106 pumpkinseed captured, 43 were males and 63 females, rendering a male:female a ratio of 0.68, which is much lower than the range of values (1.1–3.2) reported for *L. gibbosus* populations elsewhere in England and continental Europe (Cucherousset et al. 2009). Four of the specimens had damaged tails, so these were not considered further in analyses that involved body length. As is commonly observed in fishes, the total length-weight relationship for all specimens (Log Wt = 3.283LogTL – 5.274; r^2 = 0.851) was highly significant (F = 592.309, P = 0.0001). Mean (Fulton's) condition factor was virtually identical for males (1.89) and females (1.83), being slightly higher than in ponds densely populated by *L. gibbosus* (Fox et al. 2011), but considerably lower than those reported for populations in England, France and Romania (Copp et al. 2002).

The specimens were found to range in age from 1 to 3 years old, with fish of age 2 dominating the sample (86% of all fish aged). There was considerable overlap in 95% confidence limits around the mean lengths at age between the sampled fish and populations in North America and Europe, but with a pattern of similar TLs at age 1, higher TLs at age 2 in Langdon Lake, but then lower TLs at age 3 in

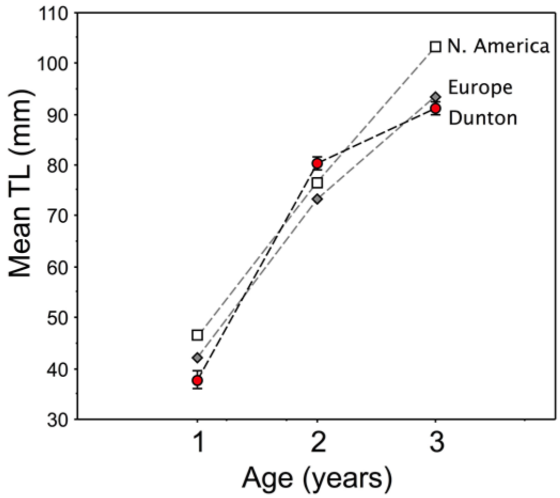

Figure 1. Mean total length (TL) at age (± 95 % confidence limits) for pumpkinseed *Lepomis gibbosus* populations in Langdon Lake (grey circles) and all available data for ages 1 to 3 from the species' native North American (black circles) and introduced European populations (clear circles).

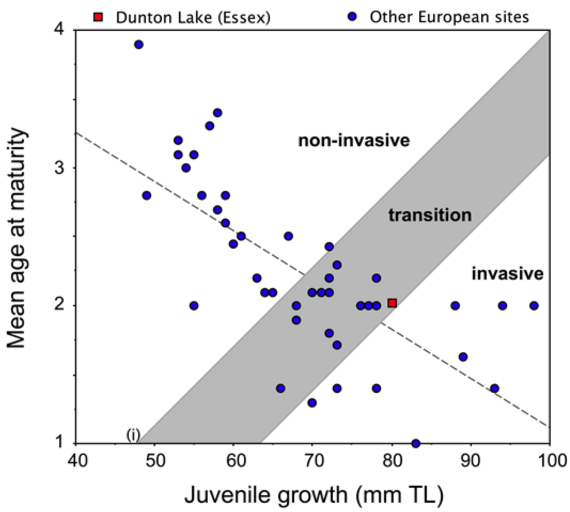

Figure 2. Mean female age at maturity (in years) as a function of mean juvenile growth (TL at age 2) for introduced pumpkinseed in European inland waters, redrafted with new data (filled square) for Langdon Lake (Essex) and for elsewhere (filled circles) reported in Copp and Fox (2007), Cucherousset et al. (2009) and Masson et al. (2015). The relationship is significant at P = 0.0001 ($\alpha = -0.036TL_{age2} + 4.738$; r^2 = 0.49, df = 42). The proposed physiological transition phase between non-invasive and invasive pumpkinseed populations is hypothesized as extending from the minimum age at maturity (the 45° line that traces from the intercept, at "i") and the end of juvenile growth (which for many pumpkinseed populations is age 2; the 45° line that traces through the age 2 intercept with the regression slope, at "ii"). To trace the transition zone, two 45° lines were traced upwards from the regression y-intercept, with one moved left until it crossed the x-axis at "i" and the other moved to the right crossed the regression line at "ii".

Langdon Lake compared with North America (Figure 1). Testing of TL at age data revealed the Langdon fish were indeed of significantly longer TL at age 2 than populations in Europe (Mann-Whitney U test: $Z = -3.17$, $P = 0.002$) and North America ($Z = -4.53$, $P < 0.001$) (Figure 1). Rapid juvenile growth, i.e. TL at age 2, is normally associated with young age at maturity both in the species' native range (Fox 1994) and its introduced European range (Copp and Fox 2007), where the relationship between juvenile growth and age at maturity has been proposed (Copp and Fox 2007) and validated (Cucherousset et al. 2009; Fobert et al. 2013; Masson et al. 2015) as a biologically-based model for predicting invasiveness. In Langdon Lake, fast juvenile growth (Figure 1) was indeed associated with relatively early juvenile growth, placing that population on the border of invasive and transitional populations (Figure 2).

Mean age at maturity for female pumpkinseed in Langdon Lake (2.02 years) is well below the mean for North America (2.98 years) and slightly lower than the mean (2.21 years) for European populations (Fox and Copp 2014). Similarly, mean female length at maturity in the Langdon Lake population (73 mm) is shorter than that for native North American populations (99.4 mm TL) and that (77.0 mm) for non-native European populations (Fox and Copp 2014).

For many non-native fish populations, the date and method of introduction is usually unknown (e.g. Welcomme 1992) and therefore difficult to determine retrospectively. However, in the present case, the age range of the *L. gibbosus* (1–3 years) and the rapid juvenile growth, especially in age 2 fish in 2015, which would have been small age 1 fish in March 2014, provide biological evidence indicative of a recently-introduced population. This is the first known incidence of a non-native fish introduction as a contaminant of an aquatic plant consignment, and it extends the introduced UK range of *L. gibbosus*. Indeed, although a few established populations of *L. gibbosus* were previously known to exist north of the River Thames (Lever 1977), more recent investigations (Copp et al. 2006, 2007; Zięba et al. 2010) found those historically-established populations to have been extirpated prior to the 21st century, including single pond populations in Scotland (Newport-on-Tay), in northeast England (just south of Newcastle) and in Cambridgeshire (just south of Cambridge; Lever 2009). The first of these populations disappeared during a hot summer in the 1980s when the pond dried out (W. Berry, Tayfield Estate, personal communication). In a subsequent fish survey of the pond, the only species observed was gudgeon *Gobio gobio* (P. Maitland, personal communication). Whereas, the latter two populations disappeared when the ponds they inhabited were filled in to construct a car park (northeast England) and a building (Cambridgeshire).

Since the demise of these more northerly populations, all known extant populations were restricted to locations south of the River Thames (Copp et al. 2007), with most of established in ponds situated in East and West Sussex (Villeneuve et al. 2005; Cucherousset et al. 2009) and only a few further west on the Isle of Wight and in the Somerset Levels (Villeneuve et al. 2005; Cucherousset et al. 2009). In most cases, established populations go unreported due to land-owner ignorance of their existence—this was the case of a newly-established population that invaded a garden pond during a flood of Batts Bridge Stream (East Sussex). This population, which was studied a few years after its introduction (Fobert et al. 2013), was found to demonstrate a similar pattern of elevated juvenile growth and early maturity as in the present study (Figure 2). As such, the *L. gibbosus* population in Langdon Lake now represents the most northerly population of *L. gibbosus* known to exist in the UK.

Management recommendations

The inadvertent, indeed negligent, introduction documented here highlights the importance of adhering to current guidelines on the movement of aquatic plants (i.e. Be Plant Wise), which aim to prevent unwanted transfer of aquatic organisms. Prevention is always more cost effective than the cure for bioinvasions (Leung et al. 2002), and there are various practices that aquaculturists and their customers can adopt in the harvest, handling, packing and shipping to reduce the risk of hitchhikers (Zajicek et al. 2009). Biosecurity plans should be in place at each step throughout the supply chain, beginning at the aquaculture facility and including the end user (i.e. the person stocking the aquatic organism into the recipient water). These biosecurity plans should involve regular inspections for diseases and nuisance species, the washing and disinfection of the rearing and transport facilities before returning to the aquaculture facility.

At the point of harvest, specific employees can be assigned to inspect visually for and remove unwanted species. Removal of smaller organisms (or propagules thereof) may be purged during the holding, grading and sorting of the intended aquatic product, such as with the use of a strong flow of water or an appropriate treatment, such as the use of salt, temperature or formaldehyde baths to remove unwanted species (e.g. Helms 1967; Carmichael and Tomasso 1983; Mitchell and Brandt 2009; Jute and Dunphy 2017).

Such treatments may be appropriate prior to transport or in some cases during transport, such as a weakly saline bath during the transport of fish or removal of free water in the transport of aquatic plants. In the latter case, plants should be washed and then either wrapped in moist paper (e.g. newspaper) or placed in air-filled, sealed plastic bags, similar to the transport of aquarium plants from the shop to the customer's home aquarium. Procedures such as these will reduce the likelihood of unwanted fish and other aquatic organisms, especially those that are not easily visible, from being inadvertently transported and introduced along with the intended aquatic organism (Padilla and Williams 2004).

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