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REGULAR PAPER

Catch-related and genetic outcome of adult northern pike *Esox lucius* stocking in a large river system

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Fédération Départementale pour la Pêche et la Protection des Milieux Aquatiques du Lot, FDPPMA 46; Region Midi-Pyrénées ; The agency for water resource management of Adour-Garonne watershed (Agence de l'Eau Adour-Garonne) Genetic introgression from stocked adult northern pike *Esox lucius* to a wild self-recruiting population was detected in a large river system and some stocked *E. lucius* survived up to two spawning seasons and dispersed over several kilometres in the river. Moreover, the catch rate of stocked *E. lucius* by anglers was low (9.6%), hence suggesting that the efficiency of stocking activity is questionable.

KEYWORDS

captive breeding, conservation, enhancement, genetic diversity, microsatellite, recreational fisheries

Stocking is a common management practice used to enhance wild populations or increase catch opportunities (Cowx, 1994; Lorenzen, 2014; Welcomme & Bartley, 1998). In contrast to the expected economic and conservation benefits from this activity (Leber et al., 1995; Uki, 2006), stocking fish into self-recruiting populations has the potential to induce deleterious effects on the wild component of the targeted stocks (Allendorf & Waples, 1996; Lorenzen et al., 2012). Among these risks, the effects of genetic introgression from stocked fish to natural populations are of critical importance (Allendorf et al., 2001). These effects may vary from barely appreciable to strong alterations of the genetic integrity of wild populations, eventually leading to fitness depression and population replacement (Araki et al., 2007; Ryman & Laikre, 1991). The degree of genetic introgression between stocked and wild fish depends on many variables, such as the survival of stocked individuals and their ability to reproduce with their wild counterparts. These variables in turn depend on many other variables such as the body size of stocked fish, their degree of domestication, their genetic relatedness to wild fish and the census size of the wild population (Lorenzen et al., 2012). As such, the long-term genetic risks induced by stocking are difficult to predict and documenting the genetic outcomes of stocking is critical to appreciate the relevance of stocking for sustainable fisheries management (*e.g.*, maintenance of local genetic characteristics and population fitness).

Species of the genus Esox L. 1758 are of major importance for fisheries in the northern hemisphere (Crane et al., 2015; Gandolfi et al., 2016). In rivers, studies investigating the genetic diversity of northern pike Esox lucius L. 1758, aquitanian pike Esox aquitanicus Denys, Dettai, Persat, Hautecœur & Keith 2014 (Denys et al., 2014), southern pike Esox cisalpinus Bianco & Delmastro 2011 (Bianco & Delmastro, 2011) suggested that stocking might be a major source of contemporary change in their genetic diversity (Denys et al., 2014; Gandolfi et al., 2017; Launey et al., 2006; Lucentini et al., 2011). Esox lucius stocking mainly involves early life stages (larvae, fry and fingerlings) reared in hatcheries, a strategy that has been demonstrated to be poorly efficient for increasing the abundance of the targeted cohort (Jansen et al., 2013; Vuorinen et al., 1998). Despite possible replacement of a wild fish component by their stocked conspecifics of the same cohort (Hühn et al., 2014), Larsen et al. (2005) found a low rate of genetic introgression from stocked E. lucius fry to a wild brackish population in the Baltic Sea, suggesting little effect of fry stocking on long-term genetic introgression. Esox lucius stocking is also carried out with adult individuals (Pierce,

2012). Larger fish suffer density-dependent growth rather than density-dependent mortality (as generally observed for juveniles) and their natural mortality is inversely related to body size (Lorenzen, 2005). Therefore, stocked adult *E. lucius* have the potential to survive in the recipient population (Snow, 1974) and hence to participate in reproduction with their wild conspecifics (Guillerault *et al.*, 2018). However, outcomes of this management practice and its genetic effects on wild populations remain poorly documented and understood.

The present study aimed at testing whether stocking of adult *E. lucius* in a self-recruiting river population affects the genetic integrity of the wild *E. lucius* population through estimation of: the propensity of stocked fish to establish and spread in the recipient waterbody using a mark-recapture approach; the propensity of stocked fish to transfer their genes to the gene pool of the wild population using microsatellite genetic markers.

The study focused on an *E. lucius* population from the Lot River in south-western France (44°26′ N, 1°26′ E). The study site was located in a 130 km reach of the river that has been receiving *c*. 1 t of *E. lucius* annually to enhance recreational angling. The *E. lucius* (30–70 cm, total lngth L_T) originated from a single fish farm (Dombes étangs SARL, Saint-Remy, France; located about 450 km away from the Lot River) for almost two decades. All fish sampling and tagging was carried out by the local angling association between 2007 and 2011. While at the time there was no requirement for official permits, the work was carried while taking guidelines for animal welfare into consideration.

For the first aim, survival of stocked E. lucius over time as well as the spatial spread of the stocked fish along the river stretch were estimated. To do so, 1839 E. lucius were stocked from 2007 to 2011 in November of each year (mean \pm SD annual number = 368 fish \pm 33 year⁻¹, L_T range = 28–82 cm) in four successive stretches of the river (mean \pm SD length = 2.9 \pm 0.9, 12.8 km long in total) bounded by weirs (c. 2.5 m height). Stocked fish were evenly spread along the study area. This represented on average 3.5 fish ha^{-1} years⁻¹ stocked fish (as usually done by local fisheries managers), whereas the average density of wild E. lucius of the same length class was estimated at c. 6 fish ha^{-1} years⁻¹ (Guillerault, unpublished data). Other characteristics of stocking, such as time of stocking, body size at stocking, fish origin and rearing conditions respected the usual stocking procedure (Cowx 1994). Before release, each E. lucius was marked with a T-bar tag on the anterior part of the dorsal fin to allow anglers to identify them and report the date and the location of their catches. Each year, c. 20% of the stocked fish were double tagged to estimate tag-shedding (30% of double tagged fish have lost one tag). The probability of losing a tag was used to correct the number of captures reported by anglers at different periods of time (see below) using a pooled approach (Seber & Felton, 1981) and to calculate a corrected catch rate, which was used as a proxy of minimum fish survival rate. Since E. lucius can reproduce from 19 cm for male and 30 cm for female (Raat, 1988), stocked E. lucius were considered mature. The stocked fish were divided in two categories based on the mandatory minimum harvest length limit (L_T = 50 cm), *i.e.*, fish protected versus those exposed to fishing mortality. Catch rate was estimated by analysing data directly reported by local anglers. Data to be reported by anglers (date, location, number of E. lucius caught with LT, number of tagged caught) were explained in a document provided with the fishing licence, informative panels placed along the banks of the river and a web document available on the website of the local angling association. A total of 89 anglers have reported information (most anglers reported their catches). A quasibinomial generalized linear model was used to test whether capture rate varied significantly among years and body-size classes at stocking (Table 1) and the significance of the resulting two-way interaction term was tested using the R 3.1.2 (www.r-project.org).

Moreover, results showed that 40.5% of the captures were recorded before the spawning season (i.e., within the 3 months after stocking), 50% of the captures were recorded after one spawning season and 9.5% were recorded after two spawning seasons (Figure 1). As a result, at least (\pm SD) 27 \pm 30 fish year⁻¹ had the opportunity to reproduce once and c. 5 \pm 5 fish year $^{-1}$ could reproduce twice (Figure 1(b)). Most E. lucius were captured near their stocking site (median distance = 0.9 km, mean distance \pm SD = 0.65 \pm 2.9 km). In most cases, captures were recorded in the river stretch where fish were released (63%) or in the next river stretch (33%; Figure 1(c)). However, captures at long distances from the stocking site, i.e. up to 14.1 km downstream and 13.4 km upstream (Figure 1(c)) were still recorded, meaning that some E. lucius were able to cross respectively four and three weirs. The proportion of E. lucius caught upstream and downstream of the stocking point was identical (46% up or downstream and 8% at the stocking site).

For the second aim of this study, the level of genetic introgression from stocked to wild *E. lucius* was assessed. In November 2013, pelvic fin clips from a sample of the stocked fish source population were collected (n = 100, size range: 38.5–75 cm L_T) to genetically characterize the stocked population. From 2013 to 2016, pelvic fin clips of each *E. lucius* captured and reported by anglers were collected (n = 51, size range: 31–109 cm L_T) to determine the level of genetic introgression. Genetic analyses were carried out on 151 fish samples. Genomic DNA was extracted using a salt-extraction protocol (Aljanabi & Martinez, 1997). PCR amplifications, genotyping and allele scoring were carried out following the protocol described in Paz-Vinaz *et al.* (2013). Seventeen microsatellite loci: Elu-252 (Miller & Kapuscinski, 1997) El-02, El-03, El-05, El-09, El-17, El-20, El-21, El-22, El-23, El-27, El-28

 TABLE 1
 Results of the generalized linear model aimed at testing the effect of the year (year) and of size (size) at stocking on the reported capture rate (Cr) of *Esox lucius* in the lot river

Parameter	Variable	Deviance	Residual deviance	df	Р
C _r	Year	68.190	1,009.41	41,834	< 0.01
	Size	74.802	934.41	11,833	< 0.01
	Size \times year	20.890	913.72	41,829	< 0.01



FIGURE 1 Main outcomes of the mark–capture experiment on stocked adult *Esox lucius* in the lot River. (a) Reported annual per cent recaptures of *E. lucius* stocked in the Lot River from 2007 to 2011. \diamond , Total annual capture rate; , capture rate of fish with $L_T < 50$ cm at stocking; , capture rate of fish with $L_T \ge 50$ cm at stocking. (b) Jitter plot of time between stocking and capture of *E. lucius* (1 point = 1 individual). (c) Jitter plot of distance from *E. lucius* stocking site to capture site

(Ouellet-Cauchon *et al.* 2014), Eluc-033, Eluc-040 (Wang *et al.* 2011), EluBe-INRA, EluB-38INRA, EluC-113INRA (Launey *et al.* 2003), were amplified using three multiplexed PCRs. The presence of null alleles was tested from the 100 *E. lucius* stocked using MICRO-CHECKER 2.2.3 (van Oosterhout *et al.*, 2004) after checking for Hardy-Weinberg equilibrium using Fstat 2.9.3.2 (Goudet, 1995). The loci with evidence of null-alleles were dropped from the analyses (El-22, El-23 and El-28), which led to a final set of 14 loci (Supporting Information Table S1).

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The identity of fish was examined (allowing 1 mismatch) using CERVUS (Kalinowski et al., 2007) to avoid double counting of fish (from catch and release, or from the capture of fish stocked in 2013). A Bayesian clustering approach was used to infer the likelihood of K = 1-4 populations (independent genetic groups) using Structure 2.3 (Falush et al., 2003; Pritchard et al., 2000). Markov chain Monte Carlo (MCMC) iterations were set to 500,000 (burn-in period was set to 100,000) with 15 replicates per K. The optimal value of K was 2 (ΔK = 333.3: Evanno et al., 2005: Supporting Information Figure S1). One of the two clusters included all 100 fish used to characterize genetically the stocked population and was therefore called C_{stocked} . The minimum assignment rate of these 100 fish to C_{stocked} (i.e., 0.91) was hereafter use as a minimum threshold values to assign E. lucius to one genetic cluster. The other cluster was hypothesized to characterize wild fish and was called Cwild. The optimal alignment of the replicates was determined using CLUMPP (Jakobsson & Rosenberg, 2007), which provides us with estimates of admixture proportions for each individual between the two clusters.

The 51 captures reported by anglers corresponded to 50 *E. lucius* (*i.e.*, one recapture according to the identity analysis). Among those 50 *E. lucius*, 21 *E. lucius* belonged to the cluster C_{stocked} (mean \pm SD affiliation to $C_{\text{stocked}} = 0.98 \pm 0.01$, range = 0.94–0.99; Figure 2(a)). According to the identity analysis, 17 of those *E. lucius* originated from the stocking carried out in November 2013, whereas the other four *E. lucius* may originate from stocking having occurred at another location or at another timing. They could also be the descendants of a past breeding event between two fish stocked. Twenty-four *E. lucius* were strongly assigned to the cluster C_{wild} (mean \pm SD affiliation to $C_{wild} = 0.98 \pm 0.02$, range = 0.94–0.99; Figure 2(a)). Five fish had mixed assignment rates (mean \pm SD affiliation to $C_{\text{stocked}} = 0.59 \pm 0.09$, range = 0.49–0.67), which suggests that these fish come from interbreeding between wild and stocked fish or hybrids.

In addition, CERVUS was used to calculate the observed heterozygosity (H_O) and expected heterozygosity (H_E) for each group of fish separately (*i.e.*, stocked *E. lucius* and wild *E. lucius*; Supporting Information Table S2). The allelic richness (A_R) and fixation index (I_{FS}) values for each group were estimated using Fstat 2.9.3.2 (Goudet, 1995; Supporting Information Table S2). A_R was significantly lower in stocked than in wild *E. lucius* (Wilcoxon test, P < 0.01), probably because of population bottlenecking during broodstock practices (*e.g.*, low A_R in the stocking founder population or small broodstock size). I_{FS} was significantly higher in wild than in stocked *E. lucius* (Wilcoxon test, P < 0.05), suggesting non-random mating in the wild population. H_O and H_E tended to be lower in the stocked *E. lucius* than in wild *E. Lucius*, although not significantly (Wilcoxon tests, P > 0.05).

Stocking of adult *E. lucius* likely resulted in reproduction between stocked and wild *E. lucius*. At least, 4.6% (mean \pm SD = 4.4% \pm 4.6 year) of the stocked fish survived until the subsequent spawning seasons. Stocked *E. lucius* also moved over several km despite the presence of weirs; Snow (1974) had similar findings in lakes. Recurrent stocking events in this population induced genetic introgression between wild and stocked *E. lucius* since five fish were considered as introgressed (15–17% of *E. lucius* from the Lot River were hybrids, depending if the four *E. lucius* from C_{stocked} which do not come from stocking of 2013 are considered as having been stocked or hatched in



FIGURE 2 Genetic discrimination of *Esox. lucius* stocked (*n*: 100) and caught by anglers (Hooked *E Lucius*; *n*: 50) in the Lot River from November 2013 to August 2016 based on 14 microsatellites. *C*_{stocked}, The genetic cluster of *E. lucius* of hatchery origin (**m**); *i.e.*, 100 individuals stocked in November 2013 including 17 pikes caught by anglers (St) and four additional individuals (event of stocking unknown) caught by anglers (St). *C*_{wild}, The genetic cluster of *E. lucius* of wild origin (**m**); *i.e.*, 24 individuals of strictly wild origin (Wi) and five individuals were hybrids (Hy)

the river (see above). The reproductive success of stocked *E. lucius* in this river, may exceed that for salmonids in which reduced competitive ability in the wild, reproductive isolation or lower survival of the offspring of stocked fish are expected to lower reproductive success compared with their wild conspecifics (Araki *et al.*, 2009; Fleming & Petersson, 2001; Hansen & Mensberg, 2009). Moreover, genetic risks associated with stocking may still increase if a high stocking pressure (*i.e.*, high frequency and quantity) is maintained (Fraser, 2008; Lorenzen *et al.*, 2012) or with other stocking sources potentially more prone to introgression. Indeed, the stocked fish came from a distant fish farm so both domestication and natural genetic differentiation could reduce their performance in the Lot River. Larsen *et al.* (2005) had similar findings with the stocking of freshwater pike in a brackish population.

Despite the lack of data about fishing pressure, the 9.6% catch rate of stocked fish was unexpectedly low. Consequently, in such recreational fisheries, it could be questionable to stock large E. lucius intended to be harvested as commonly applied for rainbow trout Oncorhynchus mykiss (Walbaum 1792) (Stanković et al., 2015). At best, E. lucius may also be stocked after the spawning period to increase their probability of being caught before the spawning season. Yet, stocking of large E. lucius may decrease the abundance of smaller fish through competition and cannibalism (Grimm, 1981, 1994; Haugen et al., 2007). In addition, it is uncertain if pond-reared pikes adapt to running water, especially in early winter before or when discharge is rising. Interestingly, the lowest C_r was observed after the stocking in 2008, which was followed by a quick and high increase of river discharge. Further studies should focus on the effect of stocking on E. lucius population genetics over time using historical genetic samples. More generally, further studies should balance the costs and benefits of stocking in comparison to alternative fisheries enhancement strategies.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

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