RESEARCH ARTICLE

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Egg drift and hatching success in European river lamprey Lampetra fluviatilis*: is egg deposition in gravel vital to spawning success?*

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ABSTRACT

1. The European river lamprey *Lampetra fluviatilis* (Linnaeus, 1758) is a threatened species, formerly widespread throughout Western Europe, for which loss and degradation of habitat is one of the main causes of decline. As with other lamprey species, areas of gravel substrate with moderate flows are considered necessary for spawning and egg development of river lamprey.

2. This study investigated the dispersal of river lamprey eggs downstream of a spawning area and the hatching success of eggs in the laboratory under a range of potential abiotic conditions (substrate type, water flow and dissolved oxygen level) which eggs could experience in nature.

3. Lamprey eggs were found to drift for a minimum of 50 m downstream of spawning excavations, facilitating dispersal in riffle habitat and to bankside depositional zones. Under conditions mimicking natural microhabitats, but without predation, median egg hatching success was 85.0% in "spawning habitat" conditions, but surprisingly, was 50.2% in "larval habitat" conditions employing natural silt.

4. The study suggests that egg dispersion out of spawning excavations may be common in this species and demonstrates that habitat located downstream of spawning areas, even larval habitat characterized by fine sediment and moderate to low flow rates, could play an important role in larval recruitment. This suggests that even small areas of gravel or degraded spawning habitat may enable a higher degree of spawning success than has previously been assumed to be necessary for conservation or recolonisation of this species.

KEY WORDS: river, dispersal, reproduction, fish, habitat management; recruitment; egg mortality; habitat restoration; siltation.

INTRODUCTION

Throughout their distribution, lampreys (Petromyzontiformes) are of significant ecological, cultural and economic importance (Hardisty, 1986a; Kelly and King, 2001; Close *et al*., 2002; Renaud, 2011). The European river lamprey *Lampetra fluviatilis* (Linnaeus, 1758), hereafter referred to as river lamprey, is typically anadromous, although freshwater-resident populations are known (Maitland *et al*., 1994; Goodwin *et al*., 2006). Formerly widespread through Western Europe (Maitland, 1980; Hardisty, 1986b), it is regarded as a threatened species (Lelek, 1987; Mateus *et al*., 2012) and receives conservation protection in Europe through the Bern Convention and the European Habitats Directive 92/43/EEC, as a species requiring the designation of Special Areas of Conservation (SACs) (EC, 1992; Mateus *et al*., 2012). Populations of river lamprey have been impacted by pollution of rivers and estuaries, overexploitation, loss of spawning and larval habitat and by physical barriers to migration (Lelek, 1987; Ojutkangas *et al*., 1995; Nunn *et al*., 2008; Lucas *et al*., 2009; Mateus *et al*., 2012).

As with other lamprey species, river lamprey spawn in areas with swift flow and gravel habitat (Jang and Lucas, 2005; Nika and Virbickas, 2010), typical of the conditions and topography in which lotic salmonids spawn (Nika and Virbickas, 2010) although the preferred particle size varies between lamprey species (Malmqvist, 1983; Hardisty, 1986a). Access to abundant, clean gravel substrate with a well-oxygenated flow of cool water has, therefore, been regarded as important to spawning success, population persistence and conservation of lamprey species, including river lamprey (Kelly and King, 2001; Maitland, 2003; Oliveira *et al*., 2004; Lucas *et al*., 2009). All lampreys excavate depressions, often referred to as 'nests' in which courtship and spawning occurs (Hardisty, 1986a). In river lamprey these can vary markedly from distinct pits, 2-12 cm deep, usually with a rim of stones deposited immediately downstream (Huggins and Thompson, 1970; Nika and Virbickas, 2010), to complex areas of excavation (Jang and Lucas, 2005) and 'fronts' of gravel-turning activity, several metres wide, with many (usually 10-100) lampreys at the upstream edge engaged in stone movement, courtship and spawning, but without forming conspicuous depressions (Morland, pers. obs.). In river lamprey, several laboratory studies, with low water flows, have described courtship and excavation of these depressions and subsequent egg deposition in them (Hagelin and Steffner, 1958; Hagelin, 1959). In frequently-consulted reviews dealing with this and other lamprey species, the sticky eggs are reported to be deposited into the nest where they adhere to sand and infiltrate into interstices, especially at the rear of the nest and that, after hatching, the young larvae drift to silt beds into which they burrow (Hardisty and Potter, 1971; Maitland, 2003; Hardisty, 2006). Larval habitat is characterized by depositional areas of fine sediment, low to moderate flow conditions and the presence of organic matter (Hardisty and Potter, 1971; Maitland, 1980; Almeida and Quintella, 2002).

Spawning habitat has been considered the optimal habitat for development of lamprey eggs as it allows for greater oxygenation than that found in fine sediment (Manion and Hanson, 1980; Hardisty, 2006). However, some studies of landlocked sea lamprey *Petromyzon marinus* Linnaeus, 1758 in the Great Lakes suggest that a high percentage of up to 85% of eggs could be washed out of nests and reach other habitats (Manion and Hanson, 1980; Smith and Marsden, 2009). In fact, the percentage of eggs that remain in the nest and hatch successfully is considered as low as between 0.4 and 7.8% (Applegate, 1950; Manion, 1968). Moreover, for Smith and Marsden's 2009 study the hatching success of eggs incubated in the laboratory on silt (69.2%) and sand (50.8%) was found to be significantly higher than survival on gravel (19.1%). Huggins and Thompson (1970) reported that most eggs spawned by river lamprey were dislodged from nests by continued spawning activities and swept downstream. In light of this, more attention needs to be paid to drift of lamprey eggs and the habitats where they can be transported to as they may play a significant role in lamprey recruitment.

This study aimed to investigate, firstly, the drift of river lamprey eggs downstream of a spawning area; and secondly, the hatching success of eggs in the laboratory under a range of potential abiotic conditions (substrate type, water flow and dissolved oxygen level) which eggs could experience in nature to better inform conservation approaches towards protection of lamprey spawning habitat.

METHODS

Study area

The field study of egg drift was carried out in April to May 2008 on the River Ure, a tributary of the Yorkshire Ouse, Northeast England, which drains into the Humber Estuary and where river lamprey are common (Whitton and Lucas, 1997; Masters *et al*., 2006). At the sampling location (54° 11' 18"N, 1° 32' 51"W) the river is approximately 30-40 m wide, has a mean discharge of about 22 $m^3 s^{-1}$, a gradient of 2 m km^{-1} and is characterised by riffle, glide and pool habitat with riparian fringes of trees and shrubs. The study site has an annual spawning population of river lamprey below a ford, at the head of a 60-m long riffle. In 2008, the main spawning area was observed to occur from

25% of channel width from the left bank to mid channel, while the strongest flow passed down the right hand half of the channel. Substrate in the spawning area was a mixture of gravel (2-64 mm diameter) and sand. Downstream of the spawning area, the substratum varied from gravel and cobble on the right side and mid channel to gravel and sand the left side, with patches of sand and silt along the margins, where willow *Salix* spp. created slow-flowing areas.

Egg drift

The dispersal of lamprey eggs from nests was sampled using sets of three drift nets (32 cm high x 28 cm wide; 0.5 mm mesh size), secured by steel rods to sample from the bed upwards. These were positioned perpendicular to the flow and across the breadth of the main spawning area, distributed evenly from near the mid channel towards the left bank with *ca.* 15 m between the outermost nets, at distances of 10, 30 and 50 m downstream from the lamprey spawning area. Only one net was placed 100 m downstream of the spawning area, close to the left bank, as the channel was too deep to sample further out. Another set of three nets was installed 10 m upstream of the spawning area as a control to determine whether eggs caught in nets below the spawning site originated from that site. Nets were set in a direction from upstream to downstream and retrieved from downstream to upstream to minimise the risk of capture of eggs disturbed from the sediment. Retrieved material was sorted and lamprey eggs were counted on site. River lamprey eggs were identified as being creamy white in colour and *ca.* 1 mm diameter (Hardisty, 1986b) and from ‘type’ eggs obtained from adult spawners; the only other fish species producing eggs of this type at this time in the river were European brook lamprey *Lampetra planeri* (Bloch, 1784), adults of which have much lower fecundity than river lamprey.

Sampling of wild egg dispersal was carried out at the study area between 29 April and 8 May 2008 during both day (mostly on occasions when river lampreys were observed spawning) and night conditions (the main spawning period, Morland pers. obs.) in order to coincide with river lamprey spawning activity at this site. Sampling was not undertaken on 1 or 2 May due to high flows causing unsafe conditions. Sampling was terminated on 8 May as spawning attempts by lamprey had ceased to be observed and the number of eggs caught reduced substantially. A total of 13 sample periods were carried out during this time with sampling duration varying between 1 and 13 hours. The sampling location was also visited daily at midnight in order to record the number of lampreys spawning, except on 30 April and 1 May due to high flows. River discharge was obtained from Environment Agency flow data at Kilgram gauging site, about 25 km upstream. Due to the lack of major tributaries between Kilgram and the study site discharge data from the gauging station was considered appropriate to provide the approximate discharge and pattern of variation over the study period at the sampling site. In order to characterize the flow regime within the sampling area, flow velocity measurements were taken (Valeport electromagnetic flow meter, model 801, 15-second samples) at six distances (upper and lower part of the spawning area and 10, 30, 50 and 100 m downstream) and transversely at three different points for each distance (left, middle, right) at flows approximating average conditions (*ca*. 11 $m^3 s^{-1}$) over the study period.

In addition to the sampling of wild egg dispersal, five sample groups of dyed eggs were placed into three pre-existing lamprey excavations located in the spawning area so that dispersal of eggs from a known excavation and of known sample size could be tracked using the sample nets. Separate releases of 500, 500, 2000, 5000 and 5000 dyed eggs respectively, at least 5 h apart, were carried out between 3 and 7 May 2008. Eggs were released into each nest using a pipette, imitating the egg release point observed for river lamprey spawners. Nets were recovered 1 h after egg release and dyed eggs were counted. Eggs used for dyeing were stripped from a female as it was not possible to obtain sufficient wild eggs; they were unfertilised.

Hatching success under different conditions

Preliminary observations in previous years indicated that river lamprey eggs may drift to habitats adjacent to spawning areas (Lucas and Morland, pers. obs). Taking into account that the dispersed eggs can reach different habitats, the survival rates of eggs were measured under different substrate, flow and dissolved oxygen conditions in the laboratory. Field experiments were not attempted due to the difficulty of maintaining such apparatus in flashy rivers such as the Ure. Wild sourced eggs were gathered from the lamprey spawning site in the last 3 days of the egg drift experiment. Because the fertilisation rates of wild eggs were unknown at the time of collection and because of the difficulty of collecting large numbers wild eggs, artificially fertilized eggs were also used in complementary, but separate, egg viability experiments alongside those of wild eggs. Eggs for artificial fertilization were obtained from lamprey caught at the spawning areas on 28 April 2008 which were temporarily held separately. On 8 May a gravid female was stripped, the eggs mixed with sperm from three males and hand-mixed in a clean bowl.

Laboratory experiments were conducted in an aquarial facility at Durham University between 8 May 2008 and 29 May 2008. The substrate, flow and dissolved oxygen conditions in the experimental treatments were similar to the conditions reported for relevant habitat types in lamprey field sites (Hardisty, 1986a; Maitland, 2003; Jang and Lucas, 2005; Nika and Virbickas, 2010) and to those measured *in situ* in the field. Ambient temperature was maintained (mean ± SE) at 14.0 ± 0.07^{o}C (range: 13.5-14.7^{o}C) for the duration of the experiment to correspond with the water temperature expected at the spawning site during development. Photoperiod was set at 15L:9D to reflect the natural photoperiod at spawning. River water, gravel and silt were sourced from the River Ure, close to the spawning site. Gravel was removed from areas away from actual nest sites to minimise disturbance to breeding lamprey. Fine sediment was sourced from three areas containing lamprey larvae at the left bank, 30-100 m downstream and mixed before use, but the few stones present and woody material larger than 1 cm^3 (decaying leaves and small twig sections remained in place) were removed.

Treatment 1, simulating spawning habitat conditions, tested hatching success in presumed optimal conditions simulating well-oxygenated riffle conditions with swift-flowing, filtered and aerated river water (maintained via an internal power pump generating a 170 L h^{-1} flow rate) flowing over mixed river gravel of various grades (4-32 mm gravel). Treatment 2 simulated larval habitat, with slow-flowing and moderately-aerated river water (maintained using an air stone with restricted air flow) over natural fine sediment sourced as described above. Treatment 3 represented stagnant conditions and comprised river water which was not circulated, aerated or filtered, and fine sediment as in treatment 2. Treatment 4, used as a control, employed dechlorinated and purified tap water which had been passed through a reverse osmosis and de-ionising filter unit, and aquarium silica sand to provide inert, clean sediment. Water was aerated, circulated and filtered as in Treatment 1 using an internal power pump (170 L h^{-1} flow rate). A total of 56 tanks (32 x 23 x 20 cm) containing 5 cm depth of sediment and 10 L of water were installed in the laboratory. Fourteen tanks were used per treatment, seven with wild eggs (n = 50 per tank) and seven with artificially fertilized eggs (n = 100 per tank). Batches of eggs were allocated randomly between treatments by placing them on the sediment using a pipette. In Treatment 1 eggs fell into interstices; in the other treatments they remained on the surface or became slightly covered by fine sediment.

Tanks were checked daily for evidence of disease or hatched prolarvae and a dissolved oxygen reading was taken. The study was halted on 29 May 2008, after 21 days (*Lampetra* eggs normally hatch after 11-12 days at 12-15 ºC; Hardisty, 2006) when a high percentage of hatched lamprey prolarvae persisted in some treatments, as these are relatively easy to remove and count, because they swim or lie on the sediment surface, but progressively change to a burrowing behaviour, where removal from sediment is much more difficult. At the end of the study the percentage of successfully hatched prolarvae, partially developed eggs (expected short term hatching success) and dead or unfertilized eggs (without any sign of development) were recorded according to Richardson *et al*. (2010).

Data analysis

For the wild egg drift study, the number of eggs caught, the eggs caught per hour, and the percentage of total eggs caught at each location were calculated for nets placed in different longitudinal (10, 30 and 50 m) as well as in transverse locations (left, middle and right side), on each sampling event. Medians and upper and lower quartiles (25-75%) were calculated for longitudinal and transverse locations respectively based on the number of net samples (3) in each category over all 13 sampling events, giving $n = 39$ for each longitudinal and $n = 39$ for each transverse category... The same pattern was follow for the dyed eggs experiment in order to calculate the number of eggs caught, the percentage of total eggs caught and the percentage of eggs caught from the total released.

Nonparametric Kruskal–Wallis *H* and Mann–Whitney *U* tests were used for egg dispersal (for both wild and dyed eggs and for transverse and longitudinal locations) and hatching success data (between different treatments) comparisons because data did not conform to normality or continuity. For all multiple comparison analyses Bonferonni corrections (Bland and Altman, 1995) were applied. Flow velocity data were normally distributed and one-way ANOVA was used for comparison. Comparisons of flow data were carried out between three transverse transects (right, middle, left) with six values taken for each location from measurements made at six different distances (upper and lower part of the spawning area and 10, 30, 50 and 100 m downstream). Analyses were performed using IBM SPSS Statistics 20.0 software.

RESULTS

Egg drift

River lamprey were recorded on the spawning site from 4 April to 6 May 2008 and were observed spawning between 19 April and 6 May, although drift sampling occurred during the later part of this period only, from 29 April to 8 May. An average (mean ± SE) of 5 ± 1.8 (range: 0-13) spawners were observed per night in the main spawning area during the egg drift sampling period, but over 50 spawning lamprey were observed on the night of 28-29 April. Only one brook lamprey was observed at the site over the study period, indicating that most egg deposition was from river lamprey. Downstream distribution of wild eggs from the spawning area was observed to occur during all sampling periods (including after lampreys were no longer observed to be spawning, after May 6). The third sampling event occurred during a high discharge conditions (≈ 35 m^3 s^{-1}), coinciding with the highest rate of eggs captured (1745 eggs h^{-1}), 219% and 6017% higher than catch rates on preceding and following sampling events (798 eggs h^{-1}; 29 eggs h^{-1}) when discharges were approximately 9 m^3 s^{-1} and 11 m^3 s^{-1} respectively. No eggs were captured in the nets located 10 m upstream or 100 m downstream of the spawning area.

The mean (± SE) discharge at the Kilgram gauging site during the study period was 11.0 ± 2.1 m^3 s^{-1} (range: 6.2-35.2 m^3 s^{-1}). The flow velocity was higher on the right side (mean 0. 62 m s^{-1}) than in the middle (0.57 m s^{-1}) and the left side (0.54 m s^{-1}) of the sampling area without reaching significant differences between them (one way ANOVA: $F_{2,\ 12}$ = 0.075, P = 0.928). Under these conditions, significant differences were recorded for percentage of eggs caught in different longitudinal (10, 30 and 50 m;

Kruskal–Wallis test, $H(2) = 31.9$, $P < 0.001$) as well as transverse locations (left, middle and right side of the sampling frame; the left half of the channel; Kruskal–Wallis test, $H(2) = 21.1$, $P < 0.001$) (Table 1). The percentage of eggs caught decreased significantly with increasing distance downstream and to the left side (Table 1).

Of the 13000 dyed eggs released in the spawning area 26.2% (3410) were caught in the drift nets, while the median capture rate for the five trials was 8.6%; high recapture rates in right-hand nets in two trials were a result of eggs being released in an excavation in line with the fixed right-hand nets. As for wild eggs, significant differences were recorded for the percentage of eggs caught in different longitudinal locations (Kruskal–Wallis test, $H(2) = 12.5$, $P = 0.002$) as well as between transverse locations (Kruskal–Wallis test, $H(2) = 12.5$, $P = 0.002$) (Table 2). Additionally, most dyed eggs were also captured in the nets located 10 m downstream of the spawning area and on the right side (Table 2). The percentage of eggs caught decreased significantly with distance downstream (Table 2).

Hatching success under different conditions

The first eggs hatched 12 days after the start of the experiment. No significant differences were recorded between artificially fertilized eggs and wild eggs for the percentage of prolarvae recovered, percentage of eggs partially developed, percentage of dead eggs, or oxygen level (Mann–Whitney tests; all $P > 0.05$), so wild and artificially fertilized data were combined for subsequent analyses. Significant differences were recorded at the end of the study between treatments (Kruskal–Wallis *H* test) for the percentage of eggs from which prolarvae were recovered ($H(3) = 41.1$, $P < 0.001$), percentage of eggs partially developed ($H(3) = 13.8$, $P = 0.003$), percentage of eggs dead ($H(3) = 40.5$, $P < 0.001$) and percentage oxygen saturation ($H(3) = 37.9$, $P < 0.001$). A high hatching rate was recorded in the spawning habitat (median of 85.0% of eggs recovered as prolarvae; Figure 1) and control treatments (median of 80.5% of eggs producing prolarvae; Figure 1), without significant differences recorded between them (Mann–Whitney test, $P = 0.581$). Although significantly less than for the spawning habitat treatment (Mann–Whitney test, $U = 15.0$, $P < 0.001$), median egg-hatching success in the larval habitat treatment remained moderate, at 52.0% (Figure 1). The lowest hatching rate (median, 7.5%) was recorded in the stagnant water and treatment, significantly less than for other treatments (Mann–Whitney test, $U = 0.0$, $P < 0.001$). The same significant differences, with a reversed pattern, were recorded for the percentage of dead eggs (Figure 1). A small percentage of eggs were observed partially developed at the end of the study in the spawning microhabitat treatment (median, 0.0%), with similar values observed in the control (median 1.0%; Mann–Whitney test, $U = 66.0$, $P = 0.086$) and significantly lower than observed in the larval habitat (median 7.0%; Mann–Whitney test, $U = 26.5$, $P < 0.001$) and stagnant water treatments (median 5.0%; Mann–Whitney test, $U = 33.5$, $P = 0.002$) (Figure 1).

The oxygen level observed in the control treatment (median 98.5%) was similar to that recorded in the spawning habitat treatment (median 98.7%, Mann–Whitney test, $U = 89.0$, $P = 0.679$), but significantly higher than for the larval habitat treatment (98.1%; $U = 33.0$, $P = 0.003$) and for stagnant water over silt (median 77.2%; $U = 0.0$, $P < 0.001$).

DISCUSSION

This study demonstrates that river lamprey eggs can drift and/or be washed out from spawning areas, even in low to moderate river discharge, causing dispersal in riffle habitat and to bankside or downstream depositional zones. In fact, studies carried out for landlocked sea lamprey populations recorded more than 85% of eggs washed out from nests (Manion and Hanson, 1980; Smith and Marsden, 2009) with a low percentage (0.4 to 7.8%) of eggs remaining and hatching successfully in the nest (Applegate, 1950; Manion, 1968). This study suggests that river lamprey eggs deposited into gravel have a high probability of hatching, while eggs deposited on silt in slow-flowing but aerated conditions typical of larval lamprey habitat have a reduced, but substantial, survival probability. Only if river lamprey eggs are deposited in non-flowing water with silt does hatching seem very unlikely. The observed frequency and extent of river lamprey egg drift in this study suggests that it may be of adaptive value, since water flow disperses a proportion of eggs away from nests towards adjacent gravel habitat (in this study, the highest egg catches were all in nets overlying gravel habitat). Dense local concentrations of small fishes such as European minnow *Phoxinus phoxinus* (Linnaeus, 1758) have been observed foraging opportunistically for river lamprey eggs in nests (Lucas and Morland, pers. obs.), so dispersal of eggs may reduce predation risk. Deposition of eggs into stagnant, depositional zones could be considered maladaptive, but the results here suggest that the majority of eggs are deposited into non-stagnant areas.

Habitat fragmentation and flow regulation in European rivers have been increasing during recent decades (Lucas and Baras, 2001; Mateus *et al*., 2012). As a consequence, the spawning habitat available for anadromous lampreys has decreased and is of a lower quality in many rivers (Ojutkangas *et al*., 1995; Jang and Lucas, 2005; Lucas *et al*., 2009). These factors may contribute towards an increase in the percentage of eggs not developing in spawning areas and being deposited in other microhabitats following spawning (Huggins and Thompson, 1970; Manion and Hanson, 1980). Flow diversion at run-of-river hydropower and other water diversion schemes may leave flow-depleted areas where gravel may become silted during periods of reduced flow (Lucas and Baras, 2001; Robson *et al*., 2011). These results suggest that river lamprey eggs deposited in such zones might be susceptible to reduced hatching success, so due consideration in environmental planning for water diversions, such as those for run-of-river hydropower at sensitive sites for lampreys, such as SACs, must be made (Robson *et al*., 2011). On the other hand, where river lamprey are recolonizing (or are restocked to) rivers to which access has been restored but gravel spawning habitat is in poor condition due, for example, to high silt transport and deposition, egg hatching success could still be moderately successful and could lead to larval recruitment.

Similar to salmonids, the optimum spawning habitat for river lamprey corresponds to areas with swift flow and gravel habitat (Malmqvist, 1983; Hardisty, 1986a; Crisp and Carling, 1989; Jang and Lucas, 2005; Nika and Virbickas, 2010). However, contrary to salmonids, this study suggests that requirements for embryonic development and hatching of river lamprey eggs are less strict and less dependent on the quality of spawning habitat and that, in this respect, conservation of river lamprey populations should be easier than for salmonids. This is demonstrated by moderate hatching success of river lamprey eggs in the larval habitat treatment, with fine sediment and low to moderate flow conditions, previously considered unsuitable for lamprey egg development (Manion and Hanson, 1980; Hardisty, 2006). Similar findings were described by Smith and Marsden (2009) for landlocked sea lamprey, showing a moderate hatching success in silt (69.2%) and sand (50.8%) sediments in the laboratory. The capacity to hatch successfully on different habitats, together with the low percentage of eggs that may remain in the nest, for sea lamprey at least (Smith and Marsden, 2009), demonstrates that habitat located downstream of spawning areas, even larval habitat characterized by fine sediment and moderate to low flow rates, could play an important role in larval lamprey recruitment. This suggests that even small areas of gravel or degraded spawning habitat may enable a higher degree of spawning success than has previously been assumed to be necessary for conservation or recolonisation by river lamprey (*cf.* Lucas *et al.*, 2009 who argued that extensive use of tiny fragments of spawning habitat by river lamprey in a river with migration barriers could act as a population bottleneck), although egg predation, not measured in the current study, may also reduce survival (Smith and Marsden, 2009).

In this study, the lack of eggs in the drift nets placed immediately upstream of the spawning zone suggests that all eggs caught during the study were from the studied spawning site. The observation of only one brook lamprey in the study area indicates that most lamprey eggs recorded originated from river lamprey. While this study was not able to enumerate wild egg washout from spawning in individual excavations, it is estimated that the first row of drift nets sampled 5.6% of the breadth of the spawning area, and so the median recovery in the first row of nets of 7.4% of dyed eggs released in natural excavations at observed lamprey spawning positions, is consistent with a high degree of natural, flow-mediated dispersal suggesting, as described for sea lamprey (Manion and Hanson, 1980; Smith and Marsden, 2009), that most eggs could be dispersed from the excavation. It is assumed that over the short, 1 h, sampling period that the unfertilized dyed eggs behaved no differently to wild eggs and although it is possible that an effect might occur, the spatial patterns of wild and dyed egg capture were similar and so suggest no difference.

Evidence from this study and from video material of natural spawning (Morland, unpublished) suggests that river lamprey may not excavate gravel-bed depressions as nests, normally defined as shelters constructed to hold eggs or young. Instead, we suggest that they are primarily sites for courtship and spawning. Eggs deposited in the excavation are easily flushed out of the depression by lamprey activity (Huggins and Thompson, 1970) or water flow (Morland, pers. obs., video records) and probably only remained in spawning excavations in Hagelin's laboratory studies (Hagelin, 1959) because of the unnaturally low flow velocities employed. The tailspill of pebbles, immediately downstream of the river lamprey spawning excavation results in reduced water depth and accelerating water flow there (Nika and Virbickas, 2010) and the eggs are commonly shed in or nearer this zone than the deepest part of the excavation (Lucas and Morland, pers. obs.). Thus, the so-called 'nests' of river lamprey may function more as egg-dispersal structures, rather than the egg-shelter structures described by Hardisty and Potter (1971) and Maitland (2003). However, the current study did not attempt to quantify the retention of eggs as well as their dispersal from individual 'nests', due to potential disturbance effects, but such an experiment is desirable in order to determine egg dispersal, retention in the 'nest' and survival rates of eggs in these categories. For river lamprey, in which multiple females may use the same 'nest', enclosure-type experiments would be needed for this, combined with drift netting and egg retrieval from the 'nest(s)' and surrounding substrate. In conclusion, it seems that egg drift is a normal, probably adaptive, phenomenon for river lamprey. Since river lamprey egg development does not rely on such stringent conditions as for salmonids, conservation efforts directed at river lamprey need not necessarily aim to ensure spawning habitat availability or quality is at the level needed for high spawning success of salmonids. However, where mixed lamprey and salmonid populations occur, ensuring the availability of high-quality salmonid spawning habitat will undoubtedly benefit lamprey, as long as plentiful stable silt habitat for lamprey larvae is readily available nearby (though the availability of plentiful silt beds within the river channel is often regarded as undesirable by salmonid stakeholders / managers). By contrast, in considering conservation of mixed salmonid and lamprey populations (discounting non-native *P. marinus* in the upper Laurentian Great Lakes), provision of upstream fish passage facilities suitable for salmonids to access spawning habitat, while commonplace, is rarely adequate for lampreys (e.g. Moser *et al*., 2002) including river lamprey (Laine *et al*., 1998; Foulds and Lucas, 2013). As regards lamprey conservation, this remains an area requiring urgent attention.

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Tables

Table 1. Median, lower and upper quartiles (25-75%) of total wild river lamprey eggs caught (*n*), number of eggs caught per hour (n h^{-1}) and percentage of wild eggs caught (%) over all sampling events. Values are for nets placed in different longitudinal (10, 30 and 50 m) and transverse (left, middle and right side) locations.

Categories	Subcategories	Eggs caught (*n*)	Eggs caught (n h^{-1})	Eggs caught (%)
Longitudinal location	10 m	184 (31-508)[a]	32.0 (13.6-338.0)[a]	71.5 (58.9-80.5)[a]
	30 m	43 (7-254)[a,b]	8.5 (3.5-161.0)[a,b]	21.4 (19.1-26.6)[b]
	50 m	2 (1-126)[b]	1.0 (0.1-62.3)[b]	6.1 (0.3-12.2)[c]
Transverse location	Left	8 (0-56)[a]	1.5 (0.0-55.5)[a]	1.1 (0.0-15.1)[a]
	Middle	124 (25-272)[b]	29.0 (7.5-183.2)[a]	44.4 (32.9-57.8)[b]
	Right	94 (17-542)[b]	17.5 (3.4-323.0)[a]	43.2 (33.3-57.9)[b]

Different letters indicate significant differences between locations (transverse and longitudinal locations analyzed separately; Mann–Whitney *U* test, with Bonferonni-corrected significance at $P = 0.016$).

Table 2. Median, lower and upper quartiles (25-75%) of total dyed river lamprey eggs caught (*n*), percentage of dyed eggs caught and percentage of eggs caught from total released (CTR). Values for nets placed in different longitudinal (10, 30 and 50 m) and transverse locations (left, middle and right side).

Categories	Subcategories	Eggs caught (*n*)	Eggs caught (%)	CTR (%)
Longitudinal location	10 m	45 (36-1402)[a]	86.0 (80.7-87.9)[a]	7.4 (4.5-28.0)[a]
	30 m	13 (5-169)[a]	10.8 (10.2-18.0)[b]	1.2 (0.7-3.4)[a,b]
	50 m	1 (0-65)[a]	1.7 (0.0-3.9)[c]	0.1 (0.0-1.3)[b]
Transverse location	Left	1 (0-30)[a]	1.5 (0.0-2.4)[a]	0.2 (0.0-0.6)[a]
	Middle	13 (11-397)[a]	22.1 (21.5-30.2)[b]	2.6 (1.2-7.9)[b]
	Right	46 (29-1209)[a]	76.4 (67.4-78.5)[c]	6.8 (3.6-24.2)[b]

Different letters indicate significant differences between locations (transverse and longitudinal locations analyzed separately; Mann–Whitney *U* test, with Bonferonni-corrected significance at $P = 0.016$).

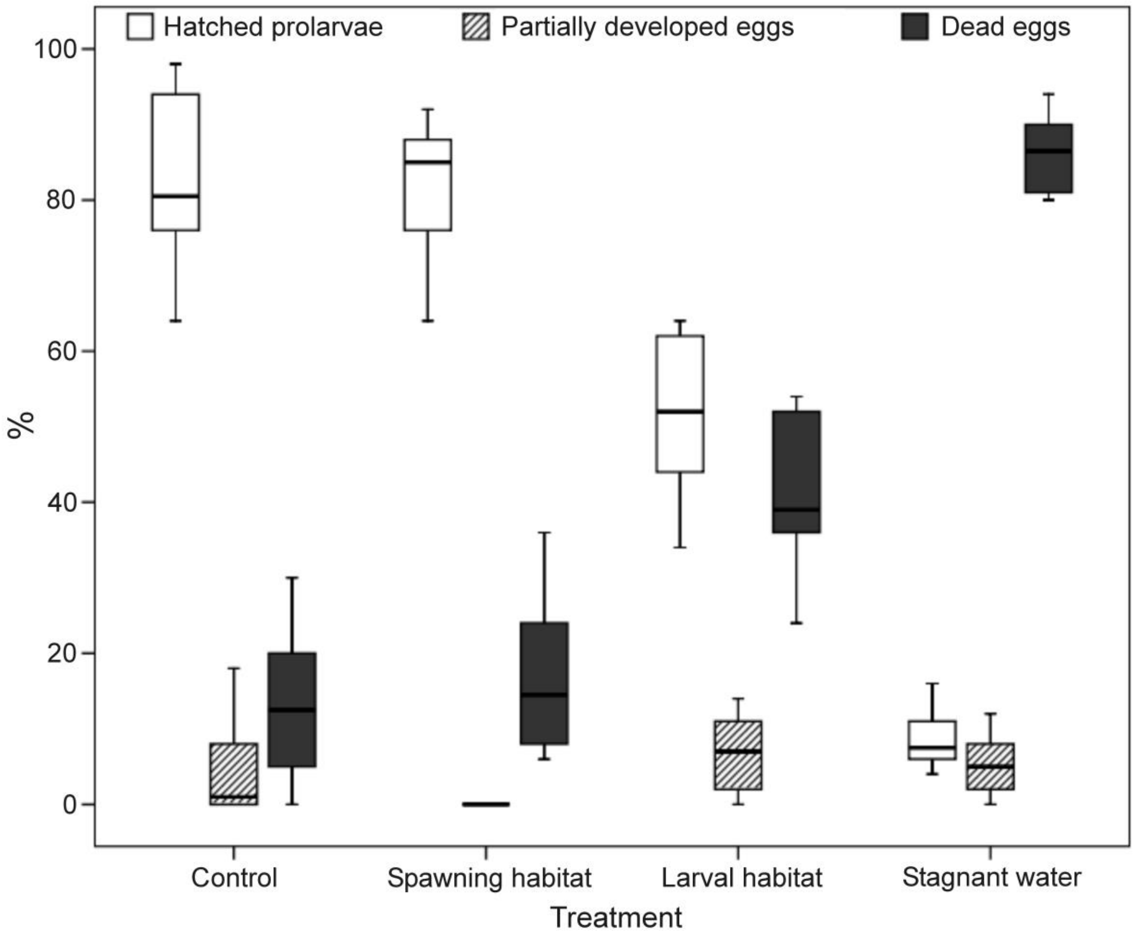

Figure 1. Box plots (maximum and minimum values, lower and upper quartiles, and median) of the percentage of hatched prolarvae, partially developed eggs and dead eggs of river lamprey observed for each treatment.