1	Rapid visual assessment of spawning activity and associated habitat utilisation of
2	sea lamprey (Petromyzon marinus Linnaeus, 1758) in a chalk stream: implications
3	for conservation monitoring
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24 Introduction

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The anadromous sea lamprey (Petromyzon marinus L.) has a native geographic range 26 27 extending across the Northern Atlantic, colonising the rivers of countries abutting coastal shores between Labrador, Canada to Florida in the West (Renaud 1997) and 28 from Norway into the western Mediterranean to the East (Kottelat & Frehof 2007). 29 30 Beyond its native range, the species has capitalised on the anthropogenically-engineered connectivity between the West Atlantic and the Great Lakes (Hartman 1972). In this 31 32 extended range, it is invasive and considered a pest (Smith and Tibbles 1980). In its native range, however, their populations are in general decline through factors including 33 river fragmentation, habitat loss and declining water quality (Renaud 1997; Almeida et 34 35 al., 2002; Maitland et al., 2015). Correspondingly, it has conservation designations 36 under Annex II of the EU Habitats Directive (Directive 92/43/EEC). These designations require their populations to be monitored regularly and conservation status evaluated. 37

38 The monitoring of *P. marinus* populations currently focuses on the cryptic, relatively sedentary and extended (~5-6 years) life stage of the ammocoetes (larvae) and thus 39 40 attempts to quantify recruitment success and nursery mortality in these early life stages (Harvey and Cowx 2003; Quintella et al., 2003). Data validity, however, remains 41 42 sensitive to the confidence associated with preferred microhabitat utilisation; most 43 studies have focused in water depths below 1m (e.g. Malmqvist 1980; Potter et al., 1980; Beamish and Jebbink 1994; Beamish and Lowartz 1996; Almeida and Quintella 44 2002; Sugiyama and Goto 2002; Torgersen and Close 2004; Lasne et al., 2010) yet the 45 46 recent development of habitat utilisation curves suggests marked preferences for deeper nursery habitats (> 2m; Taverny et al., 2012). Moreover, there is little attention on their 47 adult life-stages, despite the number of returning adults being potentially important for 48

49 the subsequent numbers of ammocoetes (Quintella et al., 2003). Whilst this may be 50 understandable when the adults are at sea, their presence in freshwater potentially 51 provides valuable monitoring opportunities that would provide complementary 52 population level data, such as adult numbers, nest counts and upstream migration 53 distances.

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55 Consequently, the aim of this study was to utilise the *P. marinus* spawning migrants 56 of an English chalk stream to provide initial assessments of (i) the value of nest counts 57 as a population and conservation monitoring tool; (ii) distances moved upstream to 58 spawn and in relation to potential blockages to migration; and (iii) identify the habitat 59 utilisation of spawning adults. The value of these data are then discussed within a 50 conservation context.

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62 Materials and Methods

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The study was completed in summer 2014 in the River Frome, a relatively small chalk river (48 km in length) in Southern England that rises in the Dorset Downs at Evershot and drains into Poole Harbour (Fig. 1). River widths are rarely greater than 15 m and depths rarely above 2 m depth.

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Spawning of adult *P. marinus* in the river commenced in May and concluded in late June. At its conclusion, intensive observations on the numbers and spatial distribution of *P. marinus* nests were completed between 1st and 7th July through direct observations completed by surveyors with high experience in salmonid redd counting. Surveys comprised of walking along the top of the river bank, starting at the river's tidal limit

74 and continuing until the upstream limit of nest distribution was confirmed by extending 75 the survey 3 km beyond the location of the last nest, which also incorporated two further potential instream barriers. During this period, river conditions were of low flow and 76 77 high water transparency, and nest identification was assisted by surveyors wearing polarised sun-glasses. This meant the majority of nests were identified without the 78 79 requirement to enter the river channel. On identification of each nest, its precise location 80 was recorded using a hand-held GPS (Garmin 60 CSx), with nest dimensions (length and width) estimated to the nearest 0.1 m. These locations were used to calculate the 81 distance of each nest from the tidal limit. Data on river discharge (m^3/s^{-1}) and water 82 temperature (°C) data were also available from an automated gauging station weir 83 (50°40'51.73"N 2°11'20.97"W) where recordings were taken every 15 minutes. These 84 85 data were used to assess their influence in the timing of the upstream spawning migration. 86

Quantitative characterisation of spawning site selection and nest structure was 87 conducted on 1st and 4th July 2014, with a sub-sample of 44 individual nests examined. 88 Geo-referenced nests, which had been vacated by adults, were subject to the following 89 measurements: Depression length (dL); Depression width (dW); water depth at 90 upstream lip (usD); maximum water depth of depression (maxD); water depth at 91 downstream lip (dsD); and excavation depth (DE). To characterise the ambient habitat 92 93 in which spawning sites were selected, the following measurements were recorded one metre upstream of the leading edge of each nest: mean water column velocity (mV); 94 mean column water temp (mT); and water depth (Dus). To explore any potential stimuli 95 96 for spatial spawning site selection, water temperature was also recorded within the interstitial gravel of each nest. All length measurements were recorded using a metal 97 rule (1m) to the nearest cm. Water velocity was recorded using an impeller flow meter 98

99 (Valeport 002) with cm/s⁻¹ averaged over 30 seconds. Temperature was recorded using a 100 hand held digital probe (Sper Scientific 800007). Excavated stones deposited at the tail 101 of each nest were then measured without physical disturbance, achieved by placing the 102 metal rule flush with the riverbed and the use of an underwater video camera (GoPro 103 Hero 3) that collected 30 seconds of high definition video footage. Each video clip was 104 then subsequently analysed on screen with the maximum axis dimension of a sub-105 sample of 10 stones measured using digital callipers, calibrated against the rule.

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107 **Results**

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109 The first P. marinus nest recorded in the River Frome in 2014 was on 16 May and the final spawners were observed on 25th June. The subsequent nest counts indicated a total 110 of 98 nests had been excavated, between 1.8 and 17.3 km upstream of the tidal limit 111 (Fig. 2). Of these nests, four were still being guarded by adult males. Spawning activity 112 113 had been concentrated within the lower 9 km reach of non-tidal river, where 88 % of nests were recorded (86 of 98). Of these, 36 were concentrated within the 1km reach 114 immediately downstream of a gauging weir (Fig. 2). Only 12 nests were observed above 115 this gauging weir; six between this weir and the next major migration impediment, and 116 a further six between this and the next major impediment (Fig. 2). The upstream limit of 117 118 the survey extended 22 km upstream of the tidal limit, with all spawning activity confirmed to be limited to the lower 19 km of non-tidal river. The abiotic characteristics 119 of the river changed markedly between March and the period of spawning activity (Fig. 120 3), with flow decreasing from a maximum of 16.16 m^3/s^{-1} to a minimum of 3.15 m^3/s^{-1} . 121 There were two notable flow peaks in this period, on 8 April (12.3 m^3/s^{-1}) and 28 April 122 (10.3 m^3/s^{-1}). Over the same period, water temperature increased from a minimum of 123

124 7.7° C to 20.1°C. Evidence of first nest construction activity corresponded with a water 125 temperature of 14.6°C and discharge of 4.6 m³ s⁻¹.

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127 A total of 44 nests, distributed downstream of East Stoke Gauging Weir (Fig. 1), 128 were examined on 1st and 3rd July. These were typically crater shaped with excavated 129 stones deposited around the nest perimeter. The physical, physicochemical and 130 hydrological parameters of the nests are provided in Table 1.

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132 Discussion

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134 Effective conservation monitoring relies on the ability of managers to detect 135 population declines within sufficient timeframes that facilitate initiation of corrective interventions, i.e. before critical population thresholds are reached (Staples et al., 2005). 136 Despite current European best-practice monitoring protocols acknowledging that annual 137 138 monitoring is required to assess recruitment success of P. marinus (Harvey & Cowx 2003), the ability to differentiate between the 0+ (<60 mm) life stages of *Petromyzon* 139 and Lampetra species has been reported to necessitate euthanasia of individuals, with 140 the identification of the smallest individuals also being constrained due to the 141 requirement for genotyping (Taverny et al., 2005). This means if destructive sampling is 142 143 to be avoided, either the costs of monitoring ammocoetes increases or imparts a minimum two year lag phase before recruitment success can be validated. This 144 constrains abilities for initiating corrective interventions on P. marinus populations and 145 146 thus other, complementary monitoring options are required. Correspondingly, our outputs suggest that annual nest counts should provide these complementary monitoring 147 options and ought to be incorporated into their monitoring toolbox forthwith; given their 148

ability to provide information on long-term patterns in returning adult numbers, theextent of their spawning migrations and their habitat utilisation.

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152 Whilst allowing for potential sources and magnitudes of sampling error (Dunham et al., 2001), the quantity and distribution of redds, the nests of salmonid fishes, have long 153 154 been recognised as providing a cost- and time-efficient method for monitoring the size 155 of their adult populations (e.g. Rieman and Myers 1997; Al-Chokhachy et al., 2005). As 156 such, they are a strong predictor of subsequent levels of part production (Beland 1996) 157 and been used to, for example, evaluate the efficacy of habitat restoration efforts (Merz and Setka 2004) and the effects of catchment management practices and instream 158 159 barriers on migration and spawning (House 1996). With spawning representing perhaps 160 the least cryptic stage of the life history of lampreys then it is perhaps surprising that examples of the use of nests as a monitoring tool are limited. Examples specific to P. 161 marinus tend to be restricted mainly to 'grey' literature sources, but include extensive 162 163 monitoring to evaluate the efficacy of a range of control treatments for invasive populations across 10 tributaries of Lake Champlain, USA (Parren and Hart 2012), 164 165 surveys which successfully confirmed the rivers supporting spawning activity in the Humber catchment rivers, UK (Bellflask Ecological Survey Team 2009), and the use of 166 167 nests to identify spawning grounds and the characterisation of spawning habitat in the 168 River Mulkear, Ireland (Igoe et al., 2004). More recently, however, Lasne et al., (in press) demonstrated the efficacy of nest counts for evaluating the effects of dam 169 removal on the colonisation of a coastal river system in France by *P. marinus*. 170

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The present study demonstrated that the rapid and cost effective collection (three 'man' days) of data can provide a temporal baseline on the spatial utilisation of

174 spawning habitats across an entire (albeit small) river catchment. Whilst the physical 175 and physiological factors determining the ability and propensity of adult P. marinus to negotiate the passage of flow control structures was beyond this study, outputs clearly 176 177 demonstrated that relatively short migrations were undertaken, with 88% of all nests distributed between the tidal limit and the first flow control structure that was only 9 km 178 179 upstream. The availability of spawning habitat upstream of this structure was observed 180 to be consistent with that downstream, and given the structure represented a relatively 181 minor migration obstacle, this suggests that where suitable habitat is available, adults 182 may consciously elect to spawn on the first appropriate habitat encountered in order to prevent unnecessary energy expenditure, so maximising investment in the reproductive 183 184 process (Quintella et al., 2004).

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186 Despite being the first study to describe spawning habitat utilisation in chalk streams, the results reported here are not dissimilar from the few previous studies extending 187 188 across the range distribution of *P. marinus*. Particularly notable is the mean nest length, reported here as 1.2 m, which is identical to that reported from Ireland by Igoe et al. 189 190 (2004). The size of gravels used for nest construction in this study ranged between 11 and 154 mm (mean = 52.3 mm). This compares to ranges reported from the Great Lakes 191 of 15 to 115 mm by Morman et al. (1980) and 9.5 to 50.8 mm by Applegate (1950). 192 193 Water depth (as recorded 1 m upstream of nests) ranged between 0.3 and 1.0 m (mean = 194 0.52 m) and compares with a preferred depth of 0.4 to 0.6 m reported by Hardesty (1986) and within the extremes 0.1 to 1.7 m reported by Applegate (1950). The 195 observed mean water velocity of 0.78 \pm 0.03cm s⁻¹ and ranges of 0.47 to 1.29 cm s⁻¹ 196 observed from this study also fit within the ranges reported from the Great Lakes 197 catchments of 39.6 to 158.5 cm s⁻¹ reported by Applegate (1950). No differences were 198

199 observed in water temperature between mean column and inter-gravel flows, suggesting

200 nest site selection was not influenced by hyporheic or groundwater flows.

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202 Whilst the adult life stage of P. marinus has to date been typically overlooked in favour of ammocoetes as providing a key indicator of population performance and 203 204 conservation status, the present work highlights the value of nest counts as either an independent or complementary monitoring tool to track temporal tends in adult lamprey 205 206 numbers in chalk streams and throughout other river catchments where nests can be 207 easily observed (Igoe et al., 2004; Bellflask Ecological Survey Team 2009). In addition to the added value associated with expanding the currently limited and much needed 208 209 knowledge of spawning behaviour across lamprey species (Johnson et al., 2015) and 210 their habitat utilisation, dismissing the efficacy and cost effectiveness of incorporating nest counts within future condition assessment methodologies will compromise the 211 protection of spawning habitats (Nunn et al., 2008) and the design of spatial sampling 212 213 strategies to monitor ammocoete numbers and distributions.

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216

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Table 1. Ranges, means and confidence (SE) associated with the physical, physicochemical and hydrological parameters recorded for 44 *P. marinus* nests in the River Frome, July 2014.

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Variable	Min	Max	Mean	SE
Depression length (dL m)	0.5	1.7	1.2	0.055
Depression width (dW m)	0.6	2	1.09	0.05
Depth at us lip (usD m)	0.25	0.99	0.57	0.03
Max depth depression (maxD)	0.43	1.1	0.7	0.02
Depth at ds lip (dsD m)	0.23	0.84	0.45	0.02
Depth excavated (DE m)	0.03	0.22	0.13	0.01
Mean column velocity (mV cm s ⁻¹)	0.47	1.29	0.78	0.03
Mean column water temp. (°C)	15.5	16.8	16.25	0.08
Inter-gravel temp. (°C)	15.5	16.8	16.3	0.11
Water depth (usD m)	0.3	1.03	0.60	0.03
Substrate size (mm)	11	154	52.3	29.42

Figure 1. Map of study site showing lower (22 km) non-tidal section of River Frome
and location of the following instream structures: (a) East Stoke Gauging Weir; (b)
Bindon Mill; (c) East Burton Hatches; (d) Moreton Weir; (e) Hurst Weir. TL indicates
upstream limit of tidal influence (tidal limit).

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Figure 2. Frequency of *P. marinus* nests recorded on the River Frome 2014, versus
distance from tidal limit. Dashed vertical lines represent the following instream flow
control structures: (a) East Stoke Gauging Weir; (b) Bindon Mill; (c) East Burton
Hatches; (d) Moreton Weir; (e) Hurst Weir. All nest counts conducted 1–7 July, 2014.

Figure 3. Daily mean values of river discharge $(m^3/s^{-1} - \text{solid line})$ and temperature (°C - dashed line) recorded at East Stoke Gauging Weir, River Frome, between March and July, 2014. Data generated from 15 minute data logs. Dashed vertical lines represent periods of: a: observed nest building activity, b: nest count survey.

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