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MONITORING POPULATIONS OF THE FRESHWATER PEARL MUSSEL, *MARGARITIFERA MARGARITIFERA*, STAGE 3 AND STAGE 4 SURVEY

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Front cover, small photographs from top row:

Limestone pavement, Bricklieve Mountains, Co. Sligo, Andy Bleasdale; **Meadow Saffron** *Colchicum autumnale*, Lorcan Scott; **Garden Tiger** *Arctia caja*, Brian Nelson; **Fulmar** *Fulmarus glacialis*, David Tierney; **Common Newt** *Lissotriton vulgaris*, Brian Nelson; **Scots Pine** *Pinus sylvestris*, Jenni Roche; **Raised bog pool**, Derrinea Bog, Co. Roscommon, Fernando Fernandez Valverde; **Coastal heath**, Howth Head, Co. Dublin, Maurice Eakin; **A deep water fly trap anemone** *Phelliactis* sp., Yvonne Leahy; **Violet Crystalwort** *Riccia huebeneriana*, Robert Thompson

Main photograph:

Monitoring the **Freshwater Pearl Mussel** *Margaritifera margaritifera,* Evelyn A. Moorkens

Monitoring Populations of the Freshwater Pearl Mussel, *Margaritifera margaritifera*, Stage 3 and Stage 4 Survey

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Executive Summary

The Freshwater Pearl Mussel (*Margaritifera margaritifera*) is a highly threatened and sensitive river bivalve that is categorised as critically endangered in Ireland and across Europe. It is in unfavourable bad status in Ireland and in the five EU biogeographic regions in which it occurs. Damage to the species' habitat, through hydrological, sedimentation, other morphological and enrichment impacts, is responsible for the decline of the Freshwater Pearl Mussel. Such habitat damage causes mussel mortality, poor adult condition (health) and failure to recruit young to populations.

Since 2004, NPWS has been conducting monitoring, or surveillance, of the Freshwater Pearl Mussel under Article 11 of the Habitats Directive. This Irish Wildlife Manual documents the monitoring methods used. These have been developed for Ireland by Freshwater Pearl Mussel experts. In 2009, Regulations (S.I. 296 of 2009) were made to set environmental quality objectives for listed Freshwater Pearl Mussel SAC populations. The schedules to the regulations, and the site-specific conservation objectives, as well as the NSAI standard, form the basis for assessment of conservation status/condition using monitoring data.

This manual provides methods for repeat monitoring of numbers of adult mussels at varying densities. It also describes the measurement of mussels, with searches for juveniles, used to produce demographic profiles that are essential to assessing the viability of a population. Searching for juveniles carries very high risk of injury or death to mussels, and damage or destruction of their habitat and, therefore, requires specific expertise.

Much of the manual is taken up with methods for monitoring the species' habitat – assessing the suitability/quality of the habitat for mussels and its condition. The key habitat parameters relate to the substratum: its physical and chemical condition, colonisation by plants and accumulation of organic matter. Flow, particularly near-bed velocity, is extremely important as a driver of the quality and condition of the habitat.

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1 Introduction

1.1 Freshwater Pearl Mussel monitoring in Ireland

Standard methods for Stage 1 and Stage 2 *Margaritifera* survey in Ireland were first published in 2004 (Anon., 2004). Since then, a series of condition monitoring assessments have been undertaken, focussing on Special Areas of Conservation (SAC) populations of the Freshwater Pearl Mussel, and the European Communities Environmental Objectives (Freshwater Pearl Mussel) Regulations 2009 (S.I. 296) have been published. A CEN (the *European Committee for Standardization*) standard has also been published (NSAI, 2017), setting out a series of requirements for achieving favourable condition for *Margaritifera* populations. Several important studies have been published, notably Moorkens & Killeen (2014) and Killeen & Moorkens (2020).

Schedules 3 and 4 of the 2009 Regulations are used as the basis for assessment of conservation condition/status during the monitoring surveys of both SAC and non-SAC populations. The monitoring methods have been tailored to provide the data necessary to assess these and additional, important population criteria and habitat elements.

This Irish Wildlife Manual describes the following monitoring methods used to inform the different aspects of condition assessment

- Stage 3 survey involves monitoring, often in permanent sites, to gain information on the status of a mussel population, and changes to that status over time. Stage 3 survey does not include handling mussels.
- Stage 4 survey includes an assessment of mussel demographic profiles, which involves handling and measuring mussels, searches for juvenile mussels, and the temporary removal of mussels from their habitat for genetic sampling, stress testing and fecundity checking.

The Freshwater Pearl Mussel (*Margaritifera margaritifera*) is a highly threatened and sensitive river bivalve that is categorised as critically endangered in Ireland (Byrne *et al.*, 2009) and across Europe (Moorkens, 2011). It is in unfavourable bad status in Ireland (NPWS, 2019 a, b), and in the five EU biogeographic regions in which it occurs. Damage to the species' habitat, through hydrological, sedimentation, other morphological and enrichment impacts, is responsible for the decline of the Freshwater Pearl Mussel. Such habitat damage causes mussel mortality, poor adult condition (health) and failure to recruit young to populations.

Freshwater Pearl Mussel monitoring, or surveillance, has been conducted since 2004 in Ireland under Article 11 of the Habitats Directive. Monitoring is undertaken at 'population' scale, which coincides with the catchment/sub-catchment. NPWS aims to routinely monitor a representative sample of *c.* 30 populations once every three years. The monitored populations cover a broad geographical range and a wide variation in population size and viability. While populations outside of SACs are monitored, the programme generally concentrates on the populations within the 19 SACs listed for the protection of the species.

1.2 Survey licensing

The Freshwater Pearl Mussel is protected under Schedule 5 of the Wildlife Acts (1976 and 2000) and, as a result, any disturbance to mussels or their habitat is an offence. Entering the species' habitat, including for the purposes of survey and monitoring, carries a risk of injury to mussels and of interference with or destruction of their habitat, so it must be licensed under Sections 9, 23 and 34. A licence is required for all survey and monitoring of the species, even where animals will not be handled. Because the species is so threatened and in such poor condition, it is only in exceptional cases that handling or

movement of animals is licenced. As well as ensuring the protection of the species and its habitat, this licensing system aims to ensure that survey does not interfere with permanent monitoring sites.

1.3 Structure of this manual

This Irish Wildlife Manual assumes a significant level of understanding of the biology and ecology of the Freshwater Pearl Mussel. For information on these topics, see Moorkens (1999), Skinner *et al*. (2003), Bauer & Wächtler (2000), amongst others. Following this brief introduction to the purpose of the manual, Chapter 2 gives an overview of the monitoring methods. Chapter 3 details methods for counting adult mussels. Chapter 4 explains the methods for monitoring the habitat of the species: measurements that must be taken during all survey and monitoring of the Freshwater Pearl Mussel. Chapter 5 details a method for mapping the quality and condition of Freshwater Pearl Mussel habitat. Chapter 6 is the method of measuring mussel shell lengths, including searching for juveniles, in order to assess population demography. It should be noted that considerable expertise is required to recognise juvenile mussel habitat and that only highly experienced surveyors are licensed to search for juvenile mussels. There is a significant risk that, by searching for juvenile mussels, the young animals will be injured or killed and their habitat will be damaged or destroyed.

2 Methodology - overview

Routine monitoring can be separated into surveillance of

1. The Freshwater Pearl Mussel population,
2. The habitat of the species, and
3. Pressures and threats.

The following sections arrange the methods under these three headings. In practice, all three should be recorded during any field survey for the species. Guidance is also provided (Section 2.5) on weather and flow conditions during monitoring. The data gathered during routine monitoring are assessed to determine the conservation status/condition of a population, based on the 3rd and 4th Schedules to S.I. 296 of 2009 and attributes and targets in the site-specific conservation objectives.

2.1 Monitoring the Freshwater Pearl Mussel population

Routine monitoring of a Freshwater Pearl Mussel population aims to quantify changes in a) adult mussel numbers/mortality, b) the distribution of the species in the river system and c) overall population structure/demographics. This manual concentrates on methods to quantify adult mortality (Chapter 3). Chapter 6 explains the method used to assess population demography, which carries high risk of injury or death to juvenile mussels and damage or destruction to their habitat. Some discussion is provided below on distribution monitoring, but the methods for this are covered in Anon. (2004).

2.1.1 Monitoring changes in adult mussel numbers/mortality

The method used depends on the overall size of the Freshwater Pearl Mussel population, and its distribution and density. In larger populations with high density, permanent marked transects (or fixed permanent counts) are established. In severely depleted and damaged populations, with small numbers of mussels and low population density, entire stretches of river are counted using sweep transects.

2.1.1.1 Permanent marked transects

Transects run perpendicular to the general flow across the full width of a river and the numbers of visible live and dead mussels are counted in contiguous 1 m x 1 m quadrats. The location of each transect is carefully mapped and marked to enable repeat monitoring and analysis of changes over time. For routine monitoring purposes a minimum of three permanent transects is counted per population.

2.1.1.2 Sweep transects

For mapped sections or sub-sections of rivers, all mussels are counted or are estimated using one count every 100 linear metres of rivers. Repeat monitoring of these sections allows analysis of changes over time.

See Chapter 3 for further information on both transect methods.

2.1.2 Monitoring changes in adult mussel distribution

The broad distribution of the population within its catchment is surveyed using Stage 1 and Stage 2 methods and monitored using spot samples to check for extensions and contractions in the range. This is a relatively rapid survey of points across the species' distribution in the river system, where the grid co-ordinates for all survey spots are recorded. Changes in distribution are measured against previous

survey reports and the Freshwater Pearl Mussel Habitat Classification features class of the NPWS *Margaritifera* GeoDatabase[1], where available.

2.1.3 Monitoring population structure/demographics

Small (0.5 m x 0.5 m = 0.25 m^2) quadrats are placed in juvenile habitat. All visible mussels are counted, and then removed, 'hidden' mussels are counted and removed, and the substratum is carefully searched for juveniles. Juvenile mussels are those that live buried in the substratum and are of approximately five years of age or younger. The shell-length of each mussel is measured to the nearest 0.1 mm using Vernier callipers, and all mussels are carefully replaced in their habitat. See Chapter 6 for further information.

2.2 Monitoring the Freshwater Pearl Mussel habitat

At all survey stations (*i.e.* permanent marked transects, sweep transects, at spot samples for population distribution and juvenile quadrats for population), the habitat of the Freshwater Pearl Mussel is examined. Measurements may include cover abundance of macroalgae, macrophytes, detritus and fine sediment, substratum characteristics, redox-potential and, where possible, flow. Chapter 4 provides further details on the methods.

2.3 Monitoring pressures and threats to the Freshwater Pearl Mussel

During sampling of the Freshwater Pearl Mussel population and its habitat, all observed pressures and likely threats must routinely be documented. Pressures and threats are documented in a way that is meaningful to the species and the relevant land-use or activity. Linkages are also made, where possible to the European Birds and Habitats Directives reference list of threats, pressures and activities. This is not always straight forward, however (see NPWS, 2013c), and the reference list has changed over time. Significant revisions were made to the 2007-2012 standard list (Ssymank, 2011) for the 2013-2018 reporting cycle (Version 2.4 - 07/05/2018, available from http://cdr.eionet.europa.eu/help/habitats_art17/).

Pressures and threats can broadly be divided into four categories

1. Direct damage or disturbance (*e.g.* trampling by livestock)
2. Hydrological changes (*e.g.* through excavation or clearance/maintenance of surface or sub-surface drains, sealing of vegetated land, land-use changes from semi-natural habitat cover)
3. Hydrogeological changes, particularly in the riparian area within 50 m either side of mussel habitat (drying out or intensive management of areas that should be contributing to seepage of valuable water and oligotrophic detritus to the river bed)
4. Morphological changes (*e.g.* erosion or armouring of the river bank, bridge works)
5. Pollution (*e.g.* slurry, nutrients, fine sediment).

It is important to keep a photographic record, where possible. Where incidents such as severe sediment pollution are recorded, an effort should be made to trace the source. Good understanding of the biology

[1] This NPWS maintained spatial data set includes the following shapefiles: 1) *Margaritifera* sensitive areas (polygons of the catchments of all known extant populations. Available at www.npws.ie and http://dahg.maps.arcgis.com/home), 2) Freshwater pearl mussel Habitat Classification (polyline of the extent of mussel habitat in SAC catchments and 3) *Margaritifera* records (point file of positive records for the species). The GeoDatabase is not provided in its entirety to the public, but data for specific catchments and sub-catchments can be requested via the NPWS data request form.

of the Freshwater Pearl Mussel and the ecology of rivers is required to recognise and understand the likely impacts of these pressures and threats.

2.4 Baseline monitoring

Full baseline monitoring has been completed for approximately 14 populations, 12 of which are amongst the 27 SAC populations listed on the First Schedule to S.I. No. 296 of 2009. In addition to establishing fixed permanent count areas (permanent marked or sweep transects) and conducting baseline population demographic surveys, baseline monitoring involves mapping the distribution and abundance of mussels throughout the catchment and collating all historical information on the Freshwater Pearl Mussel population. Abundance is mapped using a categorical scale of

1. Abundant (over 1,500 per 100 m, but may be up to 500 per metre squared)
2. Common (301 – 1,500 per 100 m)
3. Frequent (41 – 300 per 100 m)
4. Occasional (1 – 40 per 100 m)
5. Dead shells only
6. Absent (no evidence)

2.5 Weather and flow conditions during monitoring

It is important to gauge whether conditions are suitable for survey, for both safety and accuracy reasons. Clear, sunny conditions in low flows are ideal. A survey should not proceed in the following conditions

1. When the river is in flood
2. When the river is recovering from heavy rains or is highly coloured
3. When it is raining
4. When it is overcast, either more than 60% cloud cover or at dawn or dusk, or where daylight levels are naturally low (*e.g.* winter).

The most important check to make is that the river bed area can clearly be seen.

2.6 Survey equipment

In water up to 75 cm deep, survey can be carried out by wading using a bathyscope, or glass-bottomed bucket. It is recommended that the river worker wears a dry suit, closed at all times for safe floatation should the surveyor trip or become submerged in the water. Neoprene chest waders that will float rather than fill up with water can be used in lower flow situations. Wearing of a life jacket is essential for workers taking notes on the bank. The toe area of the dry suit / boot should be painted with a red non-toxic, waterproof paint in order to determine visibility levels in the river (Figure 1).

Figure 1 Boot painted to aid the assessment of river bed visibility.

In water more than 75 cm deep, wading survey is impractical on safety and accuracy grounds. In this situation the survey should be undertaken by snorkelling. Maintaining position in the water column can be challenging in the flow. As well as a dry suit, snorkel, mask and gloves, a strong stick of length a little longer than the water depth is useful to anchor the surveyor while counting mussels. A weight belt may also be needed. In general, it is not recommended to wear fins, to allow the surveyors to stand up easily when needed. Very short fins may be useful in strong flows, however long fins can cause habitat disturbance and damage. An underwater torch is useful for surveying areas under deep shade. Additional useful items include a net bag (for dead shells) and plastic 'click' counters for dealing with large numbers.

Survey should never be carried out alone. One surveyor, known as the bank manager, should remain on the bank at all times, monitoring the safety of the river worker(s) and recording the data. The bank manager is responsible for equipment, including redox and flow meters, notebooks, forms, maps, GPS and camera. The bank manager should have a charged mobile phone in case of an emergency.

2.7 Assessing the conservation condition of a Freshwater Pearl Mussel population

The data gathered on the population (*i.e.* a) adult mussel numbers/mortality, b) the distribution of the species in the river system and c) overall population structure/demographics) are assessed against the criteria and targets established in the 3rd Schedule to S.I. 296 of 2009 (Table 1), as well as the relevant attributes and targets in site-specific conservation objectives (see Appendix I) and with reference to the CEN Guidance standard on monitoring Freshwater Pearl Mussel (*Margaritifera margaritifera*) populations and their environment (NSAI, 2017). This assessment gives the conservation condition/status of the Freshwater Pearl Mussel population. Sampling small, juvenile-search quadrats provides the key information on the percentage of the population composed of young and juvenile mussel.

Table 1 Criteria for assessment of the conservation condition of Freshwater Pearl Mussel populations, as set out in the Third Schedule to S.I. No. 296 of 2009.

Criterion	Target to pass	Notes
Numbers of live adults	No recent decline	Based on comparative results from the most recent surveys
Numbers of dead shells	< 1% of population and scattered distribution	1% considered to be indicative of natural losses.
Mussels shell length ≤65 mm	At least 20% of population ≤ 65 mm in length	Field survey of 0.5 x 0.5 m quadrats must be carried out in suitable habitat areas for juveniles
Mussels shell length ≤ 30 mm	At least 5% of population ≤ 30 mm in length	Field survey of 0.5 x 0.5 m quadrats must be carried out in suitable habitat areas for juveniles

The data gathered on the species' habitat are assessed against the elements and objectives in the 4th Schedule to S.I. 296 of 2009 (Table 2), as well as the relevant attributes and targets in site-specific conservation objectives (see Appendix I), and NSAI (2017). This assessment gives the conservation condition/status of the Freshwater Pearl Mussel habitat. The macroinvertebrate (Q value) and diatom elements are not routinely used, however, as it has not been possible to establish clear relationships between these and the condition/status of the Freshwater Pearl Mussel population.

Table 2 Criteria for assessment of the conservation condition of Freshwater Pearl Mussel habitat, as set out in the Fourth Schedule to S.I. No. 296 of 2009.

Element	Objective	Notes
Macroinvertebrates	EQR ≥ 0.90	High status.
Filamentous algae (Macroalgae)	Trace or Present (< 5%)	Any filamentous algae should be wispy and ephemeral and never form mats.
Phytobenthos (Diatoms)	EQR ≥ 0.93	High status.
Macrophytes - rooted higher plants	Trace or Present (< 5%)	Rooted macrophytes should be absent or rare within the mussel habitat.
Siltation	No artificially elevated levels of siltation	No plumes of silt when substratum is disturbed.*

* In addition to the visual assessment of siltation, redox potential measurements have been used extensively since 2009 to assess sedimentation (see Section 4.2 below for further information)

Population condition/status and habitat condition/status are combined, together with consideration of the documented pressures and threats, to give an overall conservation status for each Freshwater Pearl Mussel population.

3 Population – monitoring adult mussels numbers

3.1 Permanent, marked transects – fixed permanent counts

3.1.1 Setting out a transect

Each transect is marked by more than one means (*e.g.* by rods, pegs and by landmark marking), photographed with visible landmarks, carefully described and located by GPS during the initial setup survey (many transects were established between 2004 and 2012). The transects are re-located by returning to the general locality using GPS, finding the bank landmarks, and checking against prior photographs and sketch maps until satisfied that the exact line to survey is identified.

The transect is defined by pulling a length of chain in a straight line across the river channel at right angles to the general current (perpendicular to the bank). The chain is fixed in place at both banks and also, depending on channel width, at points in the channel using metal pegs. A 30 m long tape measure is placed across the top of the chain to aid quadrat location. If snorkelling, a taut rope can also be run across the surface of the water for the surveyor to hold. A 1 m x 1 m (1 m^2) stainless steel quadrat (sub-divided into four) is placed on the downstream side of the chain at the start point bank. The exact starting point should be at bankfull width, whether wet or dry. Transects must always be repeated from the same starting point.

3.1.2 Mussel counts

The surveyor wades or snorkels across the river on the downstream side of the transect, taking great care not to trample mussels. The number of mussels visible on the substrate surface in each constituent 1 m^2 quadrat of each transect is counted across the full width of the transect. Mussels are not removed from the substrate or disturbed in any way during these transect counts. In addition to the number of mussels, the full range of habitat parameters is measured in each 1 m^2: the percentage cover of the various substrate fractions (Udden-Wentworth scale, by eye), macrophyte species and percentage cover, filamentous algal density and percentage cover, organic flocculation cover, silt surface cover, depth at the centre of the quadrat, redox measurements (or silt infiltration assessment) and velocity measurements (where required) (see Killeen & Moorkens (2020) and Chapter 4 for further information). Once all measurements are made in a 1 m^2 sample, the stainless steel quadrat is carefully turned to move on to the next metre square, and the same measurements are made across the transect until the end point at the far bank is reached. The final quadrat width is noted, and the wetted width, if part of it is dry.

As well as counting the visible, live mussels in each quadrat, note is taken of any empty shells or any dead or moribund mussels. Information on these shells should be provided in the notes. Consideration is given as to whether any empty shells originated from within the transect or have been washed-in from an upstream location. Dead shells that are buried in an upright position within the substrate are from mussels that died rapidly, and in-situ. Dead shells lying on the river bed are mussels that are likely to have died slowly. If the location of the dead shell(s) is a pool where detritus could accumulate, it is likely that the shell/s originated from further upstream. A note should be made of the likely time since death of the shells. Shells that still have a shiny pink or orange inner shell (nacre) have died very recently. Over time the acid conditions in the river erode the prismatic layer, and after a few years (depending on the river), only the periostracum remains, and can become soft over time.

3.2 Sweep transects

In small populations with sparse numbers of individuals, total counts of mussels are made for the entire section (500 m in length) or for a part of a section (100 – 200 m length). In more intermediate densities, the number of individuals in sections can be estimated by the use of Sweep Transects. One surveyor using a viewing bucket crosses the river and records every mussel seen within the surveyor's sweep (normally *c.* 2.0 m) (see Figure 2). To show the cross-channel distribution, the river-width is divided into three or four, and counts are made for each ¼ or ⅓ of the river channel width along the transect. Sweeps are made along a stretch approximately every 5 – 10 m, depending on the uniformity or otherwise of the habitat. The more diverse the habitat, the more transects are needed. The number of mussels per 100 linear metres of river can be calculated by multiplication (*e.g.* where a sweep transect is 2 m, the total number of mussels counted is multiplied by 50 and divided by the number of transects undertaken to estimate the population in a 100 m linear stretch). The abundance in each section can then be assigned to one of the six abundance categories: Abundant = > 1500 mussels/linear 100 m of channel; Common = 301 - 1500; Frequent = 41 - 300; Occasional = 1 - 40; Dead shells only; or Absent = 0.

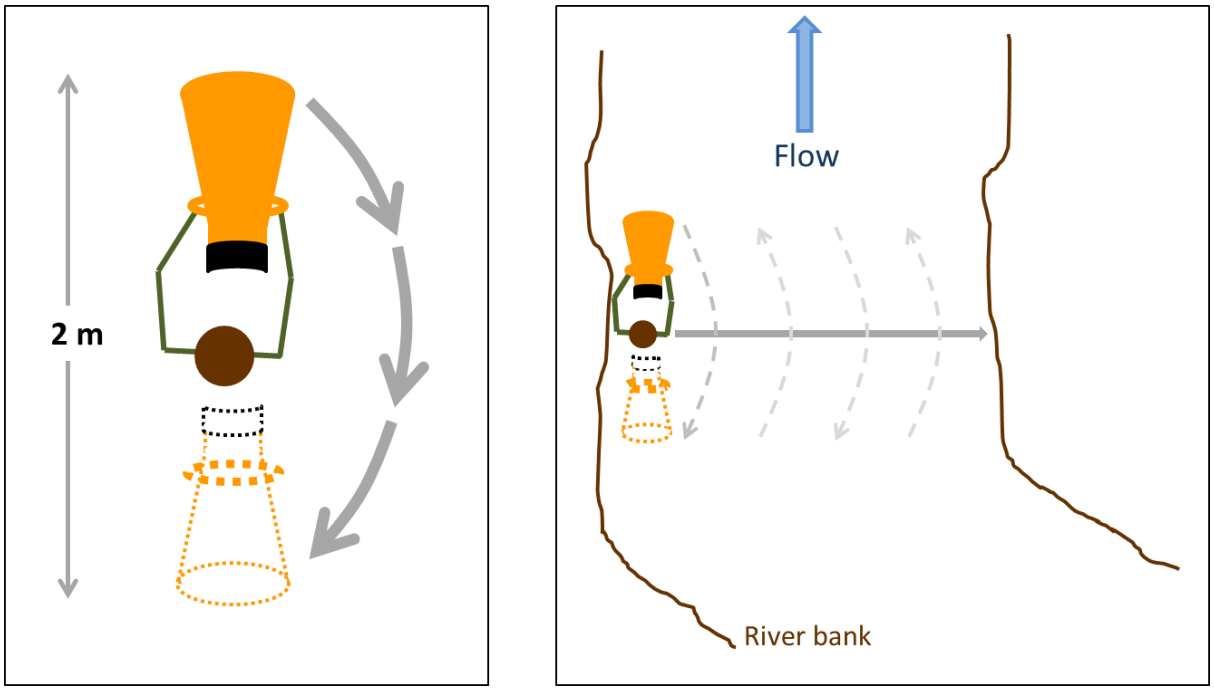


Figure 2 Sweep transects, plan view.

4 Habitat condition methods

This chapter describes the methods for assessing the condition of Freshwater Pearl Mussel habitat. The measurements are typically taken in 1 m x 1 m quadrats. The methods largely relate to the river substratum: physical substrate parameters (composition and redox), plant colonisation (algae and macrophytes) and decomposing organic matter. Methods are also given for photography and flow. See also NSAI (2017) and Killeen & Moorkens (2020).

4.1 Characterisation of substrate composition (Clast analysis)

4.1.1 Background

A significant portion of the substrate in typical mussel rivers is armoured, *i.e.* there is a vertical structure consisting of a coarse surface layer overlying finer sediment (this is an important element of stream stability). Whilst an assessment of the substrate surface composition is not an accurate representation of the bed substrate composition, it provides a very useful tool for assessing the overall suitability of the substrate as juvenile mussel habitat. A wide range of different sizes is a good reflection of a suitably stable river bed. Where the most dominant size is cobble, smaller clasts may be obscured, but where the dominant clast is pebble, it may be indicative of an area becoming destabilised.

4.1.2 Definition / methodology

In each 1 m² quadrat of a transect, the substrate surface composition is assessed visually and an approximation of the percentage by area of the surface clasts defined according to standard Udden-Wentworth Scale terminology (Udden, 1914; Wentworth, 1922, 1935) (Table 3).

Table 3 Udden-Wentworth Scale of clast sizes.

Size (mm)	Definition	Abbreviation
0.25 - 2.0	Medium to very coarse sand	MS to VCS
2 - 8	Very fine to fine gravel	VFG to FG
8 - 16	Medium gravel	MG
16 - 32	Coarse gravel (pebble)	CG
32 - 64	Very coarse gravel	VCG
64 - 128	Small cobble	SC
128 - 256	Large cobble	LC
> 256	Boulder	B

4.2 Redox measurements

4.2.1 Background

The key cause of decline in mussel populations in most rivers is lack of recruitment of young mussels into adulthood brought about by the decline into unsuitability of the stream bed habitat. The juvenile mussel stage begins after the 0.5 mm mussels fall off the gills of their host salmonids. This stage requires the safety of remaining within the river bed gravels, before growing to a size that allows the emergence

of the filtering siphons into the open water body. Juvenile mussels live between gravel particles amongst larger clast size stones, where oxygen is freely exchanged. In low flow conditions, fine sediments fall to the river bed in a higher percentage of habitat than in high flow conditions. Fine silt can become a problem due to excessive loading from various sources. Excessive nutrients in the water body lead to filamentous algal growth, which in turn decays and forms organic silt.

When fine sediments infiltrate the open coarse gravels associated with juvenile mussels, oxygen exchange is impaired. In the absence of oxygen exchange, a reducing environment exists where a microbially facilitated process of nitrate reduction occurs, and nitrate is transferred to nitrite and ammonium, both toxic to juvenile mussels (Augspurger *et al.*, 2003). Redox potential is a very useful measurement of this potential for reduction in the bed sediment, and thus provides a correlation with likely oxygen loss, and the continued loss of oxygen from oxidised nitrogen molecules.

Redox potential measurements are used to determine a reading that can be used as a 'proxy' for the ability to obtain oxygen within the river bed sediment. Where there is a loss of less than 20% in the eH between the open water and the interstitial river bed habitat at 5 cm depth, oxygen levels are unlikely to be compatible with juvenile survival, as demonstrated by Geist & Auerswald (2007). However, these readings do not simply correspond to oxygen movement from the open water into the interstices, but include the added value of measuring ongoing loss of oxygen through reduction in the sediment. Losses in redox of over 30% are generally indicative of a highly silted environment, severely depleted in interstitial oxygen to the point of anoxia. Where redox measurements fall and do not stabilise, but continue to drop, this is indicative of a highly anoxic environment.

4.2.2 Redox methodology

The Redox equipment comprises a 0.7 m long probe fitted with a platinum tipped electrode, a reference Ag/AgCl electrode and a meter with a millivolt display (see Figure 3). A reading is obtained by holding both electrodes in the water column until a stable reading is obtained (typically this would be 450 - 580 mV). With the Ag/AgCl electrode remaining in the water column, the platinum electrode is then inserted into a depth of 5 cm in the substrate and a reading taken immediately.

Four readings were taken per one metre squared quadrat, one in each 0.25 x 0.25 m sub-quadrat/quadrant.

Figure 3 Using a redox meter.

4.3 Algae (including diatoms)

4.3.1 Background

Excessive filamentous algae and unnatural levels of diatom growth consume oxygen (night-time) and smother the river bed substrate, preventing effective water and oxygen exchange to juvenile mussels. In good Freshwater Pearl Mussel habitat, algae and diatom cover is very sparse and consists of species associated with high status environments (Water Framework Directive (WFD) Trophic Diatom Index (TDI)). Where the species component still represents high status environments, but species are present in unnaturally high abundance, this is an indication of declining mussel habitat (NSAI, 2017). Where algal and diatom growth and species constituents are no longer of the abundance or species-composition associated with high status water bodies, this indicates a trophic change in the river habitats to mesotrophic or eutrophic conditions.

4.3.2 Definition / methodology

In this assessment, algae refers to the presence of green trailing filamentous algal species and diatom growth refers to the turf-forming diatom species. Presence/absence and severity are both important and this attribute is recorded as follows (Figure 4)

1. None Clean substrate surface
2. Light less than 5% cover, only a few filaments or small patches of diatom turf
3. Moderate greater than 5% cover but not extensive or luxuriant
4. Severe greater than 5% cover and extensive or luxuriant

Light Moderate Severe

Figure 4 Examples of light, moderate and severe algal cover.

4.4 Macrophytes and bryophytes

4.4.1 Background

Areas of juvenile and good adult Freshwater Pearl Mussel habitat are characterised by their oligotrophic nature, including the absence of macrophytes. The spread of rooted macrophytes is indicative of a trophic change and follows the settling of fine sediment into which the macrophytes can root. The presence of macrophytes then results in the preferential trapping of more fine sediment, thus exacerbating the trophic shift from oligotrophic to eutrophic. Macrophytes are, therefore, indicators of declining juvenile habitat conditions. In contrast, bryophytes, (in mussel rivers the positive indicator species is generally is *Fontinalis* spp.. *F. antipyretica* is replaced *F. squamosa* in western oligotrophic

rivers). *Fontinalis* spp. are good indicators of clean, un-silted, fast flowing conditions and are positive indicators of juvenile habitat. Care needs to be taken with the presence of *Myriophyllum* in peaty rivers, as it can be have naturally, slightly higher densities and is not as negative an indicator as *Ranunculus* spp. A combination of macrophyte cover and the resultant trapping of fine sediment during extended low flow conditions indicates deteriorated river bed substrate conditions.

4.4.2 Definition / methodology

Aquatic macrophytes and bryophytes mainly comprise a small number of species, river moss *Fontinalis* (*F. antipyretica* or *F. squamosa*) and water milfoil *Myriophllum* (alternate water milfoil *M. alterniflorum* and possibly spiked water milfoil *M. spicatum*). These species are associated with good flow, with *Fontinalis* preferring shaded reaches and *Myriophyllum* in more open reaches, but never luxuriant in natural situations. In less oligotrophic conditions, *Ranunculus* (mostly *R. penicillatus* var. *penicillatus*) may be present and spread to luxuriant abundances where nutrients and silt have increased. Other occasionally occurring macrophytes, generally in lower flow areas and at the upstream end of rivers close to lake outfalls, include starworts *Callitriche* (C. *hermaphroditica, C. stagnalis, C. hamulata*), shoreweed *Littorella uniflora*, and pondweeds *Potamogeton* (mainly *P. natans*, but other species are also likely). Emergent vegetation can also be found at the river edge, and the stonewort *Nitella* can also be found in margins. Macrophyte and bryophyte species, if present, are recorded as percentage cover individually by species, as the bryophytes are positive indicators and the macrophytes are generally negative indicators (Figure 5).

Figure 5 Examples of bryophytes (left) and macrophytes (right).

4.5 Organic floc

4.5.1 Background

Excessive filamentous algae and unnatural levels of diatom growth decay to form organic fine particles that often aggregate or flocculate, and the resulting material is known as 'floc'. The decaying material is consumed by growing numbers of bacteria, which in turn consume oxygen. The floc can smother the river bed substrate and infiltrate the river bed sediment, preventing effective water and oxygen exchange to juvenile mussels.

4.5.2 Definition / methodology

For the purposes of condition assessment, floc is defined as an aggregation of (mostly dead) organic material, mainly from algae and diatoms, but also with potential origins from decaying macrophytes

and associated decomposers (bacteria and fungi). The floc can form a layer at the surface of the substrate, or infiltrate the substrate, generally where there is insufficient flow to keep the material in suspension. Floc has become an unwelcome feature of parts of mussel rivers following serious nutrient pollution, particularly in summer during lower flows. Four categories can be recorded as follows (Figure 6)

1. None — No evidence on the substrate surface or from silt kick. River bed is either clean or has cover of living species only
2. Light — less than 5% cover
3. Moderate — greater than 5% cover but not extensive or luxuriant, *i.e.* not forming a more or less continuous carpet
4. Severe — greater than 5% cover and extensive or luxuriant, forming a more or less continuous carpet

Light Moderate Severe

Figure 6 Examples of light, moderate and severe floc cover.

4.6 Underwater photography

Underwater photography has proven to be a very useful tool for monitoring substrate and mussel condition and providing an image bank for future reference. In addition to general photographs of the transect and immediate vicinity and any land-use issues, an image is taken of the bed habitat and mussels in each of the 1 m x 1 m quadrats in permanent transects, using a waterproof digital camera.

4.7 Velocity

4.7.1 Background

Work on impairment of juvenile habitat has implicated the in-combination effect of impaired near-bed velocity with fine sediment load and/or nutrient load. Where abstraction and/or catchment management changes, in particular drainage works, have changed the hydrological regime of the river including within the mussel habitat, base flow velocities may be inadequate to allow oxygen movement in the river bed gravels. Lower base flow also exacerbates the volume and size-range of fine sediment deposition. Studies have shown that the near bed velocity of the river is a key issue in the survival of juvenile mussels, and should be part of investigations into the understanding of the pearl mussel's ecological status (Moorkens & Killeen, 2014).

4.7.2 Methodology

Measurements of velocity are taken in the centre of each 1 m x 1 m quadrat on each of permanent transect, or as close to as possible while ensuring that flow is unimpeded by large boulders or dense

weed. Measurements for the 2014 - 2016 monitoring project used an OTT C2 Small Current Meter. The full water depth is measured and then velocities measured at near-bed level (3 cm above the substrate surface) and at 60% depth (*i.e.* 40% from the substrate surface), in accordance with widely used techniques for measuring river velocities. The equipment is set to measure an average velocity over a 50 second duration. The number of pulses in 50 seconds is then converted to metres per second (ms^{-1}) using the factors appropriate for the size of the propeller used. The velocity measurements are relative to the discharge at the time of survey, so some indication of discharge should be noted for the day of the survey. Where there is an Office of Public Works (OPW) or Environmental Protection Agency (EPA) gauge nearby, the level on the day of the survey should be noted, and where possible, related to a flow rating curve.

5 Habitat mapping

5.1 Introduction

Fixed transect monitoring (Chapters 3 and 4) provides limited information on the quality of the wider river habitat of the population. It is generally accepted that it provides good evidence of very serious deteriorations, but is of limited use in recognising small incremental deterioration or improvement over time. A new method of river bed quality assessment for juveniles and adults was developed by Killeen & Moorkens (2020) in order to provide greater detail of the overall quality and condition of Freshwater Pearl Mussel habitat. It involves recording the more detailed information, as taken during fixed transect monitoring, every 100 metres, and at 10 m intervals between these detailed transects, undertaking more rapid transects. A map of the river bed of the surveyed stretch is then made by interpolating between the transects. Each 100 m section therefore contains 11 transects, starting at 0 m and ending at 100 m. Where habitat mapping is continuous, the last transect is also used as the first transect of the next section.

The level of marking, photography and GPS readings taken will assist interpolation of the habitat quality of the wider study area. For example, upstream and downstream photographs from each transect will provide information on the uniformity of the width, flow variation and morphology of the intermediate stretches of river. The technique provides an insight into the habitat quality at the time of the survey and its potential for improvement. It is not intended to repeat each 10 m transect in exactly the same place in the future, so if there are transects that will be used for repeat monitoring purposes, they should be marked as per Section 3.1. The stretch can be resurveyed for habitat quality after a number of years. It is expected that changes over time in a 100 m stretch would make the effort of exactly relocating 11 transects unnecessary in the context of the objectives of the study.

5.2 Methodology

A ten figure Grid Reference is obtained at the start and end point of each detailed transect (one in 10 transects) using a hand-held GPS device (surveys in Ireland have tended to use Garmin G-trex™), and at the 6th transect (*i.e.* at 50 m along the study section). The detailed transects run at right angles to the current (see Section 3.1) and the start and end points are marked by pegs. Photographs are taken across the transect line, and upstream and downstream of the line. To keep bed disturbance to a minimum and to enable fast working, the transects are not permanently marked and a chain is not laid on the riverbed. The transect line and direction are maintained by eye to be perpendicular to the start bank. A 1 m x 1 m stainless steel quadrat (sub-divided into four) is placed on the downstream side of the transect line at the start point. All data are measured by one surveyor wading or snorkelling across the river downstream of the transect, taking great care not to trample any mussels, and recorded by the second surveyor on the bank. The quadrat is carefully turned between metre squares and the measurements are made in each quadrat, until the end of the transect.

The end points of the other, intermediate 10 m transects are photographed as above and a one GPS reading taken. A 1 m x 1 m stainless steel quadrat is used to locate each metre square and a perpendicular line is maintained from the right bank.

A series of 14 ecological and other environmental parameters are assessed in the detailed transects. Five parameters are assessed in the intermediate transects. These are shown in (Table 5).

See Killeen & Moorkens (2020) for further information on all aspects of this methodology.

Table 4 Parameters surveyed in detailed transects.

No.	Parameter	Methodology	Detailed transect	Intermediate transect	See Section
1	Adult mussel numbers	Count	X	X	5.3.1
2	Water depth (cm)	Metre stick, centre of quadrat	X	X	5.3.2
3	Wide clast size range?	At least 3 clast fractions visible > 10%	X		5.3.3
4	Dominant clast size(s)	State Udden-Wentworth category that dominates	X		5.3.3
5	Sand to fine gravel?	Present or absent	X		5.3.4
6	In lee of boulders?	Gravel pockets stabilized by larger stone, present or absent	X		5.3.4
7	Compaction	Smaller clasts not easily moved - no, slight, moderate, severe	X		5.3.5
8	Silt cover	Visible on quadrat surface - no, slight, moderate, severe	X		5.3.6
9	Silt infiltration	Silt plume rise – no, slight, moderate, severe	X		5.3.7
10	Algae	Filamentous algal cover – no, slight, moderate, severe	X		5.3.8
11	Diatom	Turf forming diatom cover - no, slight, moderate, severe	X		5.3.9
12	Macrophytes	Species and condition – no, slight, moderate, severe	X		5.3.10
13	Bryophytes	No, slight, moderate, dense			5.3.10
14	Floc	Decaying organic matter – no, slight, moderate, severe	X		5.3.11
15	Coarse organic matter	Build up of leaves or twigs *etc.* in the quadrat - yes or no	X		5.3.12
16	Flow	5 categories: very fast (> 0.75 ms^{-1}) to standing (< 0.1 ms^{-1})	X	X	5.3.13
17	Scour	4 categories none (stable, dark clasts) to severe (bright clasts)	X		5.3.14
18	Photograph	Underwater photographs of river bed	X		5.3.15
19	Juvenile habitat suitability	The suitability and quality of the habitat for mussels	X	X	5.3.16
20	Habitat condition	The condition of (suitable) juvenile mussel habitat	X	X	5.3.17

5.3 Description of parameters

5.3.1 Adult mussel numbers

5.3.1.1 Background

The presence of adult mussels in stable areas of river (*i.e.* not in pools) is a good indicator that mussels were born in the area and thus, that it was suitable for juvenile recruitment in the past. The occurrence of mussels in pools indicates that the animals have been washed in from suitable habitat upstream. Pools normally have fine substrates that are not suitable juvenile habitat.

5.3.1.2 Definition/ methodology

The number of mussels visible on the substrate surface in each constituent one metre squared (1 m^2) quadrat is counted. Mussels are not removed from the substrate or disturbed in any way during these transect counts. The number of live and dead adult mussels is counted in all transects (*i.e.* in both detailed and intermediate transects).

5.3.2 Depth

5.3.2.1 Background

Depth is a good indicator of juvenile habitat. Areas of very shallow water can become exposed in extreme droughts, and very deep areas can have lower flows and be areas of preferential settling of fine solids.

5.3.2.2 Definition/ methodology

Depth (in centimetres, to the nearest whole centimetre) is measured in the centre of each one metre squared (1 m^2) quadrat using a one metre (1 m) steel rule. The water depth is measured in all transects (*i.e.* in both detailed and intermediate transects).

5.3.3 Characterisation of substrate composition (Clast analysis)

5.3.3.1 Background

A significant portion of mussel river habitat is armoured, *i.e.* there is a vertical structure consisting of a coarse surface layer overlying finer sediment (this is an important element of stream stability). Whilst an assessment of the substrate surface composition is not an accurate representation of substrate composition, it provides a very useful tool for assessing the overall suitability of the substrate as juvenile mussel habitat. A wide range of different sizes is a good reflection of a stable river bed. Where the dominant size is cobble, smaller clasts may be obscured, but where the dominant clast is pebble, it may be indicative of an area becoming destabilised.

5.3.3.2 Definition/ methodology

As in the permanent transect methodology, in each one metre squared (1 m^2) quadrat using the substrate surface composition is assessed using the standard Udden-Wentworth Scale (Table 3).

The assessment is made according to two criteria

1. Is there a wide clast size range?
    a. YES 3 or more different size categories present, or
    b. NO fewer than 3 categories present
2. What is the dominant clast size(s) present?

Substrate composition is recorded in detailed transects only. See also Section 4.1.

5.3.4 Sand/Grit/Gravel

5.3.4.1 Background

As this size range comprises the micro-habitat within which juvenile mussels are living, it is considered important to check for the presence of particles in the medium-sand to fine gravel range.

In faster flowing habitat, sand and fine gravel can be washed away. Therefore, in faster flowing water the presence of stabilising small boulders with sand accumulations behind them is a good indication that juvenile habitat is sustainable. Thus, a specific note is made of the presence or absence of the sand and grit element in the substrate in the lee (on the downstream side) of coarse clasts.

5.3.4.2 Definition/ methodology

This is additional information derived from the clast analysis and is defined as the finer (non-silted) fractions of the substrate which range from medium sand, through very coarse sand, very fine gravel to fine gravel (size range 0.25 mm to 8 mm).

The answer to the question 'sand to fine gravels present?' is assigned (following on from the clast analysis) into three categories

1. No none seen on surface, but could be present and obscured by mussels or coarse clasts (through armouring)
2. Present less than 5% of the visible substrate surface comprises material < 8 mm
3. Good greater than 5% of the visible substrate surface comprises material < 8 mm

Note is also made of where the medium sand to fine gravel factions are found in the lee of boulders. This is a yes / no answer to inform the study as to whether the habitat for mussel may depend on larger stone (often a case in high flow areas of spate rivers), or whether the sand to fine gravels are independent of large stone (often the case in shallow riffles). Sand/Grid/Gravel is recorded in detailed transects only.

5.3.5 Compaction

5.3.5.1 Background

Juvenile mussel habitat must comprise substrate through which there is an adequate flow of water. Thus any form of compaction may render the substrate unsuitable. There are three main processes leading to compaction of the substrate: armouring/packing, colmation and lithification. Whilst some degree of armouring provides stability, coarse clasts may cluster together and form tightly packed, imbricated (interlinked) or consolidated structures. Colmation refers to the retention processes that can lead to the clogging of the top layer of channel sediments. Internal colmation, clogging of the interstices directly below the armour layer, may form a thin seal that disconnects surface water from hyporheic water by inhibiting exchange processes. Finally, the substrate may become lithified whereby loose sediment is converted into sedimentary rock. Lithification is a combination of compaction and cementation

(precipitation of minerals from the water). Characterising the compaction of an area is therefore considered important.

5.3.5.2 Definition/ methodology

A true assessment of substrate compaction can only be made using techniques such as penetrometry, but a crude assessment can be made by inserting a pointed metal rod into the top 10 cm of the substrate surface amongst the smaller clast sizes, and is recorded as follows in answer to the question of whether compaction is present

1. No the finer substrate is relatively loose
2. Slight some compaction but can be loosened
3. Moderate between slight and severe
4. Severe the finer substrate is compacted and is not easily loosened

Compaction is categorised in detailed transects only.

5.3.6 Silt cover

5.3.6.1 Background

Very fine physical silt can impede water and oxygen exchange in the river bed and is a good indicator of either conditions that concentrate natural levels of fine sediment or the presence of unnaturally large loads of fine sediment.

5.3.6.2 Definition/ methodology

Defined as a layer of fine mineral material (< 0.25 mm) over the substrate material, not to be confused with organic material defined as floc, although both can be present. The surveyor looks for visible fine sediment on the river bed surface. Silt cover is recorded as one of four categories as follows

1. No clean substrate surface
2. Slight less than 5% cover, usually in small (sheltered) pockets
3. Moderate greater than 5% but less than 25% and not forming a more or less continuous layer
4. Severe greater than 25% and forming a more or less continuous layer

Silt cover is recorded in detailed transects only.

5.3.7 Silt infiltration

5.3.7.1 Background

Very fine physical/inorganic silt is a good indicator of either conditions that concentrate natural levels of fine sediment or the presence of unnaturally large loads of fine sediment. It is sometimes not visible on the surface but infiltrates into the interstices of the river bed substrate where it can impede water and oxygen exchange in the river bed and result in juvenile death.

5.3.7.2 Definition/ methodology

Silt infiltration is determined by the surveyor disturbing the top 5 - 10 cm of the substrate surface with a fine rod or their foot to check for a plume of inorganic fine sediment, if safe to do so without harming mussels. This should be done outside, but close to the quadrat. If the rising plume is organic in nature, it is recorded as floc. Inorganic silt is recorded as one of four categories as follows

1. No no plume
2. Slight a small plume which quickly dissipates
3. Moderate a small plume which is slow to dissipate
4. Severe a significant plume released from the substrate

Silt infiltration is categorised in detailed transects only.

5.3.8 Algae

5.3.8.1 Background

Excessive filamentous algae consume oxygen (at night) and smother the river bed substrate, preventing effective water and oxygen exchange to juvenile mussels.

5.3.8.2 Definition/ methodology

In this assessment, 'algae' means the green trailing filamentous type of algal growth. Presence/absence and severity are both important and the surveyor notes the condition as follows

1. None clean substrate surface
2. Light less than 5% cover, only a few filaments
3. Moderate greater than 5% but not extensive or luxuriant
4. Severe greater than 5% and extensive or luxuriant

Algal cover is recorded in detailed transects only. See also Section 4.3 and Figure 3.

5.3.9 Diatom

5.3.9.1 Background

Excessive diatom growth, even of high status species, can consume oxygen (at night) and smother the river bed substrate. Excessive diatom cover can prevent effective water and oxygen exchange to juvenile mussels.

5.3.9.2 Definition/ methodology

In this assessment, diatom means the turf forming living organism. Presence/absence and severity are both important and the surveyor notes the condition as follows

1. None clean substrate surface
2. Light less than 5% cover, only small patches of diatom turf
3. Moderate greater than 5% but not extensive or luxuriant
4. Severe greater than 5% and extensive or luxuriant

Diatom cover is recorded in detailed transects only. See also section 4.3.

5.3.10 Macrophytes and Bryophytes

5.3.10.1 Macrophytes background

Areas of juvenile and good adult Freshwater Pearl Mussel habitat are characterised by an oligotrophic nature, including the absence of macrophytes. The spread of rooted macrophytes is indicative of a trophic change and the settling of organic sediment into which the macrophytes can root. The presence of macrophytes then results in the preferential trapping of more fine sediment, thus exacerbating the trophic shift from oligotrophic to eutrophic. Macrophytes are therefore indicators of declining juvenile habitat conditions.

5.3.10.2 Macrophytes definition/ methodology

Aquatic macrophytes and bryophytes are recorded as species and percentage (%) cover.

5.3.10.3 Bryophytes background

Bryophytes such as *Fontinalis*, are good indicators of clean, unsilted, fast flowing conditions and are positive indicators of juvenile habitat.

5.3.10.4 Bryophytes definition/ methodology

Aquatic bryophytes are recorded as species and percentage (%) cover.

5.3.11 Floc

5.3.11.1 Background

Excessive filamentous algae and unnatural levels of diatom growth, as well as other organic matter, decay to form organic fine sediment that often aggregates or flocculates and the resulting material is known as floc. The decaying material is consumed by growing numbers of bacteria and fungi, which in turn consume oxygen. The floc can smother the river bed substrate and infiltrate the river bed sediment, preventing effective water and oxygen exchange to juvenile mussels.

5.3.11.2 Definition/ methodology

Floc is here defined as an aggregation of (mostly) dead, fine particulate, organic material, particularly algae and diatoms and associated decomposers, which forms a layer at the surface of the substrate, or infiltrates the substrate, generally where there is insufficient flow to keep the material in suspension. Floc has become frequent in many mussel rivers, particularly in summer during lower flows. Four categories are used to record the condition

1. None — no evidence on the substrate surface or from previous silt kick
2. Light — less than 5% cover
3. Moderate — greater than 5% but not extensive or luxuriant, *i.e.* not forming a more or less continuous carpet
4. Severe — greater than 5% and extensive or luxuriant, forming a more or less continuous carpet

Floc is recorded in detailed transects only. See also Section 4.5 and Figure 5.

5.3.12 Detritus

5.3.12.1 Background

Accumulations of debris such as leaves, twigs and other large organic fractions, and degrading humic matter can result in an environment of low oxygen and higher acidity, which is incompatible with juvenile survival. Coarse organic matter or 'detritus' is generally associated with the edges of rivers with low flows and overhanging trees.

5.3.12.2 Definition/ methodology

Detritus is here defined as organic material such as leaves or twigs that lie on the substrate surface. It is recorded as either presence or absence. Where it is present, note is taken of whether leaves, twigs or branches are the main component. Detritus is recorded in detailed transects only.

5.3.13 Flow

5.3.13.1 Background

Moorkens & Killeen (2014) demonstrated that an adequate flow was one of the most important ecological requirements for pearl mussels and, thus, is a key indicator when assessing potential juvenile habitat. Unfortunately, typical flow assessment in the absence of velocity measurement is made from the bank and describes a general flow type within a reach, such as the cascade—step pool, pool—riffle—glide, meander assessment in River Hydromorphology Assessment Technique (RHAT) surveys (NIEA, 2009), or the predominant flow type as defined within River Habitat Survey (RHS) assessments, such as broken standing waves, chaotic, rippled or smooth of RHS (Environment Agency (England), 2003). For the study of Freshwater Pearl Mussel habitat, what is needed is a relative score for every one metre square along the transect (*i.e.* across the width of the river). By comparing relative velocity across a transect, an assessment can be made as to whether there is sufficient near bed velocity in any area of the transect to support sustainable juvenile habitat within the requirements described by Moorkens & Killeen (2014). This assessment is difficult to undertake, other than by best expert judgement, as to obtain greater accuracy, near bed velocity would have to be measured (Section 4.7), which would add at least 1.5 hours to each transect.

5.3.13.2 Definition/ methodology

Each metre square across the transect is assessed for relative velocity on a five point scale from 'very fast' to 'standing' (Table 5). The approximate velocities, based on past experience are provided in Table 5. The approximate velocity ranges are based upon measurements made at a known low discharge on a regulated river in the U.K. (80 - 90 MLD), a relatively low flow, within other projects. They are merely a guide and of course, do not necessarily represent velocities at near bed level in other rivers, only a relative scale to indicate areas of preferential flow (Killeen & Moorkens, 2020).

Table 5 Flow velocity categories in transect recording.

	Assessment	Approximate Velocity (ms^{-1}) at 60% depth	Comment
	Very fast	> 0.75	Likely to be too high energy for stable juvenile mussel habitat
	Swift	0.4 - 0.75	Ideal for stable juvenile mussel habitat
	Moderate	0.25 - 0.4	Ideal for stable juvenile mussel habitat
	Slow	0.1 - 0.25	Marginal juvenile habitat
	Standing	< 0.1	Unsuitable juvenile habitat

The flow is categorised in all transects (*i.e.* in both detailed and intermediate transects). See also Section 4.7.

5.3.14 Scour

5.3.14.1 Background

In high flows, the substrate may become scoured, and thus unstable. Scour is generally recognisable by the colouration of the substrate. Scoured clasts are usually very 'bright', whereas more stable, un-scoured substrates are darker in colour (often black) often with attached algae or macrophytes. Good juvenile Freshwater Pearl Mussel habitat is associated with stable, blackened substrate.

5.3.14.2 Definition/ methodology

The level of scour is recorded by a visual assessment of the substrate – general appearance, particularly colour. Each metre square recorded scour levels as

1. None all substrate was blackened and stable
2. Slight some substrate was recently disturbed and had bright coloured elements
3. Moderate between slight and severe
4. Severe clasts very brightly coloured

Scour is categorised in detailed transects only.

5.3.15 Photography

5.3.15.1 Background

To ensure that surveyors are categorising quadrats with consistency, and to make comparisons between sites, rivers and repeat surveys undertaken at a later date, an underwater photograph of the river bed habitat is an extremely useful record.

5.3.15.2 Definition/ methodology

Underwater photographs are taken in each quadrat of a detailed transect, and as necessary to demonstrate condition in intermediate transects. A photograph is also taken in an upstream and downstream direction from each transect at approximately mid-stream to assist in interpolating the areas not surveyed. See also Section 4.6.

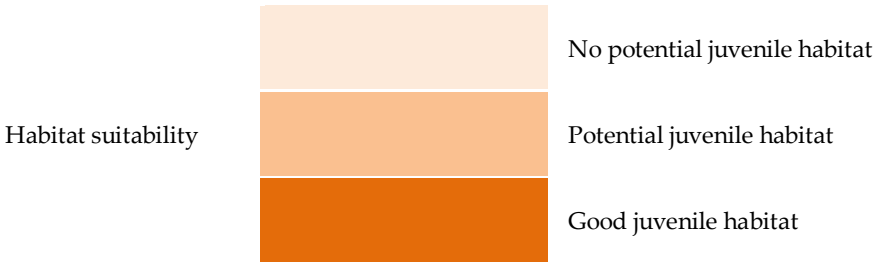
5.3.16 Juvenile habitat

5.3.16.1 Background

It is important to identify the suitability or quality of the habitat based on all the parameters documented in the detailed transects, and through observation of these parameters in the intermediate transects. This parameter is a synthesis that makes a best expert judgement on the suitability of the physical habitat (NOT its condition) to support juvenile mussels. It is important to separate the physical suitability from the condition of the habitat, as it is unlikely to be possible to make naturally unsuitable habitat or habitat that has been physically destroyed, suitable for juvenile mussels. This allows the identification of suitable habitat that can be restored for juvenile mussels.

5.3.16.2 Definition/ methodology

Juvenile habitat suitability is recorded as one of three broad categories: unsuitable habitat, potentially suitable habitat and good juvenile habitat (all regardless of the current condition). In separating the habitat suitability assessment from the habitat condition assessment, better flexibility can be achieved. Good juvenile habitat is the presence of physical habitat where an expert surveyor would expect to find juvenile mussels if it was in good condition. Potential juvenile habitat is habitat where an expert may expect to find occasional juveniles if the conditions were very good, but where the habitat is slightly compromised by slight scour, or slightly too high or low a velocity. The habitat suitability is colour coded as follows



Habitat suitability/quality is categorised in all transects (*i.e.* in both detailed and intermediate transects).

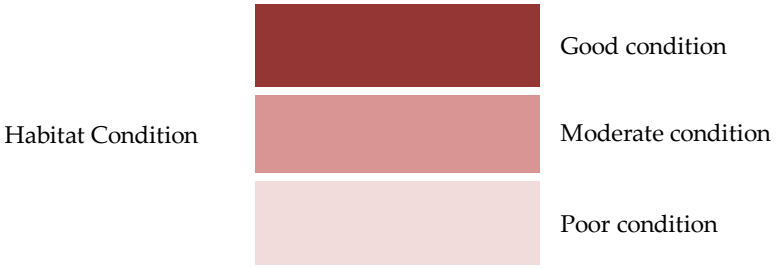
5.3.17 Juvenile habitat condition

5.3.17.1 Background

The condition of the habitat will determine whether juveniles will survive or die. It is important to separate condition from physical suitability. Where there is good physical habitat in poor condition due to *e.g.* a nutrient or sediment problem, measures can be taken to restore the habitat to good condition that can support a viable population.

5.3.17.2 Definition/ methodology

Juvenile habitat condition is recorded as one of three broad categories, good condition, moderate condition and poor condition. The habitat condition is colour coded as follows



The habitat condition is categorised in all transects (*i.e.* in both detailed and intermediate transects).

6 Mussel population demography

6.1 Introduction

Population structure is assessed by measuring the lengths of mussels including those mussels buried within the substrate. The choice of demography quadrat location is very important. Firstly, the quadrat must be representative of the section being assessed. Secondly, it must be representative of habitat where juvenile mussels would be expected to occur, or as close as possible in more impaired populations. Thirdly, it must be habitat that will not be damaged by the demographic investigations, *i.e.* stable enough to withstand the process without the habitat nearby imploding into the gaps created when the mussels and related stones have been removed. Significant expertise is required to recognise juvenile mussel habitat, and habitat that it is safe to excavate. This survey is an invasive technique that causes considerable disturbance to the mussel beds, and great care has to be taken to ensure that all mussels are replaced in correct positions and depth. It is imperative that demographic counts are carried out in very stable habitat, that measurements are made rapidly, and that mussels and substrate are replaced carefully before moving on to the next site. To avoid damage to the population, the number of quadrats examined should be minimal and adequately spaced apart.

Mussels are sedentary animals that may never move from their river bed position in their lifetime, thus removal or movement of mussels can be highly damaging (Killeen & Moorkens, 2016). Owing to the significant risk that juvenile searches will lead to the injury or death of the animals and damage or destruction of their habitat, only highly experienced surveyors are licensed to use this method.

6.2 Methodology

The size/age structure of the population is determined by removing all of the mussels from a fixed area of substrate and measuring each individual. The method requires laying a 0.5 m x 0.5 m metal quadrat on the river bed and counting the number of mussels visible from the surface. The visible mussels are then carefully removed from the quadrat with as little disturbance to the substrate as possible. The substrate is then agitated with the fingertips and any additional mussels are counted and removed. Finally, an aluminium framed sampling net equipped with a 0.5 mm nylon mesh bag is placed vertically on the downstream side of the quadrat and the substrate is further agitated to allow any remaining mussels to come to the surface and any very young (< 15 mm) individuals to be swept by the water current into the net. All mussel lengths are measured with Vernier callipers. The measured mussels are then carefully reburied in the substrate they have been taken from. Sufficient quadrats are sampled to provide between 25 and 120 mussels from any one site or habitat type. Usually at least 250 are required to provide a reasonable demographic profile for the river, but this is possible only in a large population. The numbers measured must be adjusted depending on population size, stress levels of mussels and condition of the river bed, to no more than necessary to understand the profile. The population demographic profile is established by assigning the mussels to 5 mm size classes and plotting as a histogram. The demographic profile and, thus, recruitment condition of a population can be made most accurately when it can be undertaken using the nine combinations of good, potential and no habitat in good, moderate and poor condition, *i.e.* where habitat mapping is also undertaken (Killeen & Moorkens, 2020).

Figure 7 Measuring mussel shell-length.

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Appendix 1 FPM Conservation objectives & condition monitoring

A site-specific conservation objective aims to define favourable conservation condition for a particular habitat or species of community interest at a Natura 2000 site (SAC or SPA). The maintenance/restoration of habitats and species within Natura 2000 sites at favourable conservation condition will contribute to the overall maintenance/restoration of favourable conservation status of those habitats and species at a national level.

The favourable conservation status of a species is achieved when

- population dynamics data on the species concerned indicate that it is maintaining itself on a long-term basis as a viable component of its natural habitats, and
- the natural range of the species is neither being reduced nor is likely to be reduced for the foreseeable future, and
- there is, and will probably continue to be, a sufficiently large habitat to maintain its populations on a long-term basis.

Further information on site-specific conservation objectives can be found at www.npws.ie.

The overall site-specific conservation objective for the Freshwater Pearl Mussel in each of the 19 SACs designated for its protection is

To restore the favourable conservation condition of the Freshwater Pearl Mussel in the SAC

A restore objective is necessary as all 27 populations (listed on the first schedule to S.I. 296 of 2009) are, or have been within the last decade, in unfavourable condition. Only two of the 27 populations have had favourable assessments since monitoring began in 2004.

Favourable Freshwater Pearl Mussel conservation condition is defined by a standard list of attributes, for which site-specific targets are established. The targets are determined by the results of monitoring surveys and may be maintain or restore, depending on the condition of the individual population and its habitat. Attributes and targets are based on best available information at the time of writing, and may be subject to changes as more information becomes available. Attributes are linked to three Article 17 reporting parameters: range, population and habitat for the species. The population attributes are based on the criteria in the third schedule, and the habitat for the species attributes are based on the elements in the fourth schedule to S.I. 296 of 2009.

The table overleaf lists the standard attributes and the format of the site-specific targets, and provides notes on how these link to the third and fourth schedules of S.I. 296 of 2009 and to assessing the conservation condition of a Freshwater Pearl Mussel population.

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Article 17 Parameter	Attribute	Measure	Target	Notes
Range	Distribution	Kilometres	Maintain/restore distribution at # km.	Monitoring data on the distribution and abundance of the Freshwater Pearl Mussel in a catchment are used to set this target. Distribution covers the river corridor(s) from the most upstream location(s) for the species' habitat to its most downstream extent. It is mapped using a polyline covering any gaps in the distribution of the habitat. The habitat may or may not currently be occupied. This attribute aims to maintain the full extent of the species in a catchment so as to increase the population's resilience (*i.e.* avoiding an 'all of the eggs in one basket' scenario). If a population becomes concentrated in a short stretch of channel, it is at increased risk from isolated incidents of direct damage/destruction, morphological change and pollution. Both' maintain' and 'restore' targets are used for the 27 SAC populations, the former used mainly for large populations with some level of recruitment (*i.e.* close to favourable condition). The target is for the species to be sufficiently widespread to maintain itself on a long-term basis as a viable component of the system.
Population	Population size	Number of adult mussels	Maintain/restore populations to at least: # adult mussels	Population estimates from monitoring surveys are used to set this target (NPWS, 2010). As all populations have declined in recent time, the earliest (*i.e.* largest) available estimates were generally used. These typically date from the period 1999 to 2009 (*i.e.* in most cases, the 'reference' year post-dates the 1997 transposition of the Habitats Directive). The target is for the species to be sufficiently abundant to maintain itself on a long-term basis as a viable component of the system. It has been necessary, therefore, for very small populations to set targets that are greater than the largest available estimate (*e.g.* for the Nore population, the target is set as at least 5,000). Currently, all 27 SAC populations have a 'restore' target. Monitoring provides data on the numbers, abundance (categories) and densities (numbers/m^2) of adult mussels and how these change over time. These data are used to estimate changes in population size over time.

Article 17 Parameter	Attribute	Measure	Target	Notes
Population	Population structure: recruitment	Percentage per size class	Maintain/restore to at least 20% of each population no more than 65 mm in length; and at least 5% of each population no more than 30 mm in length	This attribute and its targets are based on the third schedule to S.I. 296 of 2009 (3rd and 4th criteria). These standard targets apply to all Freshwater Pearl Mussel populations and are based on an average life-expectancy of 100 years in Ireland. Mussels ≤ 65 mm are 'young mussels' and found buried in the substratum or beneath adult mussels. Mussels ≤ 30 mm are 'juvenile mussels' and always buried in the substratum. The data from specialist mussel population demography monitoring are used to determine whether a population passes or fails these targets and hence, whether the target is to maintain or restore. Currently, all 27 SAC populations have a 'restore' target. The target is for sufficient juvenile recruitment to allow the species to maintain itself on a long-term basis as a viable component of the system.
Population	Population structure: adult mortality	Percentage	No more than 5% decline from previous number of live adults counted; dead shells less than 1% of the adult population and scattered in distribution	This attribute and its targets are based on the third schedule to S.I. 296 of 2009 (1st and 2nd criteria). These standard targets apply to all Freshwater Pearl Mussel populations and are based on an average life-expectancy of 100 years in Ireland. 5% is considered to be the cut-off between the combined errors associated with natural fluctuations and sampling methods and evidence of true population decline. 1% of dead shells is considered to be indicative of natural losses. Data from monitoring of adult mussel numbers (permanent marked and sweep transects – see Chapter 3) are used to determine whether a population passes or fails these targets and hence, whether the target is to maintain or restore. Many of the 27 SAC populations have maintain targets, as populations are failing to recruit rather than having elevated adult mortality. However, detection of elevated mortality can be challenging, even with regular monitoring. A 'restore' target indicates a population in severe decline, with stressed (unhealthy) adult mussels. The target is for sufficient survival of adults to allow the species to maintain itself on a long-term basis as a viable component of the system.

Article 17 Parameter	Attribute	Measure	Target	Notes
Habitat for the species	Suitable habitat: extent	Kilometres	Maintain/restore suitable habitat extent in # km in the [named river] and any additional stretches necessary for salmonid spawning	This attribute covers the availability of suitable habitat (Chapter 5). Mapping of mussel distribution, as well as of habitat suitability (Chapter 5) and condition (Chapters 4 and 5) have been used to determine the targets for this attribute. Site-specific targets will continue to improve as further mapping of habitat suitability becomes available. This target varies from the distribution target, in that only stretches of suitable habitat are mapped. Therefore, the suitable habitat polyline is fully contained within, and may be shorter than, the distribution polyline. Monitoring also provides information on the extent to which the available suitable habitat is occupied by adult mussels. The target is for sufficient suitable habitat in favourable condition to allow the species to maintain itself on a long-term basis as a viable component of the system.
Habitat for the species	Suitable habitat: condition	Kilometres	Maintain/restore condition of suitable habitat	This attribute covers the overall condition of the available habitat. It is a combination of the area of 1. habitat adult and juvenile mussels can occupy; 2. spawning and nursery habitats host fish can occupy. The maintain/restore target is determined by combining data available from monitoring reports on mussel habitat condition, and any evidence of reductions in juvenile salmonid numbers. As a result, this attribute can be considered as a summary of all attributes that follow. The target for all 27 SAC populations is to restore the condition of the suitable habitat. The attributes that follow identify the specific elements of habitat condition that require to be restored. Fish nursery and mussel habitat typically overlap. Fish spawning habitat is generally adjacent to mussel habitat, but may lie upstream of the generalised mussel distribution. Only spawning areas that regularly contribute juvenile fish to adult mussel habitat should be considered. Availability of mussel and fish habitat is determined by flow and substratum conditions. It is highly sensitive to hydromorphological changes, sedimentation and nutrient enrichment. Pressures throughout the catchment contribute to such impacts. The target is for sufficient habitat in favourable condition to allow the species to maintain itself on a long-term basis as a viable component of the system.

Article 17 Parameter	Attribute	Measure	Target	Notes
Habitat for the species	Water quality: macroinvertebrates and phytobenthos (diatoms)	Ecological quality ratio (EQR)	Maintain/restore water quality-macroinvertebrates: EQR greater than 0.90; phytobenthos: EQR greater than 0.93	This attribute and its targets are based on the fourth schedule to S.I. 296 of 2009 (1st and 3rd elements). The EQRs correspond to 'High Ecological Status' for these two Water Framework Directive biological quality elements (see The European Communities Environmental Objectives (Surface Waters) Regulations 2009, and The European Communities Environmental Objectives (Surface Waters) (Amendment) Regulations 2019) and represent high water quality with very low nutrient concentrations (oligotrophic conditions). As noted in Section 2.6, however, these elements are not routinely used in mussel habitat condition assessment, as there are no clear relationships between them and the condition of the Freshwater Pearl Mussel population. It is, however, useful to refer to EPA data for the nearest river monitoring stations as general indicators of water quality. The target is for sufficient habitat in favourable condition to allow the species to maintain itself on a long-term basis as a viable component of the system.
Habitat for the species	Substratum quality: filamentous algae (macroalgae); macrophytes (rooted higher plants)	Percentage	Maintain/restore substratum quality-filamentous algae: absent or trace (< 5%); macrophytes: absent or trace (< 5%)	This attribute and its targets are based on the fourth schedule to S.I. 296 of 2009 (2nd and 4th elements). Elevated algal (including diatom) and macrophyte (other than bryophyte) cover indicates increased nutrient loads (see Sections 4.3, 4.4, 5.3.8 – 5.3.10). Elevated higher plant cover also indicates deposition and infiltration of fines (organic and inorganic). The target is for sufficient habitat in favourable condition to allow the species to maintain itself on a long-term basis as a viable component of the system.
Habitat for the species	Substratum quality: sediment	Occurrence	Maintain/restore substratum quality-stable cobble and gravel substrate with very little fine material; no artificially elevated levels of fine sediment	This attribute and its targets are based on the fourth schedule to S.I. 296 of 2009 (5th element) and the various monitoring measurements of substratum condition (see Sections 4.1 and 5.3.3-5.3.7). Deposition of coarse organic particles (see Section 5.3.12) and accumulation of floc (Sections 4.5 and 5.3.11) should be considered under this attribute, as should scour (5.3.14). Consideration will be given to including the dead and decaying organic fractions, with associated decomposing communities, as a separate attribute in future revisions of SSCOs. The target is for sufficient habitat in favourable condition to allow the species to maintain itself on a long-term basis as a viable component of the system.

Article 17 Parameter	Attribute	Measure	Target	Notes
Habitat for the species	Substratum quality: oxygen availability	Redox potential	Maintain/restore to no more than 20% decline from water column to 5 cm depth in substrate	This attribute and its target are based on the redox potential monitoring data (see Section 4.2). Differences in redox potential between the water column and the substrate correlate with differences in oxygen levels. Juvenile mussels require full oxygenation while buried in gravel. In suitable habitat, there should be very little loss of redox potential between the water column and underlying gravels. The target is for sufficient habitat in favourable condition to allow the species to maintain itself on a long-term basis as a viable component of the system.
Habitat for the species	Hydrological regime: flow variability	Metres per second	Maintain/restore appropriate hydrological regime	The availability of suitable Freshwater Pearl Mussel habitat is largely determined by flow (catchment geology being the other important factor). Flow also plays a significant role in determining the condition of the habitat. In order to restore the habitat for the species, flow variability over the annual cycle must be such that: 1) high flows can wash fine sediments and organic matter from the substratum; 2) high flows are not artificially increased so as to cause excessive scour of mussel habitat; 3) low flows do not exacerbate the deposition of fines, the growth of algae/macrophytes or the accumulation of floc and other organic matter and 4) low flows do not cause stress to mussels in terms of exposure, water temperatures, food availability or aspects of the reproductive cycle. Groundwater inflow to the substratum also contributes to water-cycling and favourable habitat condition. Terrestrial wetland in the catchment play a critical role in terms of moderating the hydrological regime and supplying food to Freshwater Pearl Mussels. This attribute relates to measurement of water depth and flow, including near-bed velocity. See Sections 4.7, 5.3.2 and 5.3.13. The target is for sufficient habitat in favourable condition to allow the species to maintain itself on a long-term basis as a viable component of the system.

Article 17 Parameter	Attribute	Measure	Target	Notes
Habitat for the species	Host fish	Number	Maintain/restore sufficient juvenile salmonids to host glochidial larvae	Salmonid fish are host to the larval stage of the Freshwater Pearl Mussel and essential to completion of the life cycle. 0+ and 1+ fish are typically used, both because their habitat overlaps with that of the Freshwater Pearl Mussel and the development of immunity with age in fish. Fish presence is sufficient, as higher fish density and biomass is indicative of enriched conditions in mussel rivers. Geist *et al*. (2006) found that higher densities of host fish coincided with eutrophication, poor substrate quality for mussels and a lack of mussel recruitment, while significantly lower host fish density and biomass were associated with high juvenile mussel numbers. Fish movements must be such that 0+ fish remain in the mussel habitat until their 1+ summer. No fish stocking should occur within the mussel habitat, nor any works that may change the salmonid balance or residency time. The Irish Freshwater Pearl Mussel populations studied have been shown to utilise juvenile Atlantic salmon or juvenile trout, but larger, more restorable populations are associated with salmon (Johnston, 2009; NPWS, 2010, Johnston & Moorkens, 2018).
Habitat for the species	Fringing habitat	Hectares	Maintain/restore the area and condition of fringing habitats necessary to support the population	This attribute relates to the riparian habitats of rivers and lakes within the catchment. Riparian habitats upstream of and adjacent to Freshwater Pearl Mussel habitat are included. Riparian habitats, including those along lake fringes, even where they do not form part of a natural floodplain, are an integral part of the structure and functioning of river systems. Open wetlands, such as wet heath and blanket bog, are considered particularly critical to the hydrological regime of mussel rivers. Fringing habitats assist in the settlement of fine suspended material, protect banks from erosion and contribute to nutrient cycling, as well as contributing to the aquatic food web (*e.g.* allochthonous matter from poor fens and flushes) and providing habitat (refuge and resources) for life-stages of fish, birds and aquatic invertebrates. Equally, fringing habitats are dependent on rivers/lakes, particularly their water levels, and support wetland communities and species of conservation concern. The structure and condition of riparian habitats is documented during monitoring survey, by recording pressures and threats, describing sampling locations and mapping habitat suitability and condition. The target is for sufficient habitat in favourable condition to allow the species to maintain itself on a long-term basis as a viable component of the system.