

MAPPING JUVENILE HABITAT FOR THE FRESHWATER PEARL MUSSEL (*MARGARITIFERA MARGARITIFERA*)

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Abstract A technique has been developed to determine the extent and condition of suitable habitat for the freshwater pearl mussel *Margaritifera margaritifera* in rivers supporting this species. The habitat mapping technique combines physical measurements and adult mussel counts with best expert judgement in a dense network of transects, qualified by measured categorical habitat quality data in more dispersed transects, in order to get a mapped overview of mussel populations that are accurate enough to inform conservation agencies, river managers and researchers regarding the status of the population.

The study to develop the methodology was carried out in a river in north-west England. A 3km long section of riverbed was surveyed. Data was collected from each 1m×1m quadrat on transverse transects at 10m intervals. In addition, the results of the study were validated from results of a mussel demographic study undertaken in the habitat and condition combinations identified in the mapping study. Examples are given of GIS habitat maps derived from the study, including sample variation in habitat, condition and mussel numbers within individual 100m sections, and their relationship with flow.

Key words Freshwater pearl mussel, habitat, riverine mapping, conservation, demographic profile

INTRODUCTION

The loss of sustainable recruitment of juvenile *Margaritifera margaritifera* (L., 1758) is frequently cited as the main mechanism of the decline of the species (Geist, 2010; British Standards Institution, 2017). The death of juvenile mussels is related to impacted river bed habitats through inappropriately high sediment and/or nutrient conditions, often related to low flow impacts, which in turn are caused by changes in hydrology, morphology and land use in the wider catchment (Moorkens & Killeen, 2014). The mapping of the quality and condition of *Margaritifera* habitats is of great value in planning conservation measures.

Riverine mapping is undertaken at many spatial levels, including large scale catchment use mapping using nationally available datasets such as from Landsat imagery to generate comparative predictors, such as inundation extent and frequency (e.g. Allen *et al.*, 2016).

River and reach scale mapping can also include remote sensing, side-scan sonar (Powers *et al.*, 2015) with drone technology and increasingly affordable high resolution multispectral imagery capable of mapping river stretches at high resolution. Appropriate resolution at the river bed stone and mussel level is not yet possible by remote methods. River Habitat Survey (RHS) in conjunction with Light Detection and Ranging

(LIDAR) surveys and using Digital Elevation Models (DEM) can be a useful means of mapping river habitats to at least reach level (e.g. Bentley *et al.*, 2016).

Mapping at the scale that includes the quality and condition of the individual mussel involves detailed and time consuming searches of the river bed, and many survey techniques have been developed to make such surveys relevant both spatially and temporally (e.g. Smith *et al.*, 2011; Daniel *et al.*, 2017; Young *et al.*, 2001a; Strayer & Smith, 2003; Pooler & Smith, 2005). Freshwater mussels have been demonstrated to move over time (e.g. Zajac & Zajac, 2011), and for studies to be accurate they must be representative of the population as a whole (Zieritz *et al.*, 2014), or at least that portion of the population that is the subject of the study.

Low numbers of observations reduce the validity of habitat mapping (Parasiewicz *et al.*, 2012), and the challenge of mapping of habitat that is appropriate for both adult and juvenile *Margaritifera* is complex. The cryptic nature of black adult mussels amongst similar sized black stones, and the fact that juveniles remain buried for many years all adds to the complexity, and makes indirect methods such as sonar inappropriate. Thus the approach for this study was to increase the density of mapping to a level that would make it representative, while retaining sufficient detailed (and time consuming) survey

to allow surveyors to develop expert skills in mapping intermediate “best expert judgement” rapidly mapped areas.

The achievement of sustainable condition of the study river *Margaritifera* population (North West England) is being hampered by the lack of juvenile survival in a wide area of potential juvenile habitat, and thus adult mussels that are reaching the end of their life are not being replaced by a new generation (Killeen, 2012). The river supports the largest population of freshwater mussels in England. In order to understand the relationship between the current adult population, its habitat distribution, and the distribution and condition of juvenile mussels, 3 kilometres of the river bed habitat have been mapped for their physical potential to support juvenile *Margaritifera*, and the condition of the habitat in supporting the survival of the young mussels. The objective of this research study was to develop a methodology to determine the extent and condition of potential habitat for juvenile freshwater mussels in a river in north-west England in order to provide accurate information on the status of the population, and the differences within the population in the extent of loss of sustainable condition. The resultant map thus aims to provide the locations where improvement measures should be prioritised. Accurate mapping also provides greater information that can be subsequently related to other datasets.

Typical juvenile *Margaritifera* habitat in the context of north-west England are smaller patches of coarse sand and fine gravels stabilised by larger stones, but within environmental conditions that are fast-flowing, mid-gradient riffles without inappropriately large influxes of suspended solids or nutrients. The “goldilocks” conditions that are suitable for juvenile survival are the combination of stability and cleanliness. An oligotrophic river bed with an absence of algal and macrophyte growth is ideal, where the presence of oligotrophic bryophytes is considered a positive indicator, as previously described by Hastie *et al.* (2000), Skinner *et al.* (2003), and Moorkens & Killeen (2014).

In an unimpacted catchment, an excellent population of freshwater pearl mussel (FPM) will have a large area of appropriate juvenile habitat through natural hydrology and geomorphology of the river in its catchment surroundings. The slope of the river bed, the presence of rock

outcrops, the input of coarse sands and gravels from steep tributaries, the stability of river bed substrates and the buffering of flows and nutrients from upstream lakes and the natural climate and rainfall patterns of the area all dictate the distribution and quality of FPM juvenile habitat.

In a catchment that has been impacted over time, there are a number of levels of juvenile FPM habitat quality that are likely to be present.

The first is habitat that is present and functional, in good condition, dictated by its resilience to the current pressures and threats acting on it, through stability in the river bed combined with preferential flow velocities.

The second is habitat that is present but in poor condition, due to current pressures that have not yet resulted in the habitat being destroyed. In the case of many rivers, this represents a wide area where dense beds of adult mussels were born, but there is not sufficient free-flowing oxygen through the river bed substrate at all times of the year to support the juvenile mussels living there to survive for the 2,000 or more continuous days needed before emerging to filter feed in open water conditions. The juvenile habitat is recognisably present, and can look excellent after a period of cleansing high flow, but following low flow periods it can be seen to be impacted by the presence of physical or organic fine sediment, or living algal growth. For this reason, condition assessment and thus the methodology proposed in this paper is best undertaken during the period when poorer conditions are most likely to be present.

Another range of habitat is habitat that has been destroyed. This is often evident where adults are still living in areas where they were evidently born, but there is no habitat suitable for juvenile recruitment. A frequent cause of juvenile habitat destruction is modification of river beds and banks, such as rock armouring, where adults remain alive but sands and gravels required by juvenile mussels have been scoured from their habitat and the reflection of energy from the hard armour is such that replenishment of juvenile sized clasts are unlikely into the future. Compaction of areas of river bed from insufficient replenishment of fresh substrate can also render prior juvenile habitat physically unsuitable, regardless of the cleanliness of its condition.

The last category of habitat is unsuitable, i.e. areas of river bed that are naturally unsuitable

for juvenile recruitment. Examples of such areas are edge gravels that always dry for a few days every year, or areas scoured through natural instability, or natural hard rock outcropping. All rivers will have areas that are naturally unsuitable for juvenile and adult mussels.

METHODOLOGY

The basis for data collection was transverse transects (i.e. perpendicular to the bank) which were surveyed at 10 metre intervals. Survey was undertaken using two different transect types, a detailed transect at both ends of each 100 metres of river length, with 9 intermediate less detailed transects in between.

In each metre square of all transects, habitat suitability was assessed as having "unsuitable (= no)", "potential" and "good" juvenile habitat, and habitat condition was assessed as being "good", "moderate" or "poor". These were best expert judgements based on the ecological interpretation of the combination of parameters surveyed in the detailed transects, and observed in the intermediate transects.

Good juvenile habitat corresponds to river bed areas that are likely to support juvenile mussels that settle there if the condition of the interstitial substrate is sufficiently good. Potential juvenile habitat corresponds to river bed areas that are less favourable, but that are still likely to support juvenile mussels that settle there during some years, e.g. potential habitat that is vulnerable due to shallowness or low near bed velocity can still support juvenile mussels if there is a succession of wet summers. Potential habitat that is vulnerable to scouring may support juvenile mussels if there is a succession of winters without major floods. Good juvenile habitat in good condition should support a wide range of mussel ages, whereas potential habitat in good condition should support a non-continuous range of mussel ages. Unsuitable habitat corresponds to river bed areas where juvenile mussels do not survive. Examples of such areas are edge gravels that always dry for a few days every year, or areas scoured through natural instability, or natural hard rock outcropping, or deep pools and standing water where fine sediment accumulates. All rivers will have areas of naturally unsuitable habitat, but catchment and river modification can also lead to habitat areas becoming unsuitable.

Habitat condition was mapped into three broad categories regardless of habitat suitability, poor condition, moderate condition and good condition. Good condition refers to clean, silt-free river beds with an absence of impact from excessive algal, diatom or macrophyte growth. Moderate condition refers to river bed habitat that has intermediate levels of fine sediment or nutrient impact, enough to potentially result in juvenile death but not enough to cause severe stress to adult mussels. Poor condition refers to nutrient and/or sediment impacts that are likely to be negatively affecting adult mussels as well as killing juvenile mussels in their interstitial habitat.

A demographic study was undertaken to assist in the interpretation of the condition of the mapped population. The results of the demographic study are interpreted with respect to the mapped data.

Field methodology

The end points of each transect were marked by flags and a ten figure National Grid Reference was obtained for one or both ends using a handheld Garmin G-trex GPS. Photographs were 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 were not permanently marked and a chain was not laid on the riverbed. The transect line and direction was maintained by using a sturdy 1m×1m stainless steel quadrat (subdivided into 4). This was placed on the downstream side of the transect line at (usually) the left bank, and all data was surveyed by one worker wading across the river downstream of the transect, taking great care not to trample mussels, and recorded by a second worker at the river bank. When all the parameters needed from one quadrat were collected, the quadrat was then carefully turned (flipped from the leading edge) and the same measurements were made, until the end of the transect. Upstream and downstream photographs were also taken to be used at a later date to assist the interpretation of the 9m river sections between each transect.

Two different transect types were employed. One in every ten transects were surveyed using 14 different parameters that are relevant to the assessment of physical habitat and its condition (Table 1). From these, the Habitat and Condition categories were assigned. Photographs were

Table 1 Parameters used in detailed transects for the assessment of physical habitat and its condition

Parameter	Rationale	Relevant to	Result
Adult Mussel Numbers	The presence of adult mussels in stable areas of river (i.e. not in pools where they can be washed in) is a good indicator that mussels were born in the area and thus was suitable for juvenile recruitment in the past. The number of mussels visible on the substrate surface in each constituent 1m ² quadrat was counted. Mussels were not removed from the substrate or disturbed in any way during these transect counts.	Habitat	Number of mussels
Water Depth	Depth is a good indicator of juvenile habitat. Areas of very shallow water can get exposed in low flows, and very deep areas can have lower flows and be areas of preferential settling of fine solids. Depth (in cm, to the nearest whole cm) was measured in the centre of each 1m ² quadrat using a 1m steel rule.	Habitat	Depth in cm
Flow velocity	Moorkens & Killeen (2014) demonstrated that an adequate flow was one of the most important ecological requirements for FPM and thus is a key indicator when assessing potential juvenile habitat. The surface flow in metre square across each transect was visually assessed for relative velocity on a five point scale. The approximate velocity ranges are based upon measurements made on the River Ehen at compensation flow (80–90 MLD). They are merely a guide and do not necessarily represent velocities at near bed level which is the velocity that directly affects the juvenile habitat.	Habitat and Condition	<p>Assessment Very fast</p> <p>Approx. Velocity (ms⁻¹) at 60% depth >0.75</p> <p>Comment Likely to be too high energy for stable juvenile mussel habitat</p> <p>Swift</p> <p>Moderate</p> <p>Slow</p> <p>Standing</p> <p>0.4–0.75</p> <p>0.25–0.4</p> <p>0.1–0.25</p> <p><0.1</p> <p>Ideal for stable juvenile mussel habitat</p> <p>Ideal for stable juvenile mussel habitat</p> <p>Marginal juvenile habitat</p> <p>Unsuitable juvenile habitat</p>

Parameter	Rationale	Relevant to	Result
Characterisation of substrate composition (Clast analysis)	A significant portion of the substrate in many <i>Margaritifera</i> 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 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 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.	Habitat	In each quadrat the surface composition was assessed according to 2 criteria: 1) Is there a wide clast size range? YES=3 or more different size categories present, or NO=<3 categories present. 2) What is the dominant clast size(s) present? Answer: The dominant size from the standard Udden-Wentworth Scale (Wentworth 1922) below or NO dominant, if the clast sizes are evenly distributed. Size (mm) 0.25 to 2.0 Medium to very coarse sand 2-8 Very fine to fine gravel 8-16 Medium gravel 16-32 Coarse gravel (pebble) 32-64 Very coarse gravel 64-128 Small cobble 128-256 Large cobble >256 Boulder
Sand/fine gravel	As this size range comprises the micro-habitat within which juvenile mussels are living, it is considered important to check for its presence. This is additional information derived from the clast analysis and is defined as the finer (non-silt) fractions of the substrate which range from medium sand, through very coarse sand, very fine gravel to fine gravel (size range 0.25 to 8mm). The presence of stabilizing small boulders with sand accumulations behind them are a good indication that juvenile habitat is sustainable, as sands and gravels in the lee of stabilizing stone are not easily scoured away during floods. This is included as a specific note of the presence or absence of the sand and fine gravel element in the substrate in the lee (on the downstream side) of coarse clasts. Where large boulders are rare or absent the assessment for this category comprised clasts classed as large cobble and boulder (i.e. all >128mm)	Habitat	This was assigned (following on from the clast analysis) into 3 categories: No=None seen on surface, but could be present and obscured by mussels or coarse clasts (through armouring). Present=less than 5% of the visible substrate surface comprises material <8mm Good=More than 5% of the visible substrate surface comprises material <8mm The choice for this category is either YES or NO
In lee of boulders?		Habitat	



Parameter	Rationale	Relevant to	Result
Compaction	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 3 main processes leading to compaction of the substrate: armouring/packing, colimation and lithification. A discriminatory assessment of substrate compaction can be made using techniques such as penetrometry if there are no larger stones (Geist & Auerswald, 2007), but an assessment can be made in armoured habitats by inserting a pointed metal rod into the top 10cm of the substrate surface amongst the smaller clast sizes	Habitat and Condition	No=the finer substrate is relatively loose Slight=some compaction but can be loosened Yes=the finer substrate is compacted and is not easily loosened
Silt cover	Very fine physical silt can impede water and oxygen exchange in the river bed and a cover of visible fine sediment over the surface layer is found in areas where there is insufficient flow velocity to prevent settlement of fine solids (such as some edge quadrats), or there has been an input of unnaturally large concentrations of fine sediment that cover river bed areas that should normally be clean. Defined as a layer of fine mineral material (<0.25mm) over the substrate material, not to be confused with organic material defined as floc (see below).	Habitat and Condition	The surveyor assesses the visible fine sediment on the river bed surface as follows: No=clean substrate surface Slight=less than 5% cover, usually in small (sheltered) pockets Moderate=>5% but <25% and not forming a more or less continuous layer Severe=>25% and forming a more or less continuous layer

Parameter	Rationale	Relevant to	Result
Silt infiltration	Very fine physical silt (as above) indicates either conditions of very low flow that concentrate natural levels of fine sediment or the presence of unnaturally large concentrations of fine sediment. Fine sediment is more easily moved from the surface during higher flows than from the interstitial sediment, which is a more stable environment and less prone to scouring. Therefore fine sediment pollution events can be quickly cleared from the surface of the river bed, but remain for long periods within the interstitial layer below. Interstices impacted by fine sediment have reduced water and oxygen exchange in the river bed and result in juvenile death. This important parameter was determined by the surveyor disturbing the top 5-10cm of the substrate surface with their foot, checking for a plume. If the rising plume dissipates very quickly, it is heavier than silt and considered to be sand, which is positive rather than negative as it does not block oxygen exchange.	Habitat and Condition	The categories of this parameter are as follows: No=no plume or sand which dissipates quickly Slight=a small plume of size smaller than sand Moderate=intermediate between slight and severe Severe=a significant plume released from the substrate that is slow to clear
Scour	In high flow velocity areas without larger stabilizing boulders, the substrate is more easily moved around or pulled downstream during flood conditions. Due to their frequent movement, scoured clasts are usually a bright colour, whereas more stable, un-scoured substrates are darker in colour (often black). Good juvenile <i>Margaritifera</i> habitat is associated with stable, blackened substrate.	Habitat	The level of scour is recorded by a visual assessment of the substrate appearance and particularly colour as follows: None=all larger substrate clasts (>32mm) had some degree of blackening and with no evidence of disturbance Slight=Some indications that substrate was recently disturbed, and/or some bright coloured elements in the smaller stones (>32<64) Moderate=Intermediate between slight and severe Severe=Clasts very brightly coloured and likely to have been recently disturbed, unstable when disturbed.
Algae and diatom growth	Natural levels of diatom growth within a range of indicator species are a feature of high status rivers. Excessive filamentous algae and unnatural levels of diatom growth consume oxygen and smother the river bed substrate, preventing effective water and oxygen exchange to juvenile mussels. In the assessment, algae / diatoms are defined as the green trailing filamentous species, other algae and the turf-forming diatom species	Condition	None=Clean substrate surface Slight=less than 5% cover, only a few filaments or small patches of diatom turf Moderate=>5% but not extensive or luxuriant Severe=>5% and extensive or luxuriant

Parameter	Rationale	Relevant to	Result
Flocculating organic fine material	Algal growths will decay over time to form organic fine sediment that often aggregates or flocculates. The decaying material (floc) 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. Areas of juvenile and good adult <i>Margaritifera</i> habitat are characterised by their 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. In contrast, bryophytes such as <i>Fontinalis</i> that attach directly to rocks are good indicators of clean, unsilted, fast flowing conditions and are positive indicators of juvenile habitat.	Condition	None=No evidence on the substrate surface or from previous silt kick Slight=less than 5% cover Moderate=>5% but not extensive or luxuriant, i.e. not forming a more or less continuous carpet Severe=>5% and extensive or luxuriant, forming a more or less continuous carpet.
Macrophytes and Bryophytes		Condition	Macrophytes if present should be recorded as species and % cover of each species
Detritus	Accumulations of debris and degrading humic matter can result in an environment of low oxygen and higher acidity, which is incompatible with juvenile survival. Detritus is generally associated with edges of rivers with low flows and overhanging trees – this is not juvenile habitat. This is not to be confused with an input of microscopic detritus from oligotrophic seepages, which act as a vital element of juvenile food. Permanent record for monitoring change	Habitat and Condition	This parameter is either a YES or NO for the presence or absence of visible detritus and (if present) whether leaves, twigs or decaying larger material such branches are the main component. Non-decaying dead wood was not included.
Photography		Habitat and Condition Habitat	Underwater photograph of each quadrat ideally with a scale
Habitat Category	From the results of the above parameters relating to habitat, the appropriate category is chosen	Condition Habitat	Good, Potential or Unsuitable (no) (See descriptions in text)
Condition Category	From the results of the above parameters relating to condition, the appropriate category is chosen	Condition	Good, Moderate or Poor (See descriptions in text)

Table 2 Example of data collected in the field

a) Detailed Transect every 100m

Transect	B90		
Location	c. 100m d/s footbridge		
Description	From small birch on RB to just d/s multi-trunked alder with indented bank d/s		
Grid Reference	Not included in this example		
Date	22 June 2017		
Time	15:10		
Transect Direction	R bank to L bank		
Transect Width	12 m		
Notes	Shallow run with moderate gradient, partially tree-lined banks, backed by semi-improved grassland.	View upstream	View across

Quadrat	1	2	3	4	5	6	7	8	9	10	11	12
Water depth (cm)	30	30	35	40	44	47	41	35	35	25	27	12
Mussels	9	37	23	37	22	20	32	65	50	0	3	0
Flow	Mod	Mod	Mod	Mod	Mod	Mod	Mod	Mod	Slow	Stand	Stand	Stand
Juvenile habitat suitability	Good	Good	Good	Good	Good	Pot	Good	Good	Good	No	No	No
Habitat condition	Good	Good	Good	Good	Good	Good	Good	Good	Good	Poor	Poor	Poor
Wide clast size range?	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Dominant clast size(s)	>32	>32	>32	>64	>256	>128	>128	>128	>64	>32	>64	>64
Sand to fine gravel?	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
In lee of boulders?	Yes	No	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No	No	No
Compaction	No	No	No	No	No	Slight	No	No	No	No	Slight	Slight
Silt cover	No	No	No	No	No	No	No	No	No	No	Severe	Severe
Silt infiltration	No	No	No	No	No	No	No	No	No	Mod	Mod	Mod
Detritus	Slight	No	No	No	No	No	No	No	No	No	No	No
Algae	No	Slight	No	Yes	No	No	Slight	Slight	Slight	Mod	Mod	Mod
Floc	No	No	No	No	No	No	No	No	No	Severe	Severe	Severe
<i>Fontinalis</i> %	0	0	0	0	10	10	10	25	0	0	0	0
<i>Myriophyllum</i> %	0	0	5	0	0	0	0	0	0	0	0	5
Scour	Slight	Slight	Slight	Slight	Slight	Slight	Slight	Slight	Slight	No	No	No
Quadrat Photo	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y



taken in each quadrat of the detailed transect to provide a record. Nine intermediate transects were surveyed for 5 parameters. These consist of depth, visible mussel number, flow velocity category and a best expert judgement for both habitat and condition category.

All data was collected during low flow (Q85 or lower). In the case of the study river this equates to a compensation flow regime. This restricted the

number of field days possible to undertake the work, but ensured that the results were comparable across the entire river. An example of the data collected is provided in Table 2. The condition assessment is a snapshot and condition is likely to vary according to many factors particularly seasonality and high/low flows. Because of the restrictions of flow and seasonal requirements, the data took 3 years to collect (2015 to 2017).

Table 2 (Continued)

b) Intermediate transect every 10m between detailed transects

Transect	135		
Location	c. 10m d/s T134		
Description	From Willow on LB to 3 trunked alder on RB		
Grid Reference	Not included in this example		
Date	12 June 2016		
Time	10:35		
Transect Direction	L bank to R bank		
Transect Width	12 m		
Notes	Shallow run with moderate gradient, tree-lined on both banks.	View upstream	View across

Quadrat	1	2	3	4	5	6	7	8	9	10	11	12
Water depth (cm)	21	25	29	35	38	41	45	35	32	29	10	10
Mussels	0	1	12	5	25	1	9	29	5	0	0	0
Flow	Stand	Slow	Mod	Mod	Mod	Mod	Mod	Mod	Slow	Stand	Stand	Stand
Juvenile habitat suitability	Good	Good	Good	Good	Good	Pot	Good	Good	Good	No	No	No
Habitat condition	Good	Good	Good	Good	Good	Good	Good	Good	Good	Poor	Poor	Poor

The demographic study was undertaken separately from the mapping process. A total of five sections of the river (each c. 50m long) in which all of the 9 habitat and condition combinations occurred were chosen, to include a representation of the range of mussel habitat along the length of the river. The results of the juvenile habitat monitoring informed the choice of river locations. Within each of the 5 sections 2 replicate samples for each of the 9 combinations were undertaken, giving a total of 90 quadrats.

The size structure of the population was determined by removing all of the mussels from a fixed area of substrate and measuring them. This consisted of laying a 0.5m×0.5m metal quadrat on the river bed and counting the number of mussels visible from the surface. This provides a comparison with the number of mussels excavated to provide information on the number of hidden mussels. The visible mussels were then carefully removed from the quadrat with as little disturbance to the substrate as possible. The substrate was then disturbed with the fingertips and any additional mussels counted and removed. Finally, an aluminium framed sampling net equipped with a 0.5mm nylon mesh bag was stood vertically on the downstream side of the

quadrat and the substrate was agitated to allow any remaining mussels to come to the surface and any very young (<15mm) individuals to be swept by the water current into the net. All mussel lengths were measured with Vernier callipers. The measured mussels were then carefully reburied in the substrate they were taken from. The population demographic profile is established by assigning the mussels to 5mm size classes and plotting as a histogram.

Interpretation

The map was prepared using QGIS™ Version 2.18 as the platform to visually represent the habitat quality categories surveyed or interpreted throughout the study site. All other parameters surveyed are linked to their location in the GIS via the spreadsheet containing the full dataset.

To enable the results to be portrayed visually, a layered basemap was set up. The following layers were included:

- A high resolution aerial photography layer of the river collected at the start of the project
- The river outline as used in GIS by the UK conservation agencies
- A 1 metre by 1 metre grid to allow each quadrat to be individually located.



Figure 1 Example of a section with transects colour coded according to juvenile habitat.

The $1\text{m}\times 1\text{m}$ grid was used to determine the start and end point of each transect. This was achieved initially by use of grid references recorded from a handheld Garmin GPS in the field. However, when these co-ordinates were incorporated into the GIS, their locations in some cases were subject to a 3–5 metre error. To determine the actual start/end points, the GPS co-ordinates were used in conjunction with identified land and river features (e.g. drains, trees, field boundaries, riffle crests, beaches).

The intermediate quadrats on each transect were then allocated a $1\text{m}\times 1\text{m}$ square (Note: although the transect was always set up perpendicular to the bank, for mapping into a fixed grid divided into 1m^2 in a north-south direction, the resulting transect line in the GIS is most often staggered or zig-zag (Fig. 1), and thus there may be a portion of 1m error in each square that does not align in an exact North/South direction). The final locations on GIS are therefore at a minimum 10 times more accurate than using GPS in the field. Once all of the quadrats were identified, their unique 12 figure grid references were transferred into a new Excel spreadsheet.

The next part of the mapping process was to represent each field transect recorded as

a GIS layer. Each quadrat was colour-coded according to its suitability as juvenile mussel habitat (Green=Good, Orange=Potential, Red=Unsuitable). When all of the quadrats within each transect were colour-coded, the interpreted habitat within the intervening 9m sections between the transects was added.

The inter-transect habitat was determined from an interpretation of the habitat type likely to be present based upon the upstream and downstream patterns, information collected into field notebooks, photographs upstream and downstream of each transect, location and presence of features visible on the aerial photographs (e.g. riffles, beaches, bends).

Good habitat was considered to continue along the areas of preferentially good flow that can be clearly seen between surveyed transects. Extensions of exposed beaches and bright scoured substrate sections were used to interpret the continuum of unsuitable habitat between transects, and potential habitat was similarly interpreted from evidence of the continuation of more vulnerable habitat areas such as those of high flow (slight scour) or low flow (or pool) characteristics. From this, each intervening $1\text{m}\times 1\text{m}$ quadrat was assigned an interpreted habitat type and colour-coded in toned shades (Pale green,



Figure 2 Example of section with interpreted habitat between the transects colour coded.

Pale orange, Pale red). An example of a section of the river with interpreted habitat is shown in Fig. 2.

A sample of 4 transects were repeated in each of the three years to assess their differences. In addition, the mapping technique was taught to three surveyors with prior certification for mussel survey to examine the potential for between surveyor errors.

RESULTS

The study delivered a map of a 3km long section of the river bed with respect to habitat quality and its condition for juvenile *M. margaritifera*. The map can then be interpreted to look for patterns throughout the area. The demographic study can be used in two ways. Firstly, it can be used to validate the mapping methodology, by assessing the linkage between the permutations of mapped habitat and condition parameters with the recruitment and survival of size classes of mussels in each of the 9 habitat and condition combinations. Secondly, the demographic profile can be used in combination with the mapped areas to estimate the level of improvement needed to achieve a sustainable population.

Habitat assessment

A total of 297 transects were surveyed over 3km. For ease of interpretation, these have been divided into 30 sections of 100m length (numbered 1 to 30 from upstream to downstream).

Within the 297 transects, 3347 quadrats were surveyed. These surveyed quadrats provide a high resolution accurate dataset for 10% of the river bed, and should be considered to be the key data for cross interpretation. To provide a full map for use in a GIS context, the interpreted area can be used.

When mapped, the total area estimated was of 36,211m². Using GIS provides a more accurate measure of area, in this case the estimate is 7.1% higher than the average area estimated by scaling up from the transects alone. The estimated proportions of each habitat category were relatively evenly distributed. A total of 11,231m² was considered to be good habitat, 10,840m² was potential habitat and 10,792m² was unsuitable habitat following interpretation for the entire 3km.

The distribution of habitat classes varies considerably across the study area, as shown in Fig. 3 (shown results are for collected, not interpreted data). This variability in the proportions of each habitat suitability category is demonstrated by

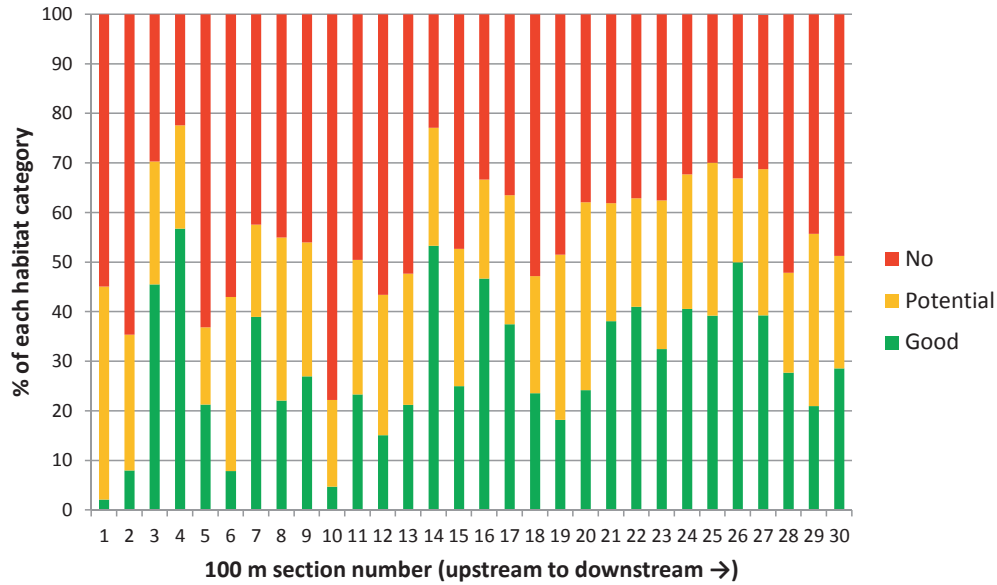


Figure 3 Distribution of habitat suitability category by 100m section.

Table 3 Comparison of transect and interpreted habitat ratios for selected 100m and 500m river lengths

Habitat Category	500m Sections 21–25		100m section 25	
	Areas mapped in transects only (%)	Estimated Area with interpretation (%)	Areas mapped in transects only (%)	Estimated Area with interpretation (%)
Good Juvenile Habitat	38.3	40.3	39.1	43.5
Potential Juvenile Habitat	27.9	36.5	31.0	38.6
No juvenile habitat (unsuitable)	33.8	23.2	29.9	17.9

comparing the results of estimated and interpreted areas of habitat from 500m and 100m sections within the overall 3km of river (Table 3). For the 500m section there is very close agreement between the percentage of Good habitat predicted from the transects alone (38.3%) with that from the interpreted plus actual transects (40.3%), whereas the estimated area for Potential habitat increases and the area of Unsuitable habitat decreases. Similar results are shown from the 100m example – there is good agreement between the percentage of Good habitat, but the estimated area for Potential habitat increases and the area of Unsuitable habitat decreases.

Habitat condition

The proportions of the 9 habitat category and habitat condition combinations are shown in Table 4. Overall 33% was recorded as being in Good condition, 22.8% in Moderate condition and 44.2% in Poor condition.

Table 4 Habitat Condition in each Habitat Category (based on 3347 quadrats)

Habitat category	% of each Condition category		
	Good	Moderate	Poor
Good	16.25	7.05	6.10
Potential	8.90	8.07	9.35
None	7.92	7.65	28.71
All habitats	33.1	22.8	44.2

The results show 55% of good habitat to be in good condition compared to 34% of the potential habitat. Only 18% of the non-habitat category was in good condition, reflecting areas of scour and flow gradients too high to support juvenile mussels. Much of the river bed (65%) that is not suitable habitat for juvenile mussels was found to be in poor condition. This is to be expected, and is normal for marginal areas that accumulate fine sediments and detritus.

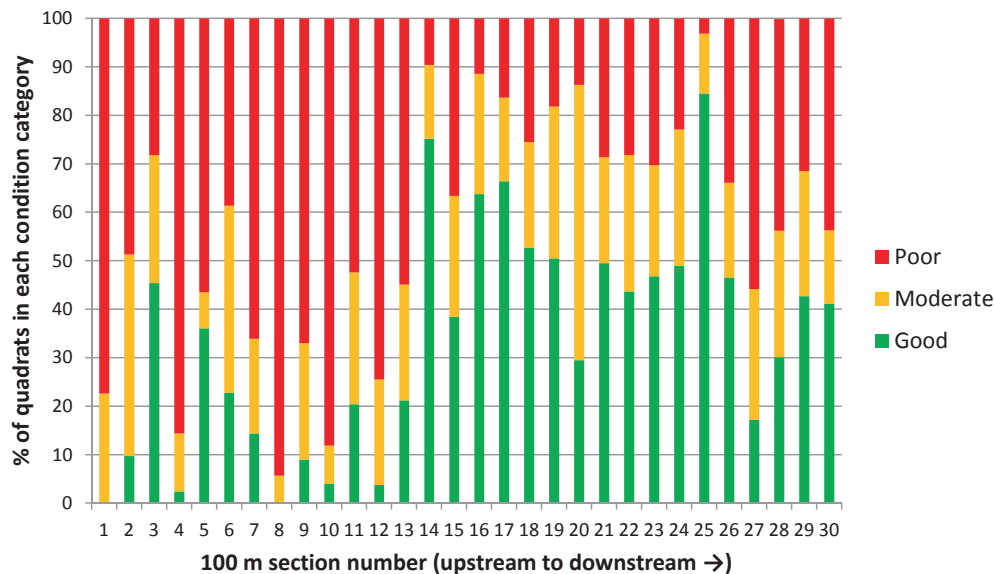


Figure 4 Distribution of Habitat Condition category in each 100m section.

There was considerable variation in the percentage of each condition category per 100m section (Fig. 4). Based upon condition, this 3km section of the river may be divided broadly into 2 halves. The upstream half (Sections 1–13) has mostly <25% habitat in Good condition and has mostly >50% of habitat in Poor condition. The downstream sections (14–30) mostly have >40% habitat in Good Condition and mostly <30% in Poor Condition. Section 8 was in the poorest condition and which, at the time of survey (June 2015) had no habitat in Good condition and had 94.3% assessed as being in poor condition.

Mussel numbers

Absolute numbers of mussels in their locations are not given in this paper due to conservation sensitivity. However, the sections with the highest densities correlate well with results found in previous detailed surveys (unpublished data). There is a high variability in mussel numbers within the different 100m sections of the river and, as has been shown in previous surveys, there are very large differences between individual quadrats both across the channel and within a short reach. The variability in mussel density per 100m section can be seen more clearly by assigning the numbers of mussels found in each quadrat to six abundance categories. The percentage of each category per 100m section is shown in Fig. 5, and the percentage for the 30 sections combined, are shown in the pie chart in Fig. 6.

The abundance category with the highest proportion of the total was zero mussels – with 39.2% of all quadrats recorded. The next highest category was 1 to 10 individuals per m^2 with 31.2% of the total. However, 5.4% of the total was represented by a single mussel. Quadrats with high mussel densities are relatively few with only 0.72% of the total with >100 individuals, and 5% with 51 to 100 individuals.

Of the total number of mussels counted in the 3,347 quadrats in the 3km study section, 71% were in good juvenile habitat, 22% were in potential juvenile habitat, and 7% of adult mussels were in habitat with no potential for juvenile survival. This equates to adult mussels that have been washed in to areas that they were not born in, such as deeper pools.

Flow

The freshwater pearl mussel is a species that occupies areas of river bed with a preferential high flow. The numbers of mussels recorded in an area of riverbed are therefore a reflection of the flow velocity regime, and it intersects with juvenile mussel habitat where conditions are suitable within the substrate as well as in the open water. Ideally mussels prefer the higher end of Moderate and the lower end of Swift (Moorkens & Killeen, 2014). A total of 54% of the mussels recorded were found in quadrats where the flow was assessed as Moderate and a further 26% of the mussels were found in quadrats where the flow was assessed as Swift. Category ranges were

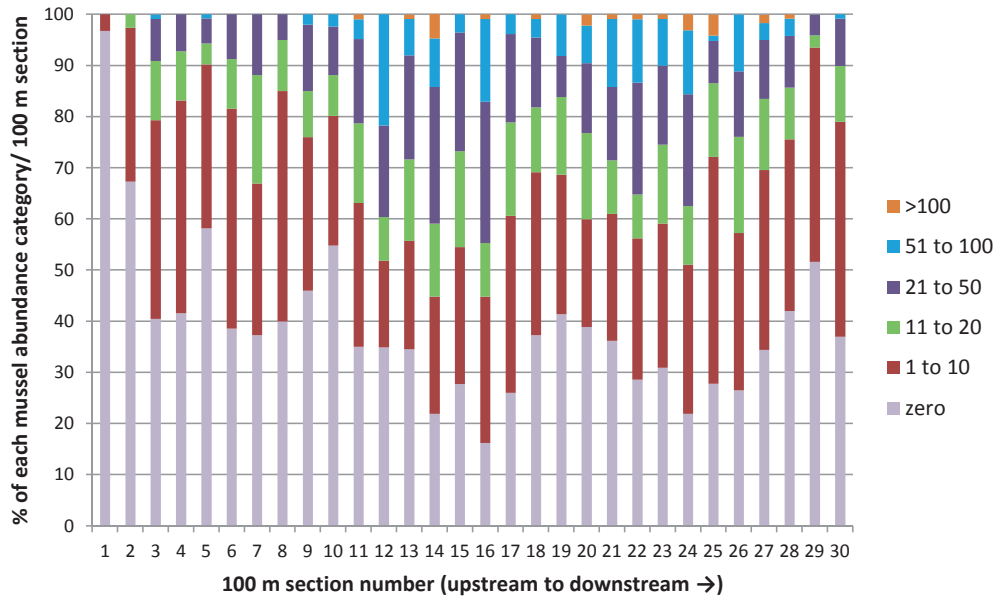


Figure 5 Distribution of mussel abundance in each 100m section.

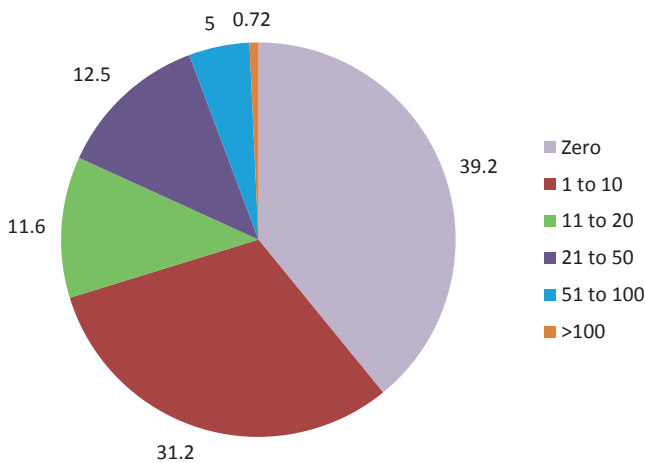


Figure 6 Percentage distribution of mussel abundance in the 30 sections.

checked using a using an OTT C2 Small Current Meter (OTT Hydromet, Kempton, Germany) during a previous study (Moorkens & Killeen, 2014), and the approximate near bed velocities associated with each category are shown in Fig. 7.

Fig. 8 shows the relationship between flow and the six adult mussel abundance categories. In lower densities, there is less association with flow velocity, but at higher mussel densities there is a much stronger relationship with higher flows.

Comparative relationships in shorter sections

Trends and relationships are very difficult to assess from a very large database such as the

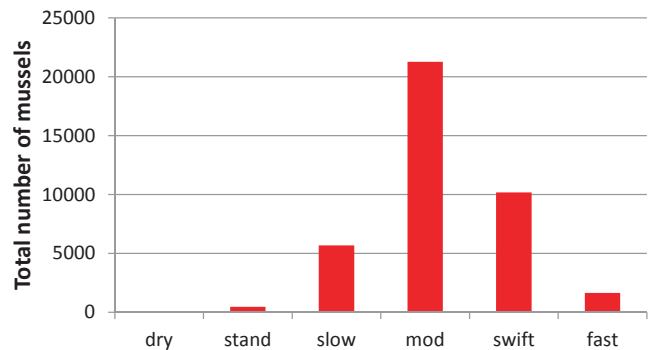


Figure 7 Numbers of mussels recorded in different flow categories (Standing velocity= $\leq 0.1\text{ms}^{-1}$; Slow= $\Rightarrow 0.1 < 0.15 \text{ms}^{-1}$; Moderate= $\Rightarrow 0.15 < 0.25 \text{ms}^{-1}$; Swift= $\Rightarrow 0.25 < 0.5 \text{ms}^{-1}$; Fast= $\Rightarrow 0.5 \text{ms}^{-1}$).

Excel spreadsheet or tables. However, a very useful method for a simple visual assessment can be achieved by preparing schematic diagrams and graphics for selected reaches of the river. Fig. 9 shows schematic diagrams of the quadrats in one 100m section (11 transects) with density of mussels, flow distribution, depth distribution, habitat suitability and habitat condition respectively.

In this example, there are high numbers of mussels distributed both across the channel width and throughout the section. Mussels are absent only from a few marginal areas. The section also has a high proportion of good juvenile mussel habitat (54%) and only 20% with no juvenile habitat compared to the 44% average for the

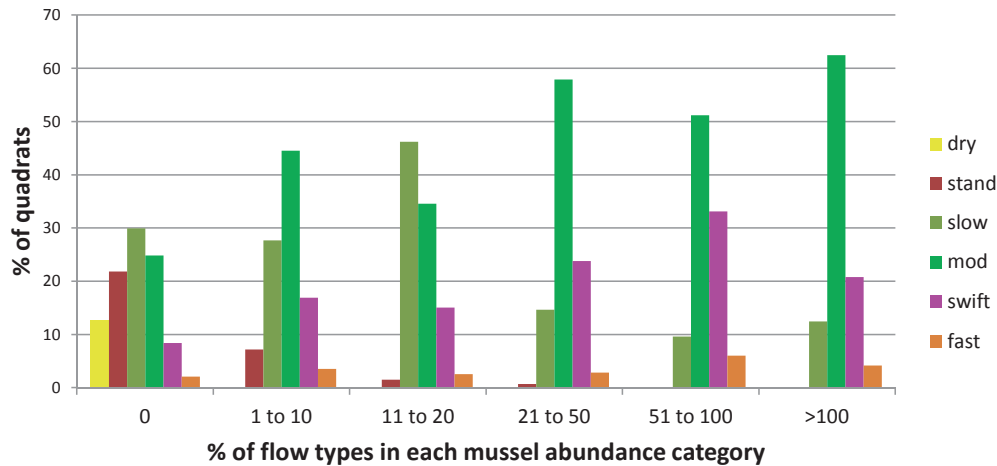


Figure 8 Adult mussel abundance in different flow categories.

whole 3km section. Similarly there is a high proportion of habitat in good condition (79%), with 14% in moderate condition and only 7% in poor condition. There is a good correlation between the quadrats with high mussel numbers categorized as having good habitat in good condition. There is a good variation in depth levels, which assists with the movement of water at near-bed level, and the flow patterns also reflect the good velocities away from the edges where there is good juvenile habitat and there are high adult mussel numbers.

Demographic study

A total of 789 mussels were found and measured in the 90 quadrats comprising: 92.5% adult mussels, 5.2% young mussels (>30mm <65mm) and 2.3% juvenile mussels (<30mm) (Table 5). The results show poor recruitment in the upper sections of river, with very few younger mussels found in the 2 furthest upstream of the 5 demography study sections. The mid-section had the highest proportion of juveniles with over 16% of the mussels measured less than 65mm in length. The 2 downstream sections had an intermediate level of recruitment. This corresponds to the quality of juvenile habitat and condition in the mapping study.

When categorised by habitat quality and condition regardless of location, the results demonstrate the influence of poor condition on habitat function (Table 6). Analysis of variance (ANOVA of numbers of juveniles <30mm and <65mm in quadrats of each habitat suitability category) was highly significant ($P < 0.005$) for quadrats taken from the different habitat and condition

combinations, which validates the visual mapping methodology used in the study.

The numbers of 5mm size classes found by habitat quality and condition are shown in Table 7. The good habitat in good condition had examples of every possible 5mm size class range, showing that the best habitats have a good continuity of recruitment, although there are many missing class sizes within individual quadrats, demonstrating that there have been many gaps in recruitment in all habitat categories. The good habitat in good condition had a size class range from 5 to 13 per quadrat, but which covered the full range of 24 size classes in the 19 quadrats investigated. Those mussels in good habitat in moderate condition also had a good range of age classes, suggesting that at least some of them were in good condition in the recent past, and thus there is good potential for recovery to good condition in these cases. The good habitat in poor condition had an absence of 9 size classes from all 17 of the quadrats examined, and had an average of 6 class sizes per quadrat (range 3 to 9) suggesting that they have not been functioning sustainably for considerable time. The results of the potential categories were as expected, with evidence of intermittent recruitment. The potential habitat in moderate condition had 5mm size classes that varied from 2 to 11 per quadrat.

The number of hidden adult mussels (mussels entirely buried below the river bed surface) is also a good indicator of good, oxygenated habitat in good flow. In 2012 (when there was a lower compensation flow regime) there were no hidden adults, as mussels had pulled themselves up high to gain the best possible flow (unpublished

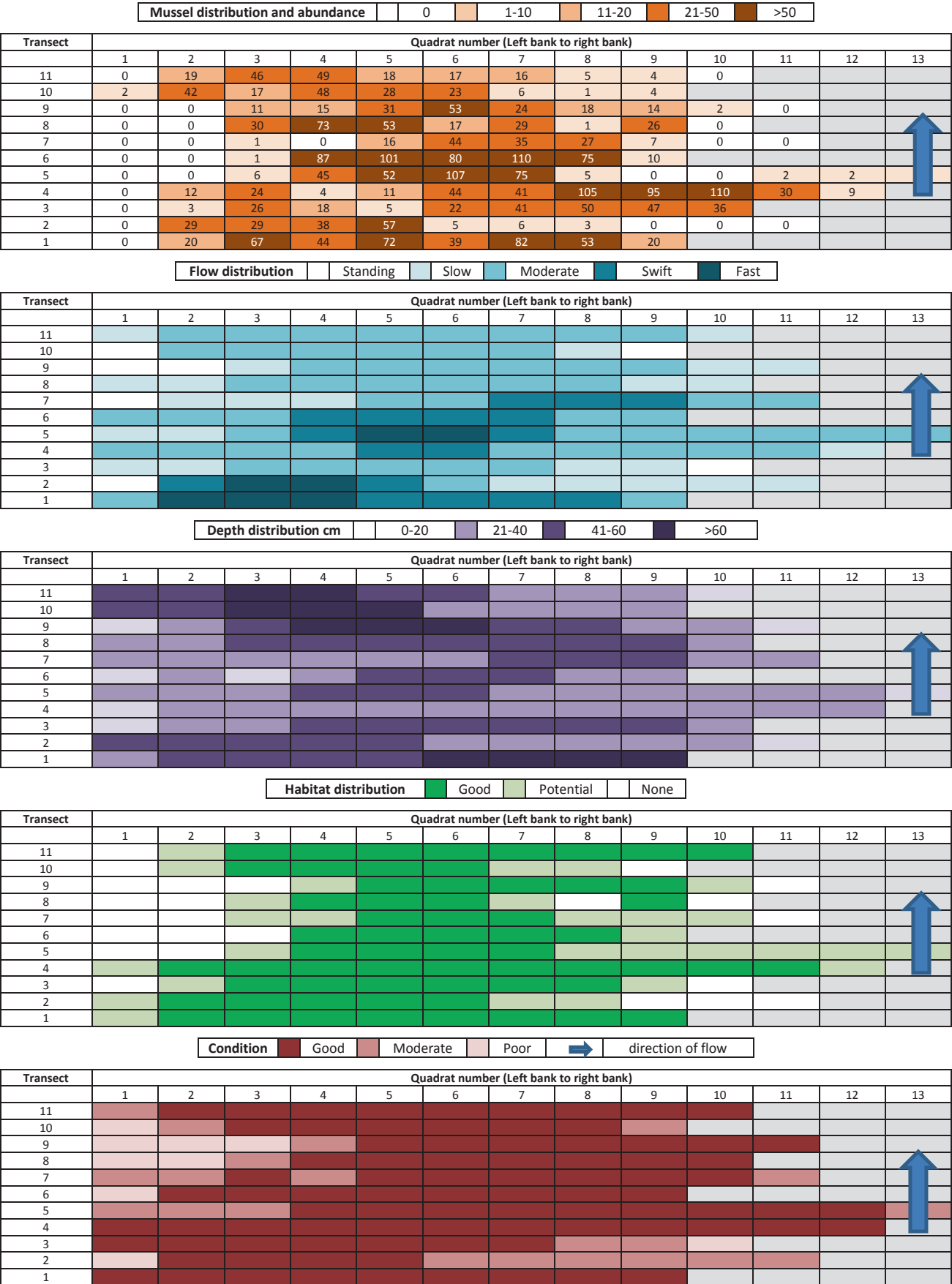


Figure 9 Patterns of mussels, flow, depth, habitat and condition in a selected 100m section.

Table 5 Number of adult, young and juvenile mussels found in each study section

Juvenile search section	1	2	3	4	5	Total
100m section number location	3	9	14	16	25	–
Mussels visible at the surface	146	125	110	144	143	668
Total number of mussels found and measured	169	139	153	166	162	789
% <65 mm	1.18%	4.32%	16.34%	7.83%	8.02%	7.48%
% <30 mm	0.59%	2.16%	5.23%	2.41%	1.23%	2.28%

Table 6 Percentage of recruitment of juvenile and young mussels in each of the 9 categories (this study)

Habitat Condition	Good (n=295)	Good Moderate (n=182)	Good Poor (n=106)	Potential Good (n=100)	Potential Moderate (n=66)	Potential Poor (n=35)	Unsuitable Good (n=1)	Unsuitable Moderate (n=2)	Unsuitable Poor (n=3)
% <30 mm	2.7	3.3	0	0	4.5	2.8	0	0	0
% <65 mm	7.1	10.4	2.8	10	7.6	2.8	0	0	0

Table 7 Number of 5mm size classes per habitat and condition categories found in the study

Juvenile habitat	Condition	No. of 5mm size classes
Good	Good	24
Good	Moderate	20
Good	Poor	15
Potential	Good	17
Potential	Moderate	16
Potential	Poor	11
Unsuitable	Good	1
Unsuitable	Moderate	2
Unsuitable	Poor	2

data). In this study there were 8% hidden adult mussels. Fig. 10 shows the overall demographic profile of the 90 quadrats studied during this investigation.

Repeatability

As the surveys were deliberately undertaken to determine the river bed condition during low flows, when they would be expected to be at their poorest, this aspect of habitat mapping is linked to the response of the river bed to low flows and higher temperatures, and these will differ from year to year. Table 8 shows the percentage of the three condition categories found in each of the three different years of study. While the differences between the results by year were found to be not significantly different from each other, there is a small trend of improvement in

condition seen each year, perhaps due to a more favourable compensation flow regime resulting in year on year cleansing of the river bed.

In terms of surveyor differences, no significant differences were found. The mapping technique was taught to two surveyors with prior certification for mussel count survey to examine the potential for between surveyor errors. Only 5 out of 466 quadrats differed from the author's categorization, i.e. 1.1% deviation between the authors and the two trained surveyors (Table 9).

DISCUSSION

The habitat mapping study developed a technique that combines physical measurements and adult mussel counts with best expert judgement in a dense network of transects, qualified by measured categorical habitat quality data in more dispersed transects, in order to get a mapped overview of mussel populations that are accurate enough to inform conservation agencies, river managers and researchers regarding the status of the population. The dataset is intended to be used in two different ways. The mapped, interpreted dataset can be used within its GIS platform, and combined with other GIS datasets from the catchment to assist with a greater understanding of how the catchment that serves the river is functioning for the mussel population. The dataset of intensively collected data can also be used as a baseline for the

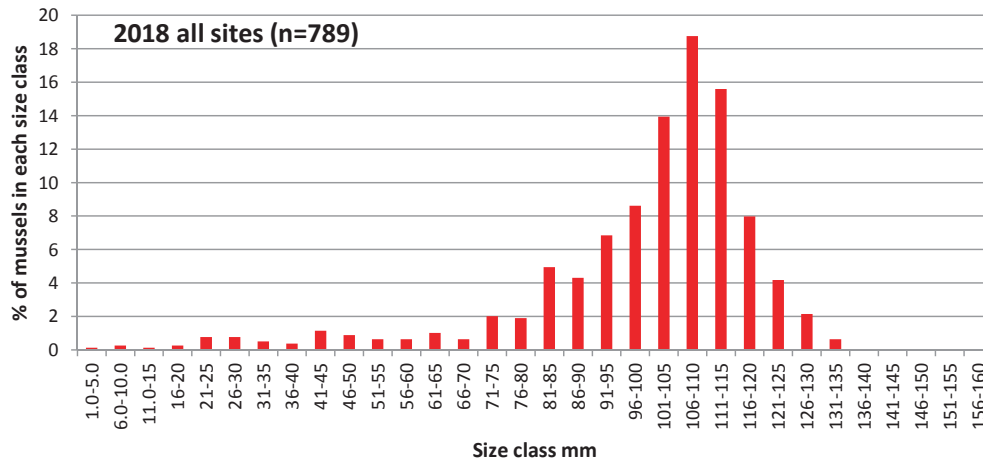


Figure 10 Mussel population demography in 2018.

Table 8 Habitat Condition percentages by year of survey

	% of each Condition category		
	Good	Moderate	Poor
2015	30.24	23.08	46.68
2016	33.81	22.79	43.39
2017	38.72	23.81	37.47
All	33.1	22.8	44.2

functional status of the population, from which change over time can be compared. This aspect provides a context that relates the available habitat area present to its condition, and to the adult and juvenile population present. In a sustainable population, there should be sufficient physical juvenile habitat in adequate condition to support the number of juveniles needed to maintain or increase the mussel population over time.

In a sustainable population where the maximum age is approximately 100 years, as is the case in this river, 20% of the mussels should be 20 years or younger (<65mm), and 5% should be 5 years or younger (<30mm) (Young *et al.*, 2001b). In the demographic study, an average of 7.28%

of the population was found to be less than 65mm and 2.28% of the population was less than 30mm.

Taking the different class sizes and assuming a life span of 100 years, a population prediction graph, also known as an “extinction curve” (Moorkens, 2010) is shown in Fig. 11. This predicts that based on a continuation of the current levels of recent recruitment, the population will keep declining to around 100,000 mussels. This graph may be falsely positive as, in reality, juvenile recruitment is also a factor of density of mussels, and recruitment rates generally decline as mussel densities decline. However, if the future recruitment levels improve to sustainable levels, the population could stabilise at over 300,000 individuals (Fig. 11).

To utilize this mapping technique to inform conservation policy and responses, it is useful to determine the level of improvement that would be needed to achieve sustainable recruitment by comparing demographic sustainability with habitat and condition categories. For example, in the case of this river, recruitment of juveniles <65mm was 7.5% instead of 20%, but recent flow improvements have resulted in an average of 2.3% mussels <30mm (46% of the 5% needed for

Table 9 Between surveyor differences

	Habitat			Condition		
	Good	Potential	None	Good	Moderate	Poor
Surveyor 1	61	115	62	55	50	123
Surveyor 2	63	111	64	55	48	123
Difference	+2	-4	+2	0	-1	+1

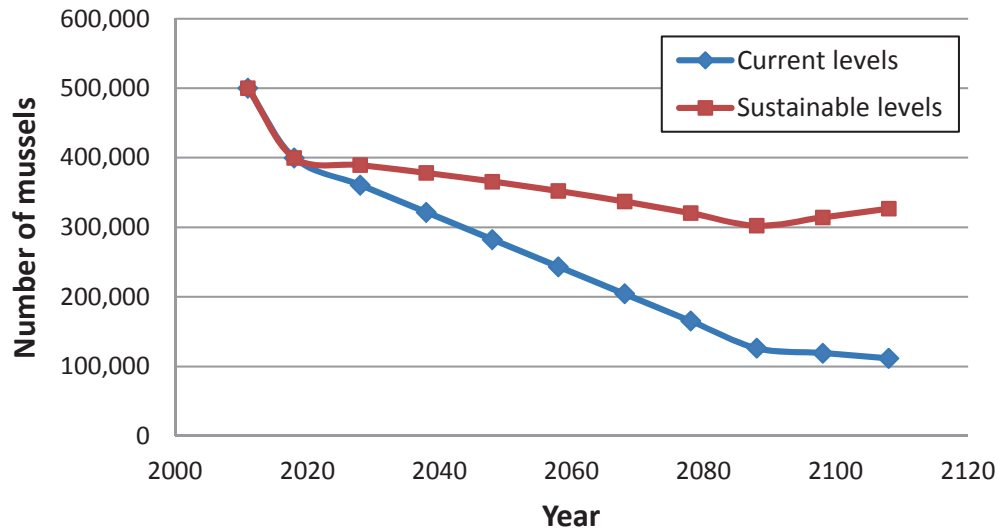


Figure 11 Population prediction graphs based on recent recruitment levels (current study) on sustainable recruitment levels.

juveniles <30mm). Thus a 54% improvement of recruitment would be needed across the population, although the improvements needed do vary considerably in the different sections. This sets a clear target for demographic improvement needed, which can be related back to where the mapping studies have shown areas of good or potential habitat that have depressed condition. In rivers where recruitment is not found at all, this should assist conservation projects in understanding the level of physical improvements needed in the river bed, as well as assisting with estimating the numbers of captive bred juveniles needed to restore a sustainable population size and indeed where to place them.

The reasons for the gap in recruitment may be at least partly explained by the results of the mapping study. In this study the percentage distribution of physical juvenile habitat in the different condition categories vary from 28.7% of the riverbed (unsuitable habitat in poor condition) to 6.1% of the riverbed (good juvenile habitat in poor condition). A total of 55.85% of the river bed has potential for juvenile recruitment (good plus potential habitat), with 29.45% being classified as good juvenile habitat.

There is considerable scope for improvement of condition in both good and potential juvenile habitat in the study river, although the situation may be much more challenging in populations with a greater level of deterioration. The mapping results show that improving moderate condition to good condition and maintaining

good condition in long term favourable quality could fill the gap towards the correct levels of juvenile survival through increasing both annual survival rates (i.e. future numbers of size classes per quadrat) and the spread of area of juvenile survival (improving the condition of good and potential habitat that is currently in moderate and bad condition).

CONCLUSIONS

The methodology developed in this three-year study has been shown to provide an accurate assessment of the physical structure and condition of the river bed in a manner that allows a numerical calculation of improvement needed. This can also be used to compare river sections and prioritize conservation measures, which in turn can be monitored for changes over time. The technique allows for data analysis from the measured areas, and mapped information to be used in relation to the GIS dataset. This study carried out over 3km of river has provided high resolution data, without the need to do intensive measurements in every transect. With the 1 in 10 detailed transects to provide a solid basis for mapping, best expert judgement can be quickly learned to allow for many intermediate transects to be undertaken. Naturally, the wider the mapping area undertaken, the more representative and accurate the results will be.

Undertaking demographic studies that are linked to the nine categories of habitat quality

and condition provides an accurate record of the recruitment status of the population. The combination of mapping and demographic knowledge provides valuable insight into where the most important habitats in the river are located, which in turn can be related to landscape, land use, water quality and flow datasets. Relating the survival of juvenile mussels to the habitat categories they are found in can alert the data users of key issues in any particular location, such as an abundance of good physical habitat in poor condition, or areas where physical habitat may be sparse. It provides information on whether pressures are likely to be associated with flow velocities or with pollution pressures in certain locations.

When interpreted together, habitat mapping and demographic studies can reduce the bias in juvenile searches, by ensuring that all combinations of habitat and condition quality are assessed. However, this is an invasive technique which 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, and the habitat within the quadrat is 'rebuilt' to reduce the risk of the mussels being scoured out. Naturally pearl mussel workers are reluctant to carry out potentially damaging work, particularly in vulnerable populations. However, in large populations, particularly where there is evidence of decline, having a reliable demographic profile is essential to inform the conservation decision making process. To avoid potential damage to the population, the number of quadrats examined should be minimal and adequately spaced apart, but be of sufficient number to give the true profile.

This new mapping methodology and the associated demographic studies are in keeping with the European CEN Standard for monitoring and assessment of the freshwater pearl mussel (British Standards Institute, 2017; Boon *et al.*, 2019), and provides critical information on the level of function of the study population. The use of juvenile habitat mapping, although time consuming, provides information on the status quo of the population, with sufficient information to form opinions with respect to the key issues that are leading to declines and improvements.

This technique provides a basis for establishing the most likely receptor sites for captive

bred young mussels, or short-term reared juvenile mussels (Moorkens, 2018). In many cases, captive rearing is only undertaken when populations have become seriously depleted, and residual adult mussels may have been washed into areas that are no longer proximal to the habitats in which they were born. For these rivers, it is important for those tasked with replenishing these depleted rivers with precious captive reared mussels to find areas where there remains good physical habitat with the potential to improve in condition over time.

Juvenile habitat mapping has the added advantage of focusing on the important aspects of river bed habitats that are most relevant to *Margaritifera*, thus increasing the knowledge and understanding of mussel habitats for field biologists. Over time, stretches of rivers in different catchments can be compared in order to provide a more balanced comparison between mussel population conditions and the progression of conservation objectives.

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