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Water quality — Guidance standard on monitoring freshwater pearl mussel (Margaritifera margaritifera) populations and their environment

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National foreword

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Wasserbeschaffenheit - Anleitung für das Monitoring von Populationen der Flussperlmuschel (Margaritifera Margaritifera) und ihrer Umwelt

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European foreword

This document (EN 16859:2017) has been prepared by Technical Committee CEN/TC 230 “Water analysis”, the secretariat of which is held by DIN.

This European Standard shall be given the status of a national standard, either by publication of an identical text or by endorsement, at the latest by August 2017, and conflicting national standards shall be withdrawn at the latest by August 2017.

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Introduction

This European Standard provides guidance on monitoring populations of freshwater pearl mussel *Margaritifera margaritifera* and the environmental features on which this species depends. Pearl mussels are endangered throughout their Holarctic range as a result of intensive land-use, pollution, river engineering, abstraction, declining populations of host fish, and exploitation by pearl fishers [1], [2], [3]. Throughout this document, use of the term *Margaritifera* refers only to the species *Margaritifera margaritifera* (Linnaeus, 1758). Within the EU, *Margaritifera* is protected under national legislation as well as by the EC Habitats Directive (Council Directive 92/43/EEC) which requires Special Areas of Conservation to be designated to safeguard this species. The presence of a population of *Margaritifera* with full juvenile recruitment is the sign of a healthy functioning river [4].

Margaritifera has a well-documented but complicated life history, with a larval glochidial stage dependent on a salmonid host. The larvae encyst within the host fish gills following release of glochidia in summer or early autumn. There they overwinter and grow before dropping off in the following spring or early summer. The few that survive initially remain buried in the river-bed substrate for several years where they interact with interstitial water. Older mussels typically have their siphons exposed to filter within the open water. The glochidial and juvenile stages are more demanding of a high-quality environment than adult mussels, emphasizing the importance of defining and maintaining appropriate ecological conditions for the young stages [5].

Margaritifera lives for an unusually long time – over 100 years in much of its range – but life spans can be much shorter at the southern extreme of its range and much longer at the northern extreme. A lack of recruitment of young mussels leads to populations becoming unsustainable, but these problems can be masked by the continued survival of older mussels for many years long after successful recruitment has ended. The requirement for a host salmonid fish to carry the mussel larval stage presents an added challenge in maintaining the condition of freshwater pearl mussel populations.

Although *Margaritifera* is highly demanding in river substrate and water quality, it occurs in a wide range of catchments from small, siliceous, oligotrophic rivers, often with a lake upstream, to large lowland mineral systems. This standard strives to encompass the range of latitudinal and geological factors that affect *Margaritifera* across its range. It is essential to take into consideration the unique pressures on each individual population when setting priorities for monitoring.

NOTE A limited number of key references are given in the Bibliography. A comprehensive list can be consulted by using the following link to the website of the Freshwater Biological Association – http://www.fba.org.uk/cen-pearl-mussel-standard-development-reference-list

The applications of the standard include the provision of site-level data that will contribute to reporting under the Habitats Directive, Article 17, undertaking environmental impact assessment, and restoring pearl mussel populations.

WARNING —Safety issues are paramount when surveying rivers. Surveyors should conform to EU and national Health and Safety legislation, and any additional guidelines appropriate for working in or near rivers.

IMPORTANT — Freshwater pearl mussel surveys are carried out under licence, and the methods used should be fully compliant with any conditions imposed.

1 Scope

This European Standard provides guidance on methods for monitoring freshwater pearl mussel (*Margaritifera margaritifera*) populations and the environmental characteristics important for maintaining populations in favourable condition. The standard is based on best practice developed and used by *Margaritifera* experts in Europe, and describes approaches that individual countries have adopted for survey, data analysis and condition assessment. While it is recommended that the causes for pearl mussel decline should be urgently investigated, standard methods for restoring populations are beyond the scope of this document.

2 Normative references

The following documents, in whole or in part, are normatively referenced in this document and are indispensable for its application. For dated references, only the edition cited applies. For undated references, the latest edition of the referenced document (including any amendments) applies.

EN ISO 14688-1:2002, *Geotechnical investigation and testing - Identification and classification of soil - Part 1: Identification and description (ISO 14688-1:2002)*

3 Terms and definitions

For the purposes of this document, the following terms and definitions apply.

3.1
acoustic doppler current profiler
ADCP
sonar device that produces a record of water current velocities for a range of depths

3.2
aquatic macrophyte
larger plant of fresh water which is easily seen with the naked eye, including all aquatic vascular plants, bryophytes, stoneworts (Characeae) and macro-algal growths

Note 1 to entry: This definition includes plants associated with open water or wetlands with shallow water.

[SOURCE: EN 14614:2004, definition 2.1]

3.3
bankfull
maximum point on banks at which floods are held within the channel before spilling over onto the floodplain

[SOURCE: EN 14614:2004, definition 2.5]

3.4
baseline survey
first survey of environmental or biological features by which progress towards rehabilitation or continuing decline can be monitored by subsequent surveys

3.5
bathyscope
bucket with a transparent bottom used for viewing freshwater pearl mussels on the river bed

3.6
biochemical oxygen demand after 5 days
BOD_5
mass concentration of dissolved oxygen consumed under specified conditions by the biochemical oxidation of organic and/or inorganic matter in water after 5 days

Note 1 to entry: For the purposes of this document, “biochemical oxidation” is taken to mean “biological oxidation”.

[SOURCE: ISO 5815-1:2003, definition 3.1 modified]

3.7
brooding period
length of time that glochidia remain within the body of a gravid pearl mussel

3.8
colmation
blockage of stream-bed interstitial spaces by the ingress of fine sediments and organic material

3.9
compaction
consolidation of the river bed through physical, chemical or biological processes

[SOURCE: EN 14614:2004, definition 2.10]

3.10
concretion
hard, compact mass of sedimentary rock formed by the precipitation of mineral cement within the spaces between the sediment grains

3.11
culvert
arched, enclosed or piped structure constructed to carry water under roads, railways and buildings

[SOURCE: EN 15843:2010, definition 3.8]

3.12
ecological quality ratio
EQR
ratio between the value of the observed biological parameter for a given surface water body and the expected value under reference conditions

3.13
encystment
process in which pearl mussel glochidia attach to the gills of their salmonid hosts

3.14
eutrophication
process by which a body of water acquires an overabundance of nutrients, especially phosphates and nitrates, leading to increased growth of algae and macrophytes

3.15
favourable condition
condition of a population compatible with contributing to 'favourable conservation status'

Note 1 to entry: As defined in Article 1 of the Habitats Directive”:- 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.”

3.16
filter feeding
process by which pearl mussels feed by straining suspended matter and food particles from water, typically passing the water over a specialized filtering structure

3.17
flow duration curve
graphical representation of a ranking of all the flows in a given period, from the lowest to the highest, where the rank is the percentage of time the flow value is equalled or exceeded

Note 1 to entry: These curves may be derived for flows in any time interval, such as daily flows, monthly flows or annual flows

3.18
fluvial audit
method for assessing the condition of a river and its associated human pressures, using information from field survey, remote sensing, historical and recent maps, scientific literature and other sources

3.19
functionally extinct (not currently viable)
pearl mussel population that is incapable of sustaining itself owing to a lack of juvenile recruitment

3.20
glide
moderately-flowing water with undisturbed surface other than occasional swirls or eddies, and with constant depth across part of the channel

[SOURCE: EN 14614:2004, definition 2.17]

3.21
glochidium (plural ‘glochidia’)
larva of *Margaritifera*

3.22
glochidial release
process by which gravid pearl mussels release glochidia into the water

3.23
gravid
carrying eggs or developing young

3.24
hydromorphology
physical and hydrological characteristics of rivers including the underlying processes from which they result

[SOURCE: EN 14614:2004, definition 2.18]

3.25
hyporheic zone
spatio-temporally dynamic ecotone between the surficial benthic sediments and the underlying aquifer

3.26
interstitial habitat
area occupied by aquatic organisms in the spaces between sediment particles

3.27
otoscope
instrument designed for examining the interior of the ear but in the context of this standard used to investigate brooding in freshwater pearl mussels

3.28
penetrometry
method for assessing the resistance of the river-bed substrate in situ using a standard cone or disc penetrometer

3.29
pool
habitat feature characterized by distinctly deeper parts of the channel that are usually no longer than one to three times the channel's bankfull width, and where the hollowed river bed profiles are sustained by scouring

[SOURCE: EN 14614:2004, definition 2.24]

3.30
recruitment
survival of juvenile pearl mussels and their addition to a population

3.31
redox potential
Eh
tendency of a substance to gain or lose electrons

Note 1 to entry: In the context of this standard, redox measurements of the stream-bed water at the typical depth of juvenile mussels are used as indicators of oxic (high Eh) or anoxic (low Eh) conditions.

3.32
reference river
river containing viable population of pearl mussels, where the associated environmental characteristics can be used to help define the species' requirements

3.33
reproductively viable
able to maintain a self-sustaining population without the addition of new genetic material from outside the system

3.34
riffle
fast-flowing shallow water with distinctly broken or disturbed surface over gravel/pebble or cobble substrate

[SOURCE: EN 14614:2004, definition 2.28]

3.35
riparian zone
area of land adjoining a river channel (including the river bank) capable of directly influencing the condition of the aquatic ecosystem (e.g. by shading and leaf litter input)

Note 1 to entry: In this European Standard, the term 'riparian zone' does not include the wider floodplain.

[SOURCE: EN 14614:2004, definition 2.29]

3.36
salmonid host
essential host for pearl mussel glochidia, in Europe usually Atlantic salmon (*Salmo salar*) or brown trout (*Salmo trutta*)

3.37
salt bridge
device containing a chemically inert electrolyte which is used to increase electrical conductivity locally

3.38
shear stress
measure of the force of friction caused by water flowing around a submerged surface or object

3.39
turbidity
reduction of transparency of a liquid caused by the presence of undissolved matter

[SOURCE: ISO 6107-2:2006, definition 145]

3.40
wade gauging
wading across the river taking measurements at regular intervals (e. g. depth, velocity)

3.41
woody material
material that falls into rivers and streams, ranging in size from leaf fragments (fine woody material) to branches or whole trees (coarse woody material)

4 Monitoring and assessing the condition of a *Margaritifera* population

4.1 Requirements for a sustainable *Margaritifera* population

4.1.1 General

Monitoring the condition of *Margaritifera* populations is carried out in three ways:

a) direct monitoring of the condition of the pearl mussel population;

b) direct monitoring of the condition of pearl mussel habitat;

c) monitoring indicators of the state of the pearl mussel environment, using databases or by new survey (b) and c) are covered in Clause 5).

Monitoring *Margaritifera* populations is needed for a wide range of purposes. The purpose of the investigation should determine the direct and indirect aspects of the environment that need to be considered. The following main types of investigation can be distinguished: detailed baseline monitoring; routine surveillance; investigating the causes of decline; ecological impact assessment; monitoring the effectiveness of management measures.

As viable *Margaritifera* populations are mainly found in undisturbed streams, water quality and other relevant data have rarely been collected and in many cases serious problems had already progressed before relevant data collection commenced. The first studies in a river with *Margaritifera* are therefore not normally a baseline of reference conditions, but a first survey by which progress towards rehabilitation or continuing decline can be monitored in subsequent surveys.

Targets for assessing whether *Margaritifera* populations are in sustainable condition are given in informative Annex B.

4.1.2 Monitoring

Adequate information on *Margaritifera* populations in good quality habitats and with successful recruitment of young mussels is essential if the aim of returning declining pearl mussel populations to favourable condition is to be met.

Monitoring should include a suite of samples and surveys recommended in Table 1 as a baseline. Once a baseline is established, the frequency of monitoring should be based on an assessment of risk to the population, with some aspects needing to be carried out at a higher frequency than others. Where negative pressures are apparent, investigative monitoring may be required to establish their cause.

Attributes of pearl mussel populations that should be monitored are shown in Table 1.

Mussel surveys should be carried out by wading using a bathyscope or by snorkelling/SCUBA diving in the river. Mussels should be counted in river stretches or by estimation using transects along or across the river and extrapolation to assess distribution and density. Standard methods developed for the country in which the survey is carried out should be used. Fixed repeatable monitoring transects should be established to assess changes in mussel distribution and density, substrate composition, and the cover of filamentous algae, macrophytes and fine sediment.

Population structure should be assessed by measuring the lengths of mussels including those mussels buried within the substrate.

NOTE 1 In some countries, standard methods do not include investigations of buried mussels.

A series of quadrats builds up a profile of the recent reproductive success in the population. 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, adequately spaced apart, and the work carried out by well trained and experienced workers during appropriate low-flow and low-turbidity conditions. Measurements should be made at random within areas where suitable mussel habitat is found. These data should form a series of separate size profiles that represent the population within the river. For repeat surveys for monitoring purposes it is appropriate to return to the same general areas. For each population profile, at least 250 mussels should be measured. In small populations this may not be possible, and measurements may need to be combined from different parts of the river. Ideally, quadrats should be linked to permanent transects by being close to but not immediately on or adjacent to them.

NOTE 2 This standard is principally concerned with surveying mussels in relatively shallow water. However, surveys of mussels in deep water can also be undertaken using an underwater camera or by diving.

Table 1 — Checklist of monitoring recommended on mussel attributes in rivers with *Margaritifera*

Aspect	Method	Output (units)	Notes
Distribution	Wading or snorkelling /SCUBA survey counts	Map	Once thoroughly to create a baseline with checks during 6 year survey
Population density	Wading or snorkelling/ SCUBA survey counts (including transects)	Number of mussels per m^2	Every 6 years, more frequently if needed for investigative monitoring (normally through repeating transect counts).
Individual mussel size	Quadrat analysis	Mussel measurement (mm)	5 mm class size grouping is recommended. Demography should be assessed every 6 years or more frequently for investigative monitoring.
Population age structure	Analysis of growth rates	Growth curve (mm per year)	Where juveniles and young mussels are present, age–size relationships should be established, particularly the range of sizes for mussels under 5 years and under 20 years for mussels with a life span of about 100 years. For longer and shorter life spans, the age–size relationships will vary accordingly. (Note that removing mussels to establish age structure could be damaging if repeated too frequently.)
Brooding levels	Visual, sub-sample of mussel adults checked using otoscope by trained expert	Age (%) of surveyed mussels with evidence of brooding, based on a sample of 20 individuals	To be undertaken where no other evidence of recruitment has been found.

4.2 Training and quality assurance for pearl mussel survey and assessment

4.2.1 Pearl mussel survey

Surveyor training is essential to ensure consistency, accuracy and precision. Surveyors need to understand the biology of the species sufficiently to appreciate the reasons for the methods used and the need for care in their application to avoid damage to pearl mussels.

Training should be structured to cover the level of survey required, from non-invasive counts of adults to specialist demographic quadrat analysis. Where relevant, a qualification in snorkelling / SCUBA diving should precede *Margaritifera* survey training. Although counting adult mussels can be taught to a wide range of surveyors, handling adults and juveniles should only be carried out by experts. In general, the more experienced that surveyors are the more likely they are to carry out an accurate survey. If survey experience is infrequent, regular refresher courses are recommended.

Content of training should include:

a) health and safety education relevant to mussel survey;

b) monitoring mussels without damaging them;

c) planning surveys, including issues of access and permission;

d) carrying out full mussel counts and population estimates, including wading with a bathyscope and snorkel/SCUBA survey;

e) setting out permanent monitoring transects and how to survey and relocate them, including the use of photography;

f) completing recording forms accurately;

g) carrying out juvenile searches and demographic profiles (expert training);

h) reporting survey results and compiling licence returns;

i) mapping mussel habitats;

j) gathering data on river corridor and land use.

Training should:

k) ideally incorporate a certification system;

l) provide regular refresher courses;

m) be carried out over the range of river types that will be encountered in the country or area covered by the certification;

n) be carried out in rivers that require wading with a bathyscope and rivers that demand snorkel / SCUBA survey;

o) be fully supported by manuals of techniques.

Before certification, the course participants should carry out a trial survey. Procedures should be put in place to test the results obtained by different surveyors on the same stretches of river. If a surveyor consistently records results that vary from those recorded by experts, the problem should be rectified by additional training, and certification should only be provided when survey results are deemed to be accurate.

4.2.2 Training manuals

Training manuals should be designed to support the objectives of this standard and incorporate national requirements. Manuals should include general background, unambiguous information on how to carry out surveys, accurate descriptions of the features to be recorded, and guidance on the format in which the data are to be maintained and presented. Text should be supported by illustrative material (e.g. photographs, videos, DVDs, CDs) to help describe survey details.

4.2.3 Data entry and validation

It is important that no errors occur when transferring data from field sheets to databases and Geographical Information Systems (GIS). Suitable quality assurance methods should be used, such as double entry of data into databases by two different operators followed by tests to ensure the results are identical. Data corruption can occur when systems are updated or during information transfer; some form of checking procedure is required following such changes. Sensitive data on *Margaritifera* populations should only be released according to national protocols on endangered species.

4.2.4 Licences

Margaritifera surveys are carried out under licence, and the methods used should be fully compliant with any conditions imposed. It is essential to maintain accurate information on the distribution and status of *Margaritifera*. A survey licence is normally provided on the understanding that data gathered are provided to the competent authority.

5 Monitoring the environmental conditions needed to support *Margaritifera margaritifera* populations

5.1 General

The assessment of water quality, hydromorphology, fish and macroinvertebrate features in pearl mussel rivers should be carried out by trained personnel. National protocols should always be used, and surveyors shall ensure that they obtain the appropriate licences before starting work.

High water quality is vitally important in maintaining sustainable *Margaritifera* populations [6]. Together with direct damage, flow changes and sedimentation, a decline in water quality is often responsible for the loss of *Margaritifera* recruitment and ultimately for the extinction of populations. To assist clarity in presentation, this standard describes the ecological requirements of *Margaritifera* in three separate sections: fish hosts, water quality, and hydromorphology (including flow and habitat structure). However, these factors do not act in isolation from each other and their combined effects need to be taken into account when determining the requirements of a specific pearl mussel population. In addition, it is important to assess the requirements of *Margaritifera* populations in a way that takes account of the differences between rivers.

Given the wide-ranging applications of the standard, it is not necessary to monitor all the following aspects for every investigation; the purpose of the monitoring should determine which aspects need to be considered. For those parameters that can be measured *in situ*, automated continuous samplers are highly recommended, particularly in rivers showing inadequate juvenile recruitment. Where water quality is consistently high, standard monitoring carried out under the EC Water Framework Directive (WFD) may be sufficient. However, it is advised that these rivers should be included in the WFD sampling programme.

Table 2, Table 3, and Table 5 provide an inventory of methods that are useful for monitoring a range of environmental parameters that can influence the condition of a *Margaritifera* population. Informative Annex B explains the rationale for monitoring the environmental features set out in Clause 4 of the standard. Informative Annex C describes the conditions under which sustainable populations of *Margaritifera* have been found to occur.

Over the years national standards bodies, as well as CEN and ISO, have produced many water quality standard methods in areas relevant to pearl mussel work. Most of these standards are specific methods of analysis in chemistry, ecology or hydrology that enable national and international comparisons to be made between different pearl mussel rivers. Such comparisons will help to increase understanding of the requirements needed for maintaining or restoring pearl mussel populations. However, because the parameters of greatest importance for individual populations may vary, relevant experts (with knowledge of water quality standards) should be consulted on the most appropriate methods to use before planning an environmental survey programme in pearl mussel rivers. In many cases such experts will be employees of the statutory environmental regulators or the nature conservation bodies responsible for implementing the WFD and the Habitats Directive.

5.2 Fish hosts

5.2.1 Fish host species

It is desirable to determine the species and density of host fish that a mussel population needs, and whether encystment is occurring. Where there are no data or the data are inadequate, electrofishing should be carried out twice using standard methods (EN 14011:2003), once in early autumn to establish the presence and density of suitable fish hosts as a proportion of the fish population just downstream of mussel beds, and again in late spring to establish the presence of yearling fish in the vicinity of permanent mussel habitat. The fish in the second survey should be checked for encystment of glochidia on the gills, which are visible on the live fish. More detailed studies of fish numbers and glochidial encystment (e.g. number of glochidia per fish) can be undertaken but the above should be considered as a minimum requirement.

Fish species composition and densities should be derived from electrofishing (catch per unit effort or efficiency) in sites where glochidial attachment is likely (i.e. downstream of the sites with pearl mussels) (Table 2). Depending on the size of the river, the current velocity, and the technical feasibility, stream sections at least 50 m long, and in areas where glochidial encystment is likely to occur should be investigated. Where blocking with nets and multiple electrofishing runs are not possible, values of minimum densities should be reported. If a valid correction factor for catch efficiency can be applied, this should also be reported.

Although the priority is to obtain data on pearl mussel host species, information on other fish species and their habitat preferences may help to identify and resolve problems when fish hosts are absent.

Table 2 — Checklist of monitoring recommended on fish hosts in rivers with *Margaritifera*

Aspect	Method	Output (units)	Notes
Numbers of 0+ fish in autumn	Electrofishing	Numbers per 100 m^2	Baseline in all rivers, with fish density surveyed every 3 to 6 years. Electrofished site should be downstream of large beds of mussels.
Numbers of yearling fish in spring	Electrofishing	Numbers per 100 m^2	
Numbers of encysted fish in spring	Electrofishing and visual check of gills	Percentage of host fish caught that are encysted. Estimated density of glochidia per fish (and by fish species)	Inspection should be done at a time when the cysts are visible to the naked eye, so that fish do not need to be harmed.

5.2.2 Barriers to fish migration

A survey and evaluation of fish barriers within the catchment should be undertaken as part of a risk assessment/fluvial audit (see 5.4.3), especially where 0+ host fish are infrequent or absent. Such barriers can be identified using morphological or water quality survey methods.

5.2.3 Host suitability

Where there is evidence that resident fish are not suitable hosts for the mussel population an investigation should be carried out. The resident fish and other potential fish hosts should be tested for compatibility with the mussels.

5.3 Water quality

5.3.1 General

A summary of water quality monitoring recommendations is given in Table 3.

5.3.2 Phosphorus

Phosphorus (P) monitoring in rivers is usually carried out as part of a more general programme of water quality monitoring, with samples often taken monthly or at longer intervals. Annual mean or median results are often quoted in order to make inter- or intra-catchment comparisons. Routine water quality monitoring is useful in establishing background levels, and when combined with ecological monitoring can give a useful, time-integrated view. However, mean or median values and monthly or less frequent monitoring have limited uses in *Margaritifera* monitoring, as one incident of elevated phosphorus can result in a period of high algal productivity, a reduction in oxygen in the river-bed gravels, and potentially with a loss of several years' recruitment effort.

Phosphorus monitoring should be designed to suit the needs of the individual catchment. Where risk of elevated levels is low, regular sampling may be sufficient. More detailed investigative monitoring may be needed if there is evidence of occasional or frequent deviations from oligotrophic conditions.

To assist the effective interpretation of the results, phosphorus should be measured both as total P (as an indicator of eutrophication) and as dissolved P (molybdate-reactive phosphorus (MRP) concentration, as an indicator of P available for uptake) and reported as mg/l P. Filtered and unfiltered total P samples provide different information and methods should be selected that are appropriate to each investigation.

Phosphorus monitoring should be done in conjunction with periodic assessments of filamentous algae and macrophytes. Evidence of excessive algal and macrophyte growth should trigger investigative monitoring. In order to trace the nutrient sources, a series of water samples should be taken. Where nutrient inputs are from point sources such as septic tanks, urban wastewater plants or direct discharges, sampling at low flows is recommended as concentrations are highest at these times. Where inputs are more diffuse (e.g. from agriculture) sampling on a rising flood is preferable, as in these conditions phosphorus will be released at levels that will be detectable before all of the MRP is absorbed into algal and plant growth. As natural levels of MRP in oligotrophic systems can be extremely low (<0,001 mg/l) it is important that methods are suitably sensitive, otherwise a considerable rise in nutrient levels can occur before this is observed in a general monitoring programme.

Early indicators of eutrophication can also be detected by measuring redox potential (see 5.4.4) and from the results of biological monitoring (see 5.3.12 and Annex A).

5.3.3 Nitrogen, including ammonia

Where concentrations are consistently low and detection limits are appropriate for *Margaritifera* (Informative Annex A) standard WFD monitoring may be sufficient. However, if there is evidence of enhanced levels or there is evidence of nutrient enrichment, investigative monitoring should be undertaken. Owing to the toxicity of ammonia to aquatic life and its fluctuation at different times of year, investigative sampling should include regular or continuous sampling when nitrification processes are unlikely to occur. Nitrogen concentrations are reported differently in different regions; however, nitrogen should be reported as mg/l nitrate N and mg/l ammoniacal N.

5.3.4 BOD_5 / dissolved oxygen

It is particularly important to check BOD levels (measured as BOD_5) downstream of potentially polluting activities and upstream of pearl mussel locations, to an appropriate detection limit.

5.3.5 pH

The natural pH range of the rivers of each *Margaritifera* population should be determined by regular monitoring throughout the year, including periods of snow melt, if appropriate. For investigative monitoring, pH levels should be monitored regularly, especially where pulses of low or high pH are believed to be occurring. Measurements should be taken *in situ* rather than as part of a suite of laboratory measurements.

5.3.6 Calcium

A baseline calcium level for each *Margaritifera* river should be determined using regular water quality survey data if there is a long time series, or by direct investigation under a conservation monitoring scheme. Calcium can be measured in the laboratory or in the field using an ion-selective electrode. The baseline calcium levels can be related to the total hardness levels of the river system being monitored, if hardness is the more regularly monitored parameter.

5.3.7 Alkalinity

A baseline alkalinity range should be determined and regular monitoring of alkalinity should be carried out (including during periods of snow melt) using water quality survey data if there is a long time series, or by direct investigation.

Natural levels of alkalinity vary between rivers so it is important to understand the baseline and natural variation. Any unexpected changes should be taken as warnings and used to trigger further investigative monitoring.

5.3.8 Electrical conductivity

A baseline electrical conductivity (Ec) range should be determined using water quality survey data, if there is a long time series, or by direct investigation. Ec can be measured easily in the field using a conductivity probe. The reading is influenced by temperature (and can change by up to 3 % per °C) so measurements should be temperature compensated to correspond to µS/cm at 20° C.

Given the variation in Ec levels experienced by European *Margaritifera* populations, no conductivity limits are proposed, but any elevated levels compared with the normal baseline for each river, or the predicted baseline if the river is to return to favourable condition, should be investigated for pollution sources.

5.3.9 Temperature

A baseline temperature regime for each river with *Margaritifera* should be established using water quality survey data, if there is a long time series, or using autologgers if other data are unavailable. Temperature levels should be monitored to 0,1 °C preferably using instruments that record continuously. Care should be taken to position recorders appropriately with regard to shade and water depth, in order to be representative of the mussel habitat. More detailed investigative monitoring may be needed where abstraction, impoundment or other management may be affecting the temperature profile of the river.

5.3.10 Contaminants

For rivers with *Margaritifera*, those toxic substances listed in the WFD should be analysed according to the risk of their occurrence, with more frequent sampling where the presence of toxic substances seems more likely. Other pressures, such as radionuclides, should be assessed case by case according to their potential risk.

5.3.11 Turbidity, suspended solids

Water samples can be analysed for suspended solids and correlated with turbidity for each pearl mussel river. Continuously sampled turbidity is valuable for monitoring the success of measures to mitigate construction work or other events such as tree felling in sensitive areas. The equipment can also be fitted with alarm triggers so that construction ceases until the source of the problem is removed. Turbidity meters should be used upstream and downstream of areas at risk, with the alarm set to respond when a significant difference between the two readings occurs.

Suspended solids should be analysed at a range of particle sizes that are environmentally relevant to the risk to *Margaritifera*.

Table 3 — Checklist of monitoring recommended on water quality parameters in rivers with *Margaritifera*

Aspect	Method	Output (units)
Phosphorus MRP	Water sample Regular monitoring until means and extremes are established; thereafter repeated at a frequency dependent on risk.	mg/l P
Phosphorus TP	Water sample	mg/l P
Nitrate N	Water sample	mg/l N
Ammoniacal N	Water sample	mg/l N
Dissolved oxygen	Dissolved oxygen probe / autologger	% saturation
BOD	Water samples	mg/l O_2
pH	Autologger / point samples	pH units
Calcium	Water sample	mg/l Ca
Hardness	Water sample	mg/l $CaCO_3$
Alkalinity	Water sample	mEq/l
Electrical conductivity	Autologger / point samples	µS/cm
Temperature	Autologger	°C
Contaminants	Water samples	µg/l of substance
Turbidity	Autologger	NTU / FNU
Suspended solids (total)	Water samples	mg/l

5.3.12 Biotic indicators of water quality

5.3.12.1 General

A summary of monitoring requirements for biotic indicators of water quality is given in Table 4.

5.3.12.2 Macroinvertebrates

Regular macroinvertebrate sampling using standard multi-habitat methods for each country should be undertaken in rivers with *Margaritifera* populations, taking care not to disturb or damage pearl mussel beds. This should be done according to national procedures.

5.3.12.3 Diatoms

Standard methods of diatom sampling should be used. Naturally occurring moveable hard surfaces (e.g. cobbles) in the vicinity of mussels are recommended for sampling.

5.3.12.4 Filamentous algae

Visual assessments should be made of *Margaritifera* habitat during the algal growing season and estimates made of the percentage of filamentous algal cover. A standard area of a mussel bed that is considered to be vulnerable to algal growth (i.e. an unshaded, shallow area) and is easily accessible (e.g. visible from a bridge or stopping point) should be chosen and used for regularly assessing algal growth. Filamentous algal cover should also be recorded as a standard part of any pearl mussel survey, as should any fungal and bacterial growths observed as these can have similar impacts. Fixed point photography can be very useful for monitoring purposes.

5.3.12.5 Macrophytes

Visual assessments should be made of *Margaritifera* habitat during the macrophyte growing season, and carried out in permanent transects used for *Margaritifera* monitoring. Fixed point photography is useful for monitoring purposes.

Table 4 — Checklist of monitoring recommended on biotic indicators in rivers with *Margaritifera*

Feature	Aspect	Method	Output (units)	Notes
Macroinvertebrates	Taxon assemblage	Kick samples	biotic index, EQR	Samples need to be in areas relevant to mussel locations
Diatoms	Species assemblage	Samples from cobbles / stones	biotic index, EQR	
Filamentous algae	Cover	Observations during growing season	% cover	Where elevated nutrient levels have been recorded, regular visits to check for algal growth should be carried out. This can be reduced to annual surveys where rivers have consistently < 5 % cover
Macrophytes	Cover	Observations during growing season	% cover	

5.4 Hydromorphology

5.4.1 Monitoring requirements

A summary of hydromorphological monitoring recommendations is given in Table 5.

Table 5 — Checklist of monitoring recommended on flow and physical environmental parameters in rivers with *Margaritifera*

Feature	Aspect	Method	Output (units)	Notes
Flow	Discharge	Various standard methods available, most commonly conversion from recorded level data to flow via stage discharge equation using V or rectangular notches where available	m^3/s	Establish a baseline (where needed) for each river. See 5.4.2 if no level data are available for river
	Discharge (flow duration curve)	Derived from continuous flow data	% time flow exceeded	

Feature	Aspect	Method	Output (units)	Notes
	Cross-section depths and velocities (including near bed level), spot flow measurements	Wade gauging (or Acoustic Doppler Current Profiler would be less intrusive for mussels)	m; m/s	Establish a baseline (where needed) for each river. See 5.4.2 if no level data are available for river
Physical habitat structure	Substrate composition	Classification by EN ISO 14688-1:2002 (see Table 6)	% each size clast	As part of mussel monitoring
	Fine sediment, surface	Visual estimate	% cover	As part of mussel monitoring
Substrate quality	Fine sediment, infiltrated	Silt plume or redox potential	Severity of silt plume, % loss of mV from open water (temperature corrected)	Close to area of mussel monitoring
	Penetrometry7	Penetrometer	kg/cm^2	Investigative monitoring
	Sedimentation rate	Sediment traps	Size range of settled solids, quantity and time of accumulation % size classes kg/m^2 per month	Investigative monitoring
Substrate stability	Shear stress	Calculated from particle size and velocity	N/m^2	Risk-based survey

5.4.2 Flow

The overall aim should be to ensure that discharges, velocities and depths protect the mussel population as a whole, and especially its most vulnerable mussel beds. For conservation management and for impact assessment, a flow regime monitoring programme should be established if the natural flow regime is proposed to be or is being affected.

The information required varies greatly between rivers. Before carrying out work on river flow, users should determine what information is already available, and consult a hydrologist who can recommend the most appropriate methods for providing additional information needed in each river.

Depth and velocity data should be linked with the discharge in the river to which they apply. As river discharge is constantly changing due to precipitation it is essential also to relate this discharge rate to the flow 'state' using a flow duration curve.

The following are suggested options, the level of detail should be related to risk:

1) Measurements of discharge (m^3/s) should be made at the same time as depth and velocity (including velocity near the bed of the river). If possible, a flow exceedance value should be derived from a duration curve.

2) Data on velocities and depths should be collected from a minimum of five cross-sections with the number appropriate to the river length and location of tributaries. The results should be expressed as minimum, median, maximum and mean values for each cross-section at a specific flow. The mean and median values for all the cross-sections should also be given. Data should be collected at a minimum of three different discharges. This relatively simple approach should not cover too wide a range of flows or extrapolation would not be appropriate.

3) Where a development proposal is likely to have a significant effect on flow, an appropriate hydraulic model should be produced to assess the potential effects on the mussel population. The outputs of the model should be related to mussel habitat.

4) Data should be presented as flow per metre bankfull width as a standard for all mussel populations that do not have continuous level or discharge recorders. Flow per metre bankfull width requires estimated discharge data and a number of river width measurements and may sometimes be undertaken without entering the river. A range of mussel habitats can also be compared using this simple standardized approach. The width is based on bank-top features that indicate the level of the bankfull flow and a mean of 10 measurements at any one site should be taken. The flow of the river or the volume of water is then divided by the number of metres in the bankfull width ($m^3/s \cdot m$) to provide a hydraulic indicator of the quality of the flow characteristics over the mussels. Although this technique has some limitations it provides within- and between-river comparisons for all sizes of river simply and inexpensively where more sophisticated monitoring is missing.

5) Discharge or level measurements should be made continuously using non-intrusive methods.

5.4.3 Physical habitat structure

A series of transects across the river width in *Margaritifera* habitat are needed to determine substrate composition. The number of transects needed will depend on the heterogeneity of the river and the size of the population, but should be sufficient to be representative of the river or the stretch of river of interest.

A relatively simple field method is to estimate by eye the percentage cover of boulders, cobbles, gravel and sand for each 1 m^2 quadrat of each transect. This can be difficult to do accurately and inter-surveyor variability may also lead to results that are not comparable. These problems are compounded where the substrate is obscured by large numbers of mussels, by macrophytes or filamentous algae, or by a layer of surface silt. The percentage cover of any overlying layer of silt should be analysed separately. Substrate should be separated into clast sizes as expressed in the relevant range for *Margaritifera* in EN ISO 14688-1:2002, (Table 6). Photographic survey is useful here, as it will reflect the changes in the habitat over time.

Other methods are commonly used to assess composition, but these are less suitable for rivers with *Margaritifera* as they require removal of samples of substrate.

Table 6 — Clast sizes relevant to *Margaritifera* habitat (according to EN ISO 14688-1:2002, Table 1)

Category	Size mm
Tree root /other	Any
Silt	> 0,002 to 0,063
Sand	> 0,063 to 2,0
Gravel (fine)	> 2 to 6,3
Gravel (medium)	> 6,3 to 20
Gravel (coarse)	> 20 to 63
Cobble	> 63 to 200
Boulder	> 200 to 630
Large boulder	> 630
Bedrock	Exposed bedrock

In rivers with predominantly sand and gravel substrates, or where there is poor replenishment of large clast size, compaction may occur and should be measured using penetrometry [7]. Low resistance indicates unconsolidated fine sediment, whereas high values can either indicate consolidation, e.g. by colmation, or an extremely coarse substrate.

Several penetration resistance readings should be taken at each site, spread across the river width, in order to take account of spatial variability. Sites that are obviously not potential mussel habitat, such as outcrops of bedrock or boulders, should be excluded. Two different tip types (cone or disc) and adapter sizes can be used. Whichever method is chosen it should be used consistently. The disc adapter is typically more useful on substrates with a high proportion of small clast sizes, whereas the cone penetrometer is useful on substrates with a wider range of clast sizes.

Both methods of penetrometry can be used to establish whether the substrate compaction is appropriate for a *Margaritifera* population or may have been adversely affected in the past by dredging or boulder removal, and for monitoring changes over time.

NOTE Fluvial audit (FA) [8], although not a monitoring tool, is an extremely useful method for understanding geomorphological problems unattainable by any other means. FAs (which can only be undertaken by experienced geomorphologists) combine information on field survey, remote sensing, historical and recent maps, scientific literature and other sources to help assess the condition of the river and its associated human pressures. FAs are recommended where specific problems have been identified, e.g. where there is a perceived risk of damage to pearl mussel habitat from siltation, or where a pearl mussel population is already believed to be in decline owing to unnaturally high inputs of sediment to the river.

5.4.4 Substrate quality

Basic measurements of sedimentation can be made in a *Margaritifera* catchment, such as looking for the presence of a silt plume rising from a kick sample near to but downstream of a mussel bed, and carrying out a visual assessment of silt cover within transects in pearl mussel habitat. Even where fine sediment cannot be seen on the surface, sediment deposition on the substrate can be measured directly by using sediment traps [9],[10]. River-bed substrate can appear clean at the surface but the substrate can still be infiltrated by fine sediment. A more comprehensive understanding of sedimentation problems can be obtained by carrying out a fluvial audit (see 5.4.3).

The infiltration of high loads of fine sediment (often linked with eutrophication) typically results in low oxygen supply to the interstices of the substrate. Redox measurements provide a way of determining the reduction of available oxygen within the substrate compared with the open water, and the removal of oxygen from oxidized nitrogen molecules [7]. As the technique measures the continued reduction in the sediment, it is more useful than direct oxygen measurement. This is a method that allows a large amount of data to be gathered comparatively quickly. It can be used to measure improvement or deterioration in river-bed quality over time. Surveys should be undertaken during low-flow periods during the summer months to capture the most adverse conditions.

The principle is to measure a millivolt difference between a platinum electrode that can be directly inserted into the substrate, and a reference $Ag/AgCl_2$ electrode held within the water column.

Separate readings should be obtained for substrate depths typically ranging from 2 cm to 10 cm. At conditions of very low conductivity, it may take several minutes for readings to stabilize. In such cases, a salt bridge should be used.

Large differences in redox potential (temperature-corrected values) between the open water and the substrate indicate habitat of poor quality for juvenile *Margaritifera*.

5.4.5 Substrate stability

Critical shear stress levels should be assessed if there is evidence to suggest that a *Margaritifera* population is at risk from any activities that can affect substrate composition, mobilization or cleansing. The critical shear stress can be calculated from particle size and velocity.

5.4.6 Trees and wood

For small mussel populations, areas of dead wood and trees at risk of falling should be mapped in association with mussel beds.

5.4.7 Instream modifications

A survey of artificial structures within the river corridor should be undertaken as part of a risk assessment/ fluvial audit. Such structures can be identified using morphological survey methods (e.g. River Habitat Survey (RHS), fluvial audit). Where assessments identify structures that are causing damage, mitigation measures should be investigated and implemented.

6 Monitoring environmental pressures

Table 7 provides guidance on the environmental pressures that should be monitored in rivers with *Margaritifera*. Monitoring is only necessary where a risk assessment indicates that one or more of the pressures listed is likely to exert a significant impact on the river. In most instances the output from monitoring will be a map showing the location of the pressures observed.

Table 7 — Checklist of environmental pressures recommended for risk-based monitoring in rivers with *Margaritifera*

Pressures	Monitoring method
Trees and wood (but only where a risk to mussel beds - see 5.4.6)	Walk-over survey
Grazing / stocking/access/ trampling damage	Walk-over survey, fluvial audit
Farm pollution	Walk-over survey, cross-compliance inspections, fluvial audit
Forestry	Walk-over survey, WFD data set of % of catchment, catchment level felling plans, fluvial audit
Dams, weirs, culverts and other obstacles to migration	Walk-over survey, fluvial audit
Drains	Walk-over survey, fluvial audit
On-site sewage systems	WFD data set, local authority inspections, walk-over survey, fluvial audit
Licensed outfalls	Walk-over survey, WFD data sets, competent licensing authority data sets
Riparian land-use	Walk-over survey, remote sensing, aerial photography over time, desk based study, fluvial audit
Catchment land-use	Walk-over survey, remote sensing, aerial photography over time, desk based study, fluvial audit
River engineering works (e.g. bridges, weirs),	Walk-over survey, fluvial audit
Building works in riparian zone	Walk-over survey, planning authority data sets, fluvial audit
Quarries	Walk-over survey, planning authority data sets, fluvial audit
Sheep dipping	Walk-over survey
Road drainage	Walk-over survey, fluvial audit
Power lines, wind turbines, utility crossings	Walk-over survey, fluvial audit
Fisheries management (e.g. over-fishing, stocking levels)	Liaise with fisheries authorities
Others – any other activity that could lead to sedimentation, reduction in water quality, changes in flow regime or physical destruction of habitat.	Walk-over survey, fluvial audit
Invasion by non-native species	Walk-over survey, fluvial audit

7 Information needed to assess plans or projects on rivers with *Margaritifera*

Table 8 is a checklist of questions that should be asked where short-term activities or long-term plans or projects are being assessed for potential damaging effects on a *Margaritifera* population. The same questions may also be helpful in diagnosing adverse impacts of current activities.

Table 8 — Checklist of questions that should be addressed to ensure that plans or projects do not damage *Margaritifera* populations

These questions apply to activities in the catchment, where they could affect the river.	
Aspect	**Question**
Mussel population	Will the plan or project result in humans, animals or equipment entering the river?
	Has the plan or project the potential to affect the annual reproductive cycle of the mussels?
	Will the plan or project increase the risk of pearl fishing, or direct disturbance to mussel beds?
Fish hosts	Has the plan or project the potential to affect the upstream or downstream migration of salmonids, including the timing of their movements?
	Has the plan or project the potential to affect the distribution or numbers of salmonid fish in the catchment?
	Has the plan or project the potential to affect the quality and distribution of salmonid spawning habitat?
	Has the plan or project the potential to affect the species composition of fish in the river?
Non-native species	Has the plan or project the potential to introduce or encourage the spread of non-native species to the river or catchment?
Water quality	Will there be a new outfall or changes to an established outfall entering the river?
	Will changes to land management have the potential to increase nutrient loading to the river?
	Will the plan or project result in the concentration of nutrients that are currently more dispersed?
	Will any aspect of the plan or project potentially affect the temperature regime of the river?
	Will the plan or project change the pH of the water?
	Will any fertilizers be needed to establish or continue the project?
	Will the plan or project result in more intensive use of the catchment?
	Will the plan or project result in greater wastewater production in the catchment (increased human or animal loading)?
	Will any pesticides be needed to establish or continue the project?
	Will any potentially toxic substances be used in or generated by the project that would be damaging if they were to enter the river?
	Has the plan or project the potential to change the water quality of the river in any

These questions apply to activities in the catchment, where they could affect the river.	
Aspect	**Question**
	other way?
Flow	Are there planned abstractions, or changes to abstraction levels or compensation flows?
	Will any planned changes in land management indirectly result in changes to the flow regime of the river?
	Is there any modification to drainage, or dewatering associated with the plan or project?
	Will any modification have the potential to change the stability conditions of the river bed?
	Has the plan or project the potential to affect the flow regime in the river in any other way?
Substrate quality	Has the plan or project the potential to increase fine sediment loading to the river or within the river?
	Could works affect the supply of coarse sediment to the river?
	Will the plan or project potentially lead to erosion or bare soil in the catchment or directly adjacent to the river?
	Is there any new drainage or drainage maintenance associated with the plan or project?
	Are any instream works planned (e.g. gravel removal)?
	Are any structures planned close to the river, within or across the river (e.g. installing flow deflectors)?
	Are there any bank reprofiling or bank engineering plans?
Riparian land-use	Has the plan or project the potential to affect the nature of the riparian habitat in the river?
	Has the plan or project the potential to affect the nature of the floodplain?
Vibration and drilling / blasting /noise	Has the plan or project the potential to affect the mussels or their hosts through damage arising from vibration and drilling / blasting /noise?

Annex A
(informative)

Background information on the environmental characteristics important for maintaining populations of *Margaritifera margaritifera*

NOTE This annex is to be read in conjunction with the guidance on monitoring given in Clause 5.

A.1 Fish hosts

A.1.1 Fish host species

Salmonid fish native to each catchment are essential hosts for freshwater pearl mussel. Glochidia released from the female mussel are viable for a short period, needing to find a host fish within approximately 1 or 2 days. Glochidia snap shut when they encounter the gills of their fish host.

Glochidia will attach to a range of fish species, but are quickly lost from species other than those that are suitable hosts. Suitable hosts in Europe are native salmonid fish, such as Atlantic salmon (*Salmo salar*) and brown trout (*Salmo trutta*). Studies have shown that, in general, survival of glochidia to become juvenile mussels is often restricted either to trout or to salmon, depending on the mussel population.

Densities of fish should be typical for the natural trophic status of each individual river. Where there are specific problems affecting fish populations, such as barriers to migration, high summer temperatures or acidification, low host numbers can be a limiting factor for mussel population recruitment. However, increased numbers of resident host fish, and increased numbers of species and individuals of other fish, can be indicative of nutrient enrichment and a decreased survival of juvenile mussels.

Fish need to be in close proximity to mussels (for encystment) and mussel habitat (for juvenile drop-off). It is unlikely that host fish move large distances and return to the mussel beds. However, the movement of encysted fish may help to expand the distribution of mussels.

A.1.2 Barriers to fish migration

Barriers to fish migration may be natural or artificial, physical or chemical. Natural barriers (such as waterfalls) that inhibit fish movement upstream and downstream should never be removed or circumvented. Artificial barriers may have adverse effects on *Margaritifera* populations. Impacts of barriers such as culverts on spawning tributaries can be severe. These are often recorded during hydromorphological surveys or fluvial audits. Where there are adverse consequences, attempts should be made to restore connectivity.

A.1.3 Host suitability and stocking practices

Even within host fish species glochidial development varies according to different genetic strains. A precautionary approach is needed to ensure that fish native to a catchment are not disrupted by the introduction of different genetic strains. A policy of artificially stocking fish in catchments with *Margaritifera* should be subject to an appropriate risk assessment, and if required should only be with the local native strain that is known to support the pearl mussels of that population. In general, supporting natural recovery of fish stocks is a better policy than artificial stocking, even with native salmonids. Care shall be taken in attributing the cause of genetic host incompatibility to poor recruitment, especially where mussel numbers are sparse.

A.2 Water quality

A.2.1 Phosphorus

Sustainable populations of freshwater pearl mussels are generally associated with extremely low levels of nutrients and are thus usually found within conditions of very low productivity. Phosphorus levels are a key concern in *Margaritifera* catchments, as this is normally the limiting nutrient for algal and plant productivity. While phosphorus in any of its forms is not directly toxic to pearl mussels, it is directly linked to eutrophication, resulting in several adverse effects for the species. These include increased organic sedimentation, colmation, oxygen depletion in the substrate, changes in fish communities, and increased fluctuations in pH values.

Slight increases in phosphorus can have adverse effects in naturally oligotrophic, upland rivers, whereas lowland rivers may be less susceptible to small changes, especially where these rivers are deep and shaded. Since iron oxides bind MRP under aerobic conditions and release it under anaerobic conditions, the maintenance of aerobic substrate conditions is also crucial for juvenile survival.

A.2.2 Nitrogen, including ammonia

Nitrogen, together with phosphorus, is important in enhancing productivity and elevated levels are an important factor in eutrophication. In general, nitrate levels in rivers with *Margaritifera* populations are lower than those in areas of intensive agriculture. It is important to note that while phosphorus is normally the limiting factor for eutrophication, excessive nitrogen is an indicator that a sensitive catchment is being too intensively managed and there are likely to be adverse, combined effects. In addition, at low oxygen concentrations, nitrate can become reduced to nitrite which is a potent neurotoxin.

As pH and temperature increase, the ratio of ammonium (NH_4^+) to ammonia (NH_3) is shifted towards higher NH_3 concentrations. Where nitrogen is present as ammonia it is particularly toxic to aquatic organisms. It is mainly generated from agricultural sources such as decomposing manure, but also from human wastewater treatment plants. In general, ammonia levels are extremely low in oligotrophic rivers.

A.2.3 BOD_5/dissolved oxygen

A supply of dissolved oxygen is important for *Margaritifera*, both in open water and in the juvenile interstitial habitat. Oxygen levels should remain high and never be subject to excessive fluctuations through daytime photosynthesis and night-time respiration.

An elevated BOD is usually caused by high levels of organic pollution, often downstream from inadequately treated wastewater from human or agricultural sources. Elevated BOD levels are particularly likely to have detrimental effects on juvenile mussel survival, where adequate oxygen levels are extremely important.

An excess of organic matter leading to elevated BOD in the substrate can also be detected by measuring redox potential (see 5.4.4).

A.2.4 pH

Margaritifera normally lives in naturally slightly acid to neutral waters, at a mean pH between 6.2 and 7.3 although each river has its own natural range. Acidification is damaging to mussel populations, as well as being a well-documented threat to their salmonid hosts. A lowering of pH directly influences pearl mussels through a gradual destruction of their calcareous shell, and through problems with regulation of acid–base mantle fluid homoeostasis. The strong interaction between low pH and more toxic forms of monomeric and polymeric aluminium may lead to gill tissue damage in host fish. In some rivers there is also a problem with pH being too high. During spring, when macrophytes and algae remove large quantities of CO_2 from the water, pH can rise above natural levels. Water treatment plants may also use alkaline materials for coagulation purposes, which can lead to unsuitably high pH levels downstream in excess of the river's natural range.

A.2.5 Calcium

The ecology of *Margaritifera* is particularly notable in that individuals can grow to very large sizes compared with other freshwater molluscs, building up thick calcareous valves, in rivers which have soft water with low levels of calcium. Their shell building is consequently very slow, and individuals in natural conditions are extremely long-lived. The relationship of *Margaritifera* with calcium is therefore important.

Where there is too little calcium, shell growth is reduced and thinner shells may result. Where heavy metals are elevated, calcium metabolism can be impaired resulting in brittle shell development. In extreme cases, calcium carbonate has been added to highly acidified catchments in order to increase pH to within the range suitable for *Margaritifera*. Any liming programme for conservation purposes should be strictly monitored.

Where calcium levels are artificially elevated, such as by agricultural liming or through the release of high-calcium sediment from quarrying, *Margaritifera* can be killed through direct toxic effects. Where high calcium levels persist, pearl mussel populations shift toward increased growth rates, leading to shortened life expectancy and, thus, loss of reproductive years. In these cases, the optimum life history strategy of very slow growth and extensive opportunities to reproduce is severely impaired.

Calcium levels are also linked with overall levels of hardness in river water, where hardness is a measure of both calcium and magnesium cations in water, or in many cases only calcium carbonate ($CaCO_3$). Many monitoring regimes measure total hardness rather than calcium levels.

A.2.6 Alkalinity

Alkalinity is a measure of the river's capacity to buffer against large changes in pH, and is generally determined by the amount of minerals present such as calcium and magnesium carbonate. Low alkalinity in relation to the calcium content indicates acidified water. Alkalinity provides an indication of a stream's biological productivity, so the relationship of *Margaritifera* with alkalinity is important.

Alkalinity is strongly linked with calcium and pH levels in waters with *Margaritifera*. Alkalinity should be part of the suite of parameters measured for conservation management as it helps provide an indication of possible sources of problems in the catchment. Elevated alkalinity in the absence of increased calcium levels can be an indicator of fertilizer or detergent runoff, and can be useful in isolating the sources of nutrient problems such as under-functioning wastewater treatment units, inappropriate farmyard management and small point sources of pollution [11].

A.2.7 Electrical conductivity

Electrical conductivity is the measure of how easily water can serve as a channel or medium for electricity. Water with higher levels of dissolved salts conducts electricity more readily than water with lower levels. Thus, conductivity is strongly related to total dissolved solids. The normal environment of *Margaritifera* is water of low conductivity.

Sources of dissolved salts that lead to elevated levels of conductivity include urban and rural run-off containing salt, fertilizers and organic matter. Land use related to high levels of Ec includes clearing of vegetation and the resultant rise in the water table, excessive irrigation, groundwater seepage and runoff containing dissolved solids from industry, sewage, agriculture and stormwater. Where pearl mussels occur close to the tidal limit of rivers that flow into the sea they may experience fluctuations in salinity during very high tides.

A.2.8 Temperature

Margaritifera rivers have a wide geographical range and therefore temperatures in mussel habitats vary regionally, with temperatures normally between 0 °C and 25 °C being recorded. However, prolonged high temperature is likely to be one of the limiting factors for the species. Maximum temperatures rarely exceed 20 °C through most of the species range, although in southern Europe maximum temperatures may be higher. Changes in the normal temperature regime can lead to oxygen depletion, difficulties with salmonid host survival, glochidial survival, or a change in development periods in the reproductive cycle (such as brooding periods and glochidial release), and in the long term can lead to faster growth levels in adult mussels [12] [13]. There are some indications that the mean temperature during summer is higher in streams without recruitment than in streams with recruitment, although in both cases mussels may be gravid. At the other extreme, water abstractions and low water flows during winter can reduce temperature to levels where ice forms. Mussels cannot tolerate freezing unless there is adequate water flow under the ice and through the substrate.

Impoundment of rivers, abstraction, flood relief, removal of bankside trees and other works that create ponding can have adverse effects on mussels through prolonged temperature increases in both the water column and the substrate where juveniles live, and these are likely to be exacerbated by climate change. The requirement for *Margaritifera* populations is for the prevailing temperature regime not to deviate from the conditions in which successful recruitment occurred.

A.2.9 Contaminants

Maintaining low levels of metals is important, particularly because of the acid nature of mussel rivers, where aluminium, zinc, lead, copper and other metals can become mobilized and damage all stages of mussels and their fish hosts (see 5.3.5 on pH). Metal pollution in the water or substrate can have acute or chronic effects. Unionoid mussels have been shown to bioaccumulate heavy metals and thus long-term, low concentrations of pollutants may cause problems over time.

Toxic pollution can have very serious and long-term effects on pearl mussel rivers. Organophosphates and synthetic pyrethroids used in sheep dipping are highly toxic to species that are considered to be much less sensitive to pollution than *Margaritifera*. Pearl mussels are too endangered to justify specific laboratory toxicity testing, but this should not be used as a reason to be ambiguous about the threat such pesticides present to *Margaritifera*. Evidence from surveys of glochidial and juvenile stages of unionoid mussels have demonstrated lethal effects from very low doses of chlorpyrifos and permethrin, the fungicides chlorothalonil, pyraclostrobin and propiconazole, and glyphosate. Of particular concern are the severe deleterious effects of these substances in combination with surfactant blends, as found in various commercial products. The combined product is often far more toxic than the individual ingredients. The use of rotenone in *Margaritifera* catchments upstream of, or close to, mussel beds should not be considered without an assessment of potential impacts on the mussels.

Endocrine disrupters can potentially affect reproduction in molluscs. Investigative monitoring may need to be undertaken where brooding is found to be impaired and there is significant sewage entering the river.

There is no evidence of damage to *Margaritifera* from radionuclides, although this may be due to the fact that they are an uncommon type of pressure. The lack of evidence should not lead to the conclusion that there would be no adverse effect on mussels from such a source. In general, a precautionary approach should apply where there is no information on the effects of potential pollutants, owing to the sensitivity of *Margaritifera*.

A.2.10 Turbidity, suspended solids

Turbidity and suspended solids, both chronic and episodic, represent one of the greatest threats to *Margaritifera* populations in many rivers. Levels of turbidity and suspended solids in rivers with sustainable *Margaritifera* populations are extremely low, with only minor peaks of very short duration occurring during periods of heavy rainfall or snowmelt. Suspended solids in the smaller size range (2 µm to 20 µm) rarely fall out of suspension, and therefore are a threat to adult mussels rather than juvenile mussels. As they are within the range of food particles ingested by filter-feeding mussels, excessive fine particles in this size range can stress mussels when separating food from inert matter. Suspended solids larger than 20 µm have adverse effects on adult and juvenile mussels, causing them to clam up (they close their shells tightly and do not filter water through their siphons) leading to severe stress and death. These larger particles can fall out of suspension and infiltrate the river-bed gravels, thus preventing oxygen exchange with the waters used by juvenile mussels. The effects of infiltration by fine sediment may be investigated by measurements of redox potential (see 5.4.4). Changes in the flow regime can result in lowered velocities and greater settlement of suspended solids on the substrate (see A.1); this combined effect needs to be considered.

A.3 Biotic indicators of water quality

A.3.1 Macroinvertebrates

The composition of the benthic macroinvertebrate community in rivers containing sustainable *Margaritifera* populations is considered to be a good indicator of the health of the mussel population, but the mere presence of *Margaritifera* adults is not. Most countries have their own individual systems for macroinvertebrate quality assessment, and these have been intercalibrated to produce the Ecological Quality Ratios (EQRs) used for WFD monitoring. Problems can occur where standard macroinvertebrate assessment is made in the fastest riffle areas, as this can give a false indication that river habitat quality is high. In general, the macroinvertebrate assemblage should fall within the highest class of river quality, and there should be no significant difference between the suite of species found in mussel beds and in riffles. This is because the highest EQR level is derived from the presence of invertebrates that have a high demand for oxygen, and these, like juvenile mussels, are lost when rivers are affected by sedimentation and eutrophication.

A.3.2 Diatoms

Diatoms sampled near to mussel beds can provide additional information on water, sediment and flow quality in pearl mussel rivers. A diatom index, an EQR or an ecological status class can be used as diagnostic tools. A dominance of motile diatoms in mussel beds is an indicator of excessive sedimentation of the substrate.

A.3.3 Filamentous algae

In oligotrophic conditions nutrient levels should never be high enough to allow dense mats of filamentous algae to grow. The persistence of filamentous algae is an indication that nutrient levels may be too high for sustainable *Margaritifera* populations, but may also indicate low flow problems. Chronic problems can lead to recycling of nutrients, organic sedimentation and colmation.

A.3.4 Macrophytes

While all rivers are different, and while macrophytes may provide a detrital food source, it is generally accepted that increases in vascular plant cover in rivers are often associated with increased trophic status [14] [15], which is undesirable in *Margaritifera* habitats. An increase in trophic status in oligotrophic rivers can lead to significant habitat changes, especially a change from a *Fontinalis*-dominated flora to one dominated by *Myriophyllum* and *Ranunculus* where nutrient pollution is accompanied by siltation. Dense macrophyte growth is indicative of poor *Margaritifera* habitat and provides conditions for trapping further silt and continued loss of habitat as a result of changes in flow, sediment and nutrient dynamics. The phosphorus that brought about an increase in macrophyte growth continues to be released and mobilized as the macrophytes decompose.

A.4 Hydromorphology

A.4.1 Flow

Flow and its characteristics are a complex part of river hydromorphology. This is because water interacts with the river-bed structure to produce a variety of habitats such as riffles, pools and glides, and within each of these is a complex mosaic of depths, velocities and directions of flow. Many different factors determine flow regimes, including rainfall patterns, catchment size, geology, gradient and land use. Flow requirements are usually described in terms of depth and velocity values for a given species, but as these vary significantly between river habitat types and according to the state of flow no single figure can be provided for what constitutes suitable habitat. The rise and fall of flow is also essential in influencing geomorphological processes, fish migration and other functions. Maintaining natural flow variability is essential, including enough high flows to cleanse river-bed substrates.

The issue of flow is further complicated by its interaction with pollution problems that cause the deposition of fine particulate matter or algal growth owing to increased nutrient levels. The most appropriate way of ensuring adequate flow in *Margaritifera* populations is to maintain a natural, abstraction-free regime in the sub-catchment influencing the population, and to manage the surrounding catchment in a manner that does not affect the natural flow regime (e.g. by avoiding artificial drainage, coniferous afforestation, wetland removal, installation of weirs and dams). Similarly, if there are plans or projects being proposed in a catchment with *Margaritifera*, their potential effects on the flow regime should be assessed fully.

For either of these scenarios (i.e. present damage or proposed development) sufficient information (such as discharge patterns and velocity) should be generated to be confident that the flow requirements of *Margaritifera* are not being compromised. Adult pearl mussels require enough water to cover them and a velocity at bed level that permits adequate filter feeding, while the substrate needs sufficient oxygen supply in the areas where juveniles are living. The area occupied by mussels should not be reduced by loss of adult or juvenile habitat through inadequate flows [16] [17].

The effects of climate change on the discharge pattern should be taken into consideration when assessing potential threats to river flow.

A.4.2 Physical habitat structure

Freshwater pearl mussel has very specific substrate requirements that are becoming increasingly rare. In general, rivers are dynamic ecosystems and areas where mussels live will change over time. However, a combination of stability, high exchange rates between free-flowing and interstitial water and a lack of infiltration of fine sediment are critical for juvenile survival. The stability of the substrate favourable for both adults and juveniles is manifested in a physical structure with a wide range of clast sizes from boulders to cobble to fine gravels and some sand pockets. These habitats are most often associated with riffle areas and plane beds. Adult mussels can often be found in fine sediments in deep pools but this may be caused by individuals becoming washed in.

Where mussels are dense, a wide range of clast sizes are present from gravel to boulders, and substrate is never well sorted. Mussels concentrate in gaps between boulders and cobble and are buried in gravel found between the larger clasts. Examples of poor habitat are scoured areas of even-sized gravel that are clean but unstable, and muddy or silty backwaters where sediment accumulates. In very dense populations, pearl mussels form part of the stable structure of the river bed. Interfering with these mussels (e.g. to measure population age structure) can cause a destabilization of the surrounding area. Therefore, investigative work in mussel habitat needs to be carried out with great care.

The physical characteristics of the interstices between the larger clast sizes from pebbles upwards are particularly important. The smallest sediment sizes need to be coarse enough to allow sufficient oxygen exchange between the open water and the substrate, where the juvenile *Margaritifera* are buried. Different sizes of sediment become mobilized depending on the energy of the water, with large floods resulting in considerable movement of clasts up to boulder size downstream. Therefore, a source of replenishment of all sizes of river bed substrate is very important.

Sedimentation and changes to the supply of coarse sediment can result in compaction, colmation or concretion of river beds. This affects oxygen supply and exchange within the substrate as well as the ability of juvenile mussels to burrow.

A.4.3 Substrate quality

Infiltration by fine sediments is one of the main causes of decline in juvenile recruitment. Intensification of the catchment can disturb the natural processes of erosion and sedimentation. Even small increases in fine sediments can cause serious problems. Inorganic silt enters the river through a number of sources. Bank erosion can reach high levels where unsustainable numbers of animals graze. Inorganic silt levels are high where land is ploughed or otherwise disturbed (e.g. clear-felling of forests, forest fires, road runoff, changes to river channels/ river engineering, abstraction), and can be transported and deposited in large quantities where drains run directly into the river. Organic silt is produced as a result of decaying macrophytes and algae where excessive nutrients have resulted in their growth in the river.

Where heavy rain leads to large inputs of fine sediment just before the release of glochidia, this may cause glochidia to be released while under-developed resulting in lower levels of fish infestation. Each time infiltration of river bed gravels occurs, juvenile mussels living in the substrate are likely to be killed, and in rivers with chronic sedimentation juvenile recruitment is rare and unsustainable. In these populations, considerable numbers of adult mussels may still be present; however, when the older mussels die they will not be replaced by a younger generation. If the habitat of the river bed is not restored, these populations will inevitably become extinct. The status of these populations is described as 'functionally extinct'.

Fine sediment, once introduced to a pearl mussel river, can continue to cause very serious effects in the long term. Direct ingestion of silt by adult mussels can lead to rapid death. Turbidity, particularly from fine peat entering the water, causes adult mussels to clam up, a response that provides protection against ingesting damaging fine particles. If the river water remains strongly turbid for a number of days, mussels can die from oxygen starvation, either from remaining closed, or from ingesting turbid water while stressed. During a time of year when water temperatures are high, oxygen depletion in the body occurs more rapidly, and mussels die more quickly.

In flood conditions, silt becomes remobilized, only to settle downstream – if this area is also a site of juvenile mussels a further kill can occur. Increases in fine material in the bed and suspended in the water column, and consequent changes in channel form, may affect mussels in many ways and at various stages in their life cycle. Sediment that infiltrates the substrate decreases oxygen supply in the juvenile habitat, which prevents recruitment of the next generation. The sediment subsequently provides a medium for macrophyte growth, a negative indicator in pearl mussel habitats. Macrophytes then smother the juvenile habitat even further, and the macrophytes trap more sediment, exacerbating the problem in the long term. Silt infiltration of river bed gravels can also have a negative effect on the species of fish that host the mussel glochidial stage.

Once fine sediment in excess of the natural rate enters a river it has potential to cause harm from the site of entry all the way to the sea. It is important that excessive fine sediment does not enter any part of the river upstream of *Margaritifera*, and that mussels are protected from damaging activities in all parts of the catchment.

Where there is excessive fine sediment entering a river, small sediment traps may be used to investigate their sources and infiltration rates.

A.4.4 Substrate stability

A *Margaritifera* population depends on habitat stability to persist at a location. Substrate stability is a function of the threshold of shear stress that shall be reached before entrainment can occur, referred to as critical shear stress. A particle will move only when the shear stress acting on it is greater than the resistance of the particle to movement. Particle entrainment will vary depending on its size, its size relative to surrounding particles, how it is oriented and the degree to which it is embedded; it also depends on the flow velocity.

High levels of shear stress can lead to adult mussels being carried into less favourable downstream habitats and result in additional energy expenses for the mussels to bury or re-anchor themselves in the substrate. Similarly, buried juvenile mussels can be seriously affected by high shear stress resulting in substrate mobilization, by abrasive effects from mobile gravel and sand, and by passive translocation into less favourable areas of sediment deposition.

On the other hand, high flows can help to flush out deposits of fine sediments and to re-create clean gravel banks and pockets. These in turn can become important habitats for juvenile mussels with high exchange rates between free-flowing water and interstitial zones. Critical shear-stress values are highly stream-specific with geomorphology, texture, bed roughness and grain shape being important variables. In addition, the stream-specific flow regimes (e.g. differences between high and low flows) and their interaction with the spatial arrangement of pool and riffle structures, and the spatial distribution and size of the mussel population, all influence the levels at which shear stress can exceed threshold levels. The dynamics and spatial patterns of shear stress over time are likely to be linked to the fluctuation of recruitment in many pearl mussel populations. The flow regimes and the spatial distribution of shear stress should be maintained close to the conditions at which pearl mussel reproduction has been found to be successful.

Where levels of shear stress have been altered, it is likely to be caused by instream modifications or changes in catchment management resulting in changed velocities at high and/or low flows. Impairment caused by increased uniformity of river-bed substrates can occur through blockage of coarse sediment transport into the river.

A.4.5 Trees and wood

Trees are an important part of riparian habitats and in *Margaritifera* rivers both living trees and dead wood help to create variation in riverine habitat and provide shelter, shading and, indirectly, food for their salmonid hosts. In general, therefore, woody material should not be removed from the water.

Non-native plantations (e.g. conifers, eucalyptus) in a catchment may exacerbate acidification and sedimentation, and when planted close to river banks may pose a significant threat to mussel populations through loss of needles into the river, or through falling onto mussel beds.

It is important that a fallen tree does not constitute a severe impact on suitable pearl mussel habitat. Where a tree falls directly onto a mussel bed it can cause damage by impeding water flow and causing river-bed changes through scouring or weir effects.

A.4.6 Instream modifications

Artificial structures placed instream or abutting directly along the river bank result in erosion where the hard material meets the softer sediment. This can lead to erosion down to bedrock and deep pools depending on the depth of substrate in the area. In rivers with *Margaritifera* more natural protection schemes should be considered for areas of bank erosion, such as planting appropriate trees in the riparian zone. Bridges planned in *Margaritifera* catchments at or upstream of pearl mussels should never have instream piers, and piers should never be so close to the bank to be in direct contact with the flowing water, even during high floods. Natural instream modifications include beaver dams, where the natural ranges of the species overlap. These are normally not a problem but under exceptional circumstances may have an adverse effect on important mussel beds.

Dredging the river channel containing a pearl mussel population causes the direct destruction of mussels where the *Margaritifera* habitat is directly affected. Serious damage may also occur where dredging upstream of mussels brings about the release of fine sediment and changes to the flow regime, resulting in changes in discharge and velocity to the mussel beds downstream. New drains, or cleaning drains upstream of mussel populations, are similarly damaging through modifying discharges and velocities and releasing fine sediment.

A.5 Biotic factors and other interactions

A.5.1 Pressures and interferences

The sections below describe a range of threats that are likely to have an adverse impact on pearl mussel populations. Methods for minimizing threats and managing them will be unique to every case and population. In all situations, education and awareness campaigns, publication and distribution of results and records should follow national guidelines for *Margaritifera*, particularly to safeguard against pearl fishing. Human pressure on catchments has resulted in a range of activities that increase the risk of habitat deterioration, loss of juveniles and, in extreme cases, the deaths of adult mussels. In most catchments effects are cumulative from a wide range of pressures arising from intensification of land use over time. Many pressures will be observed during the process of fluvial audit.

A.5.2 Human interference

In some rivers there has been a long tradition of pearl fishing and damage from this activity continues to be a threat in some countries. There is no method of extracting pearls from pearl mussels without damage and therefore pearl fishing is illegal across jurisdictions where pearl mussels have protection.

Where *Margaritifera* surveys, conservation efforts or impact assessments are being carried out, a balance needs to be achieved between raising awareness of this sensitive species and attracting attention from illegal pearl fishing. In countries with policies on the publication of pearl mussel distribution data these policies should be followed. Where pearl fishing is a problem one option is to restrict information on pearl mussel location to a 10 km^2 level. Conversely, where pearl fishing is not a threat at present an alternative approach is to publicize information on distribution to encourage local communities to help protect mussel populations.

Apart from pearl fishing, other direct human activities such as canoeing, instream angling and maintenance of fishing pools can also disturb mussels.

A.5.3 Invasive non-native species

Any conservation plan or development project should ensure that non-native species are not aided in their spread or in their ability to reach beds of mussels.

A.5.4 Non-native fish

Invasive non-native fish species may compete with native host fish, particularly in southern European rivers where some non-native species are top predators.

A.5.5 Non-native molluscs

Non-native species of molluscs can interfere with unionoids. The main species that have spread in Europe are zebra mussel (*Dreissena polymorpha*) and Asian clam (*Corbicula fluminea*). The former can smother the larger native mussels while the latter changes the nature of the substrate it colonizes, making it less suitable for native species.

Most non-native mollusc species typically occur in more calcareous waters than *Margaritifera*.

A.5.6 Non-native crayfish

There is evidence that invasive non-native crayfish e.g. American signal crayfish (*Pacifastacus leniusculus*) can damage a pearl mussel population through gnawing of shell edges, sometimes to the point where mussels can no longer clam.

A.5.7 Non-native plants

Exotic species of plants, both freshwater macrophytes and riparian species, can change the nature of the substrate and riparian habitats, respectively. Spread of macrophytes, whether native or exotic, increases fine sediment accumulation and is a negative indicator when within naturally suitable habitat for pearl mussels. The spread of riparian alien species such as Japanese knotweed (*Fallopia japonica*) or Himalayan balsam (*Impatiens glandulifera*) reduces species richness and can change the nature of river banks. Hydraulic effects of riparian vegetation are important in enhancing flow resistance and sediment cohesion within the riparian zone. Non-native plants that spread widely and colonize gravel banks can render them too stable compared with natural rates of gravel movement. Conversely, non-native species can prevent native woodland species from colonizing, causing river banks to become more susceptible to erosion in flood conditions. In addition, changes in bank vegetation may have effects on detritus and food composition for juvenile mussels.

The spread of exotic macrophytes and riparian plants should be recorded when monitoring *Margaritifera* populations. Any eradication should be done with care as removal of these species can also lead to instability and bank erosion, which can lead to sedimentation.

A.5.8 Non-native mammals

The muskrat (*Ondatra zibethicus*) was first introduced to Europe in 1905 from its native North American range, and has spread across continental Europe, where it is a voracious predator of large unionoids. Middens from muskrat show that it is capable of destroying hundreds of individual mussels once a bed of mussels is targeted.

While it is impractical to exterminate muskrat from a catchment, *Margaritifera* populations benefit from control of muskrat numbers.

The coypu (*Myocastor coypus*) is a suspected predator of *Margaritifera* in France.

Annex B
(informative)

Targets for assessing whether *Margaritifera* populations are in favourable condition

Protocols differ in different jurisdictions and the notes present possible approaches. The following targets should be met in order to achieve a sustainable *Margaritifera* population.

Table B.1 — Criteria and targets to achieve sustainable *Margaritifera* populations

Criterion	Target to pass	Notes
Numbers of live adults	No recent decline (best expert judgement)	Based on comparative results from the most recent surveys (e.g. monitoring transects).
Numbers of dead shells	< 1 % of population per year and scattered distribution	1 % (based on a 100 year lifespan) considered to be indicative of natural losses for survey sites and for the entire river population per year. Where > 1 % dead shells are found, an investigation into the cause should be carried out to assess whether it may be an exceptional natural event or an indication of an unnatural kill. The dead shells should be examined for freshness (by checking the colour of the nacre) to help assess the likelihood of a problem.
Recent recruitment (20 years or less)	At least 20 % of population ≤ 20 years old, based on a population with a typical life span of ~100 years. Individual targets should reflect the maximum age for each population. (Note: Sizes of mussels vary considerably by region and by river – it is advised to establish the size range of mussels under 20 years).	Quadrat-based assessment (e.g. 0,5 m^2 or 1 m^2 quadrats) shall be carried out in suitable habitat areas for juveniles if allowed, otherwise survey appropriate to the local region. Where digging for juvenile mussels is not part of a national protocol, the presence or absence of mussels under 10 years old should be used.
Very recent recruitment (5 years or less)	At least 5 % of population ≤ 5 years of age, based on a population with a typical life span of ~100 years. Individual targets should reflect the maximum age for each population. (Note: Sizes of mussels vary considerably by region and by river – it is advised to establish the size range of mussels under 5 years).	

Annex C
(informative)

Range of environmental conditions supporting sustainable populations of *Margaritifera*

The levels presented in Table C.1 have been derived from available studies across Europe. It is important that levels are not taken out of context and are appropriate to the location and river type for the population being studied. Note that these specific levels should not be interpreted as water quality targets but are presented to provide assistance in target-setting

Table C.1 — Range of environmental conditions supporting sustainable populations of *Margaritifera* (with referenced work on which levels are based)

Attribute	Levels	References
Phosphorus	A time series with consistently very low MRP, total P in conjunction with no evidence of eutrophication (e.g. algal growth). A mean or median MRP or total P level for all rivers with freshwater pearl mussel populations should be consistent with high status under the WFD, with the following exceptions: 1) Where the present phosphorus level is at a lower concentration than the high/good (H/G) boundary, it is recommended that this lower concentration should be maintained. 2) Where evidence shows that a phosphorus concentration lower than that of the H/G boundary has been recorded consistently in the past, it is recommended that future restoration should aim to achieve this lower level. 3) Where the H/G boundary level has been achieved but this has not resulted in the *Margaritifera* population reaching a sustainable condition, a lower P concentration may be required in future. Remarks: 1. Undetectable levels of MRP are not necessarily a guarantee of good health; if all the available phosphorus is being transferred into filamentous algae then it will not be detectable as MRP in open water. A combination of very low MRP with the absence of filamentous algae is considered to indicate nutrient levels conducive to *Margaritifera* populations in favourable condition. 2. Naturally occurring levels of phosphorus vary both from country to country and at a local scale. In general,	Moorkens, 2006 [18] Degerman, 2013 [19], Lois, 2015 [20], Killeen 2012 [21]

Attribute	**Levels**	**References**
	phosphorus in pearl mussel rivers in northern and western Europe (e.g. Norway, Sweden, Republic of Ireland, UK) are lower than those in central or southern Europe. The following are examples of studies where specific ranges of phosphorus have been associated with reproducing pearl mussel populations: Ireland [18], Sweden [19], Spain [20], UK [21]. Further work is under way in other parts of Europe; when available, the results of these studies should be used locally to assist in selecting the appropriate P targets for pearl mussel rivers. For example, pearl mussel populations in upland, low alkalinity rivers are especially sensitive to enrichment by phosphorus, and sustainable populations are associated with P levels at the higher end of high status.	
Nitrogen nitrate	Few data are available specifically on the relationship between pearl mussels and nitrogen nitrate. However, nitrate levels are a measure of the naturalness of the surrounding catchment, and comparatively low values of nitrate appear to be associated with sustainable pearl mussel populations: - 0,5 mg/l N in central Europe [22] - annual mean of 0,35 mg/l N in Spain [20] (derived from the four rivers in Spain with recruitment) - annual median of 0,125 mg/l N for Ireland [18] (derived from measurements for 560 sites in 126 rivers). - 0,338 mg/l N mean for a 16 year data set for England [21]	Bauer, 1988 [22] Lois, 2015 [20] Moorkens, 2006 [18] Killeen [21]
Nitrogen – ammoniacal N	Rivers in Ireland with sustainably reproducing *Margaritifera* populations have ammoniacal N levels never exceeding the detection limit of 0,01mg/l N [18]. Rivers in southern Europe with higher temperatures and higher productivity have higher levels of ammoniacal nitrogen, with means from 0,04 mg/l N to 0,05 mg/l N [23].	Moorkens, 2006 [18] Varandas et al., 2013 [23]
BOD/ dissolved oxygen	Elevated BOD_5 (>1,4 mg/l) has been linked with poor juvenile survival in Central Europe. Rivers with reproducing populations in the UK, Ireland and Spain have BOD_5 levels consistently < 1,0 mg/l. Dissolved oxygen levels in rivers with *Margaritifera* populations should be consistently high, where productivity is insufficient to produce extremes either of supersaturation or exhaustion of oxygen supply. Saturation levels should consistently reflect the natural range (i.e. be near to 100 %)	Bauer, 1988 [22] Unpublished data

Attribute	**Levels**	**References**
pH	Natural river conditions Rivers with sustainable recruitment have been reported with typical pH levels of: ≤ 7,5, Central Europe [22] ≥ 6,2, Sweden and Norway [24] ≤ 7,45, Portugal (single sample from each of two recruiting rivers) [25]	Bauer, 1988 [22] Degerman et al., 2009 [24] Reis, 2003 [25]
Calcium	Given the variation in calcium levels experienced by European *Margaritifera* populations, no calcium thresholds are proposed, but any artificial changes proposed to the calcium levels in a catchment, whether for direct conservation purposes, or indirectly through proposed development changes, should be thoroughly assessed and the implications for pearl mussel clearly identified.	
Contaminants	Owing to the high sensitivity of the species, WFD limits for priority substances and specific pollutants should be strictly adhered to [26].	WFD, 2003 [26]
Turbidity, suspended solids	Levels of turbidity, and suspended solids contributing to turbidity, are extremely low in rivers with sustainable *Margaritifera* populations with only minor peaks of very short duration occurring during periods of heavy rainfall. Whereas data on suspended solids are sparse, turbidity is more often measured. In oligotrophic catchments with low-intensity management turbidity levels have medians from undetectable (consistently 0 NTU) to < 0,3 NTU with peaks < 10 NTU [21]. Mean turbidity in 11 streams with recruiting pearl mussels was 0,96 NTU [27]	Killeen, 2012 [21] Österling et al., 2010 [27]
Biotic indicators of water quality – macroinvertebrates	While an EQR of 0,9 or higher denotes high ecological status under the WFD, invertebrate populations in rivers with sustainable pearl mussel populations generally have EQRs closer to 1,0 than 0,9, i.e. they are at the higher end of high status [28]. NOTE The standard methods for many macroinvertebrate metrics require kick-sampling in riffles with higher flow and more unstable habitat than where mussels are found. In that case, EQR results may be higher than they would have been in mussel habitat. It is important to find a realistic balance between obtaining data relating to *Margaritifera* habitat, and disturbing dense beds of mussels by kick sampling.	European Commission, 2007 [28]

Attribute	Levels	References
Diatoms	In the absence of specific data, as an interim measure WFD high status should be considered as the requirement for *Margaritifera* populations [29], [30], as was consistent with a study of Irish rivers with recruiting mussels [31].	WFD UKTAG, no date [29], Kelly et al., 2006 [30] Department of the Environment, Heritage and Local Government (Ireland), 2010 [31]
Filamentous algae	In Ireland, mussel habitat in oligotrophic rivers has been shown to have filamentous algal cover of < 5 % and this level is used in regulation [31], [32].	Department of the Environment, Heritage and Local Government (Ireland), 2010 [31] Government Publications (Ireland), 2009 [32]
Macrophytes	Rooted macrophytes should be absent or rare. In Ireland, mussel habitat in oligotrophic rivers has been shown to have macrophyte cover of < 5 % and this level is used in regulation [31], [32]. In southern and central European rivers, higher macrophyte cover may occur	Department of the Environment, Heritage and Local Government (Ireland), 2010 [31]Government Publications (Ireland), 2009 [32]
Substrate quality	Redox potential should indicate oxic conditions at all times, with temperature- corrected values < 300 mV typically indicating anoxic conditions. There should be no pronounced difference (typically < 20 %) between open water and interstitial water at 5 cm depth [7]. Silt plume should be small and quickly dissipated [33]	Geist and Auerswald, 2007 [7] North South 2 Project, 2009 [33]
Fish	Functional pearl mussel habitats are typically characterized by a low number of fish species and comparatively low densities of fish. Examples: The mean density range of brown trout in European pearl mussel streams (functional and non-functional) found by Geist et al. (2006) [34] was 29 individuals per 100 m^2, with an average of 31 % 0+ fish. Due to the low productivity in functional pearl mussel populations, the density of hosts was lower compared with non-functional populations – typically fewer than 15 individuals per 100 m^2. Other authors have also proposed brown trout densities in the order of 10 individuals (Ziuganov et al., 1994 [35]) or lower (Degerman et al., 2013 [19]), and 10 to 20 individuals per 100 m^2 (Bauer et al., 1991 [36]). Information for a number of years is necessary as fish numbers fluctuate naturally over time. At least five 0+ brown trout per 100 m^2	Geist et al., 2006 [34] Ziuganov et al., 1994 [35] Degerman et al., 2013 [19] Bauer et al., 1991 [36]

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