COMMISSIONED REPORT

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A literature review of the water quality requirements of the freshwater pearl mussel *(Margaritifera margaritifera)* and related freshwater bivalves

(ROAME No. F01AC609d)

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SCOTTISH NATURAL HERITAGE

COMMISSIONED REPORT Summary

A literature review of the water quality requirements of the freshwater pearl mussel *(Margaritifera margaritifera)* and related freshwater bivalves

Commissioned Report No. 084 (ROAME No. F01AC609d)
Contractor: Dr. Mark Young, University of Aberdeen
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Background

A literature search from 1981 to present has shown that little work has been carried out on the chemical parameters that influence the survival and distribution of the freshwater pearl mussel *(Margaritifera margaritifera)* and not much more on related unionid mussels, either in Europe, North America or elsewhere. What has been achieved is difficult to interpret, because it is clear that many factors influence the effects observed; including 'internal' factors, such as the stage in the life cycle, or the physiological status of a mussel, and external ones, such as pH or oxygen levels. There is an urgent need for more critical investigation of this topic.

Main findings

- Juvenile mussels and glochidia are often more susceptible than adults to poor water conditions.
- Interstitial water chemistry is of crucial importance to juvenile mussels but only one study has been carried out on the requirements of juvenile freshwater pearl mussels and apparently none for other species.
- From general studies in Germany and Scotland, it has been possible to assemble a list of water quality objectives, allowing *M. margaritifera* to survive and reproduce, however, these are neither certain nor comprehensive.
- It appears that there are no similar objectives for other Unionidae.
- For *M. margaritifera* it is known that unnaturally high levels of nutrients, conductivity, nitrates, phosphates, BOD, metals and some pesticides are detrimental, as well as unnaturally high and low pH.
- It is also known that metals are toxic to many other mussels and that the decreasing order of toxicity is Cu>Cd>Zn and Ni. Cu is especially toxic to Mollusca.
- Eutrophication is widely regarded as very damaging to mussel populations but few studies have quantified this problem.
- Biocides have frequently been shown to be toxic to mussels of all species, but with great variation in the level of toxicity in relation to the species of mussel concerned, life cycle stage, physiological status, and water quality variation.
- Careful and realistic studies are urgently needed to set water quality objectives for mussels and to quantify the field effects of potentially toxic factors.

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1 INTRODUCTION AND AIMS

The freshwater pearl mussel (*Margaritifera margaritifera*) is endangered throughout its holarctic range (Young *et al.*, 2001), as are many related Unionid mussels, especially in North America, which is the centre of radiation of the group. Despite numerous studies on the ecology of these mussels (eg Hastie *et al.*, 2000) very little is known of the water quality requirements of these species. The purpose of this short contract is to carry out a literature search for information on this topic. The scope of the review is set out in Annex A of the contract and is as follows:

> "Available literature in the UK on the tolerance of freshwater bivalve molluscs to a variety of nutrients, metals and chemicals should be reviewed and collated. Available literature from North America should also be reviewed, but due to the likely extent of literature the search should be confined to freshwater Unionid molluscs. Where information exists, reference from available European literature should also be examined.
>
> The chemicals for which available information should be collected should be sheep dips (including pyrethroid chemicals), herbicides and pesticides approved for use in and near water (incl. Asulam and glyphosate), metals (incl. copper)…"

A later instruction called for the inclusion of information on Bronopol.

The finances available for this contract limited the work to eight days and this has necessitated a strict restriction to the extent of the literature review and the exclusion of selected topics, as set out in the Methods.

In view of the above, the aim of this contract is to review the effect of selected water quality parameters on *Margaritifera margaritifera* and related freshwater bivalves.

2 METHODS USED IN THE REVIEW

Information on the general water quality requirements of *M. margaritifera* was gleaned from Purser (1985), Bauer (1988) and Oliver (2000). No further attempt has been made to collate the minor, mostly anecdotal information in recent studies on the general ecology of *M. margaritifera* and there have been no recent studies specifically on water quality. Data on preferred flow regimes, substrate characteristics and other physical factors are beyond the scope of this review.

Information on the responses of other mussels to various specific chemical parameters was gained from literature searches carried out principally using 'Web of Science' WoS (Copyright 2003 Institute for Scientific Information). Further data on the effects of metals on Unionid mussels was obtained from Naimo (1995). Web of Science searches the Science Citation Index from 1981 to present, which includes over 17 million references. It does not include access to the 'grey' literature, such as Environment Agency internal reports, and time constraints did not permit a specific search for such sources.

A decision was made to exclude laboratory studies (unless directly related to field conditions), and the use of mussels as biomonitors. Furthermore the extensive literature on *Dreissena* and *Corbicula* was also excluded.

Literature searches from WoS are conducted using key words linked by Boolean logic operators and the key word searches made here are set out in Table 1, together with the number of papers recovered by each search and the number of these that included relevant data. There is considerable overlap between the more general searches, and they are very wide-ranging, so it is believed that most, if not all, relevant references have been found. However, there may be brief reference to water quality issues in papers whose title does not indicate this and these have not been included. Selected references are referred to in the text, with a wider listing in the Bibliography.

Table 1 The key words used in the literature searches, as well as the number of references and relevant studies recovered by each search (Boolean operators are shown in capitals)

Key words used	Number of references recovered	Number of relevant references used
Taxonomic key words		
Margaritifera	183	4
Unionidae OR Margaritifera OR Anodonta OR Unio OR Pseudanodonta	847	54
Freshwater AND Bivalv*	532	All present in lists above
Pisidium AND Sphaerium	16	4
Key words for parameters		
(Eutrophication OR nutrients) AND Bivalv* AND freshwater	17	0
Eutrophication AND mussels	40	All present above
Metals AND freshwater AND (Unionidae OR Margaritifera)	12	All present above
Asulam	112	0
Sheep AND dip	79	0
Glyphosate AND freshwater	8	0
Bronopol	80	0
(Herbicide OR pesticide) AND freshwater	154	4
Rotenone OR piscicide OR Malathione AND (freshwater)	14	2

3 GENERAL WATER QUALITY REQUIREMENTS FOR *M. MARGARITIFERA*

There have been generalised studies of the habitat requirements for *M. margaritifera* on many occasions, typified by Boycott (1936), but the data included in such studies are too vague to be useful. Very few studies have provided detailed data and this remains a crucial gap in our knowledge of the requirements of this mussel.

Purser (1985) recorded the value of chemical parameters from mussel rivers from many parts of Britain, but concentrated on the north west of Scotland, where most viable mussel populations remain. He relied mainly on data from River Purification Boards (now incorporated into the Scottish Environment Protection Agency), and the data are rather generalised and are expressed as annual means. However, he did provide a clear view of the normal range of values in mussel rivers for commonly measured parameters. He also showed conclusively that values varied throughout Britain, implying that mussels show local adaptation and therefore that standards from one area may not apply elsewhere. Bauer (1988) also provided data, this time from Germany, which were broadly similar to those of Purser (see Section 4.2 below).

Buddenseik *et al.* (1993) measured the interstitial water chemistry of rivers in central Germany, believing rightly that these are the conditions that would be encountered by juvenile mussels, but their measurements have not been repeated elsewhere, and they noted that the rivers concerned were certainly somewhat enriched. They included sites for *Unio crassus* and *U. tumidus* as well as *M. margaritifera*, and their main conclusion was that, at sites where juvenile mussels were present, there was little difference between the water chemistry in the water column and the substrate interstices. They considered that this indicated that juvenile mussels can only thrive where the interstitial spaces are open, allowing free movement of water amongst them.

Oliver (2000) produced recommendations for favourable Water Quality Objectives for *M. margaritifera*. He consulted Bauer (1988) and other mussel workers but finally based his water chemistry data mainly on Purser's values from northern Scotland. These are shown in Table 2. He noted the caution needed in the use of these data but recommended that they would provide a 'safe' level. They agree broadly with the 'best' qualities listed by SEPA in its assessment of the pollution status of Scottish rivers and with values suggested for salmon rivers. However, the value for BOD (<1.3mg/l) seems to be rather high, compared with typical values for many clean Highland rivers and this needs further clarification.

Table 2 The Water Quality Objectives for *M. margaritifera* suggested in Oliver (2000) and Bauer (1988)

Specific Attribute	Target (Oliver, 2000)	Target (Bauer, 1988)
Nitrate	<1.0 mg/l	<0.5 mg/l
Phosphate	<0.03 mg/l	<0.03 mg/l
pH	6.5-7.2	N/A
Conductivity	<100 μs/cm	<70 μs/cm
Calcium	<10 mg/l Ca CO_3	2 mg/l
BOD	<1.3 mg/l	1.4mg/l
Dissolved oxygen	90-110% saturation	N/A

There are a small number of *M. margaritifera* populations that live in very different water conditions, notably in the calcium-rich River Nore in Ireland. No attempt has been made to include these here.

4 THE EFFECTS OF SELECTED CHEMICAL PARAMETERS

4.1 The factors that influence the toxicity of chemical parameters to freshwater mussels

There is a wide range of factors that influence the effect of varying water quality on mussels in field conditions and these must be borne in mind when considering likely impacts. These are listed here, with only very brief explanation, but extra detail for many is included in Naimo (1995).

a) Adults, glochidia and juvenile mussels vary in sensitivity. Juveniles are often the most sensitive.

b) Well fed mussels are less sensitive than starving mussels and it is likely that other physiological states, such as gravidity, also affect sensitivity.

c) There is known to be regional adaptation to different water quality conditions.

d) Pollutants may be either bound in the substrate, or onto suspended particles, or may be dissolved and their respective effects may differ greatly.

e) Some pollutants, such as metals, may be present in toxic or non-toxic states, depending on other factors such as pH.

f) As well as pH, other factors, such as oxygen levels and water hardness, may influence toxicity.

g) Pollutants may be taken up by plankton, before being filtered by mussels, so leading to a 'multiplier' effect.

h) When studying toxicity in the laboratory it makes a great difference whether static or through-flow water regimes are used.

i) Acute and chronic effects need to be considered.

j) Lethal and sub-lethal effects must be considered. Sub-lethal effects may include reduced valve opening; reduced filtering; reduced foot movement; and reduced glochidial production.

k) Assessing death in mussels is difficult. Gaping shells and lack of response to stimuli are a guide.

l) Adult mussels reduce uptake of toxins in the short term by valve closure.

m) Toxicity of metals may be reduced by the binding of these metals in protein complexes called metallothioneins.

n) Toxicity may be reduced by incorporation of toxins into crystalline concretions, which are Ca based in freshwater mussels.

o) The site of accumulation of toxins in mussels is toxin-specific but most accumulate in the gills and mantle cavity.

p) Many mussels accumulate materials in a direct relationship with environmental concentrations and are used as biomonitors. However, there is often a threshold above which no more is accumulated.

4.2 The effects of chemical parameters on *Margaritifera margaritifera*

There are few studies that specifically link pollutants with the survival of populations of *M. margaritifera*, except those of Bauer and his co-workers in Germany in the 1970s and early 1980s. An early link was established between the failure of juvenile mussels to establish in the substrate and nutrient enrichment and it was also noted that Cr was toxic to *M. margaritifera* (Bauer *et al.*, 1980). This was explored further and Bauer (1988) summarises the findings. Mussel mortality was found to rise with increasing nitrate values, with 'natural' mortality levels observed only at sites where nitrate concentration was <0.5 mg/l and increased mortality at sites where nitrate values were c.1.5 mg/l. No correlation was found between fertility of adults, the survival of glochidia or the numbers of encysted glochidia on their fish hosts and enrichment, suggesting that the effect was the failure of juvenile settlement into the substrate.

Bauer also found that values of BOD, Ca, PO_4 and conductivity were higher in rivers which held only old mussels than they were at sites where juveniles were also present.

Based on his findings, Bauer (1988) suggested that in Germany reproducing populations of *M. margaritifera* could only occur when enrichment was not affecting its rivers. He suggested target values for various parameters (see Table 2) and these are very similar to those listed by Oliver (2000). In addition, he notes that metal pollution must be absent.

Many other papers refer in general terms to the adverse effects of pollutants on *M. margaritifera* but most do not quote precise values for the pollutants. Young *et al.* (2001) summarise the status of *M. margaritifera* and note the suggested causes of decline for each country which it inhabits. Eutrophication is proposed as a cause of decline for 11 of the 15 countries included and industrial pollution for nine of them. Acidification is also a commonly suggested problem in countries lying down-wind of major industrial areas.

4.3 The effects on related Unionid mussels

4.3.1 Metals

Naimo (1995) summarises what was then known about the effects of metals on mussels and she includes many topics of general relevance, as well as some specific examples. Her general conclusions are reviewed here, followed by reference to some more recent studies.

As Naimo (1995) makes clear, some metals can be acutely toxic to unionid mussels, despite the fact that they can close their shells and stop filtering, to reduce the intake of polluted water. Glochidia that are free living and juvenile mussels may be much more susceptible than adults, and their loss will prevent a mussel population reproducing successfully. However, sub-lethal effects on adults are also likely to be important. Fortunately, modern advances in analytical techniques have demonstrated that concentrations of metals in rivers are often orders of magnitude lower than once thought and are generally in the µg/l range.

Despite their clear toxic effect, few comprehensive studies on the effects of metals on mussels have been carried out, especially in realistic field conditions. There have been laboratory projects examining effects on enzyme functioning and DNA integrity, but almost none that have measured chronic sub-lethal responses. Consequently, we are a long way from being able to predict the real effect of an actual metal pollution incident.

Naimo also lists the EC_{50}s for Cu, Cd, Ni and Zn, determined in a range of conditions. The varied conditions make it almost impossible to compare and summarise the results but for juvenile mussels, in moderately hard water, the respective EC_{50}s are as follows:

80 µg/l, 150 µg/l, 450 µg/l and 500 µg/l.

Further studies have confirmed Naimo's review, as illustrated by the following selected references.

Hansten *et al*. (1996), Pynnonen (1995) and Huebner and Pynnonen (1992) all confirm that the toxicity rank is as follows: Cu>Cd>Zn, and they all also note that free glochidia and juveniles are far more susceptible than adult mussels. However, many other factors influence toxicity, including synergistic and antagonistic effects between metals.

Masnado *et al*. (1995) studied a mixed mine effluent and found synergistic toxicity but also noted that adult *Anodonta imbecilis* were less susceptible than other invertebrates. However, McKinney and Wade (1996) showed that juvenile *Anodonta imbecilis* were actually more susceptible to mixed metal effluents than most other invertebrates.

pH has a particularly marked influence. Toxicity of most metals is increased at low pH and Al is generally only released into solution to become toxic in acid conditions. As well as this, low pH is itself toxic and Makela and Oikari (1992) found that *Anodonta anatina* could tolerate pH 4.8 for short episodes but was killed by pH <3. Pynnonen and Huebner (1995) showed that as acidity increased, so normal patterns of valve opening and filtering were progressively disrupted.

Iron is generally not very toxic to mussels and Milam and Farris (1998) found very little effect when they tried to use *Quadrula quadrula* as a biomonitor at the site of an Fe polluted mine discharge. Pb and Hg are both much more toxic. Beckvar *et al*. (2000) showed that *Elliptio complanata* grew more slowly as Hg levels increased in a river in Massachusetts and Black *et al*. (1996) observed damage to DNA when *Anodonta grandis* was exposed to 50µg/l Pb, a concentration below that which produced acute symptoms in the mussels.

Most studies show that Cu has a high toxicity to mussels. In addition to the direct toxicity studies quoted by Naimo (1995), Jacobsen *et al*. (1997) found that glochidia of *Lampsilis* sp. were killed by levels of 26-48 µg/l Cu, whereas glochidia still in the marsupia were almost immune to the toxic effect. Doyotte *et al*. (1997) used 30 µg/l exposure on *Unio tumidus* and showed that the rapid toxic effect was accompanied by much reduced enzyme function.

Naimo's conclusions remain valid and indicate a serious need for further studies. "[There are]…several areas where information on the effects of metals on freshwater mussels is sparse. The largest data gaps pertain to the effects of sub-lethal contaminant concentrations on processes such as reproduction and growth. Furthermore, the majority of data … are laboratory derived. There are few data on the effects of existing metal concentrations on freshwater mussels in the field."

4.3.2 Nutrients

Although there are many general studies on the status of unionid mussels, which refer to the problems caused by nutrient enrichment, there are apparently none that include specific levels of nutrients that can be related to mussel decline. This lack of information is very worrying, because it is clear that eutrophication is regarded as one of the main problems facing mussels. In consequence, it is desirable to know levels of nutrients, such as phosphate, that give rise to concern in various rivers. Since each river will differ in its 'natural' nutrient loading, and there are mussel species adapted to many different levels of productivity, it is likely that 'problem' nutrients levels will be site specific. This makes the task of establishing recommended safe levels very complicated. Almost all that can be said is that, as for *M. margaritifera* above, mussels are generally very sensitive to the clogging of interstitial spaces, particularly when in their buried juvenile phase. Consequently, even mild enrichment is likely to be a serious problem for almost all mussel communities. The many general studies all support this view.

4.3.3 Piscicides

Hart *et al.* (2001) compared the mussel fauna of a river in Minnesota, that had received **rotenone** treatment to remove coarse fish, with that of nearby untreated rivers. No pre- and post-treatment study was made. They found that there was no real difference between the mussel communities in treated and untreated rivers. Some dead mussels were found in all rivers, and juveniles, as well as old mussels, were found throughout. They concluded that there had been no measurable effect.

Similarly Waller *et al.* (1998), in laboratory tests, found no acute toxicity to *Obliquaria reflexa* and *Fusconaia flava* from exposure to the **piscicide 3-trifluoromethyl-4-nitrophenol (TFM)** at levels used to control sea lampreys, which are a serious pest species in the American Great Lakes, where they threaten native fish species. However, they speculated that there may be long-term sub-lethal effects.

4.3.4 Herbicides

There are two studies on the effects of specific herbicides that are relevant. Cheney *et al.* (1997) observed the effect of **atrazine, 2,4-D, paraquat** and synthetic **estrogen** on excised gill tissue from *Elliptio complanata* and found that there was some alteration of function, including inhibited metabolism. However, they did not relate this to likely field effects.

Nordone *et al.* (1998) studied **acrolein**, a herbicide used in canals at between 1-15ppm. In laboratory tests they found no effects on *Elliptio complanata* at typical field concentrations.

4.3.5 Pesticides

Keller (1993) showed that some pesticides were acutely toxic to juveniles of a typical unionid mussel, *Anodonta imbecilis*, at concentrations considered typical for field conditions. She exposed the juveniles in static tests for 48h (7d for a sewage effluent sample) at a range of concentrations of selected organic compounds and two pesticides. LC_{50}s ranged from 35 mg/l for methanol and acetone to <1 mg/l for the pesticides, chlordane and toxaphene. However, many factors influence the actual toxicity of biocides, especially including the nature of static testing and other studies have differed in their findings.

Koppar *et al.* (1993) also observed toxic effects, in this case of the pesticide **methyl parathion** on *Parreysia favidens* and *P. caerulea*, but this was in laboratory tests at typical field concentrations, with *P. caerulea* proving most sensitive.

In contrast, Cossu *et al*. (2000) did not find acute lethality when *Unio tumidus* was transplanted into French rivers polluted by a mixture of **PAH, PCB** and **organochlorine pesticides**. However, they did find evidence of reduced enzyme function, which is likely to have long term consequences.

Moulton *et al*. (1996) studied two **pesticides**, namely **aldicarb** and **acephate**, and their effect on *Elliptio complanata*. At 21°C they found no direct mortality at field concentrations, but did find sufficient physiological changes to suggest chronic effects. At 30°C there was acute mortality at 5mg/l. They suggested that monitoring changes in the activity of cholinesterase was a useful way of detecting sublethal effects.

Doran *et al*. (2001) also suggested using cholinesterase activity as a measure of the effect of pesticides, following a study showing reduction in activity in *Amblema plicata* exposed for seven days to typical field concentrations of **chlorpyrifos**.

Keller and Ruessler (1997) measured the toxicity of **malathion** to glochidia, juveniles and adults of three mussel species at differing pHs, levels of water hardness and temperatures and found that adults were the most tolerant. However, they also found that normal field levels of malathion should not be lethal to these mussels.

In contrast Kontreczky *et al*. (1997) observed that **deltamethrin** disrupted the filtering activity of *Anodonta cygnea* at typical field concentrations, even if it was not acutely toxic, and so they speculated that this might lead to serious sub-lethal effects on mussel communities.

Although not a pesticide, **fluoranthene** is a common pollutant in freshwaters, being derived from all sorts of combustion processes and Weinstein (2001) showed that it was highly toxic to the glochidia of *Utterbackia (=Anodonta) imbecilis*.

4.3.6 Asulam

There are apparently no studies that report the effect of **Asulam** on freshwater bivalve molluscs.

4.3.7 Bronopol

There are apparently no studies that report the effect of **Bronopol** on freshwater bivalve molluscs.

4.3.8 Glyphosate

There are apparently no studies that report the effect of **glyphosate** on freshwater bivalve molluscs.

4.3.9 Sheep dip

There are apparently no studies that report the effect of **sheep dipping** on freshwater bivalve molluscs. However, there have been occasional anecdotal reports of mussel kills following sheep-dip disposal in Scotland (eg Cosgrove and Young, 1998).

4.4 The effects on *Pisidium* spp. and *Sphaerium* spp.

Heinonen, Penttinen and their co-workers are the only scientists to have studied the effects of environmental chemicals on *Pisidium* spp. and *Sphaerium* spp.. Penttinen *et al*. (1996) noted that *Sphaerium* could shut its shell valves and stop filtering in the presence of 2,4,5-trichlorophenol (TCP), so reducing its toxicity, but eventually there is a toxic effect. In hypoxic conditions the length of shell closure is even longer (Heinonen *et al*., 1997), further reducing toxicity. In *Pisidium* spp. shell closure also occurs and the toxicity of TCP, bisphenol and benzopyrene varies directly with the temperature, although infection with a digenean parasite did not influence the response (Heinonen *et al*., 2000, 2002).

5 CONCLUSIONS

Little work has been carried out on the chemical parameters that influence the survival and distribution of the freshwater pearl mussel (*Margaritifera margaritifera*) and not much more on related unionid mussels, either in Europe, North America or elsewhere. What has been achieved is difficult to interpret, because it is clear that many factors influence the effects observed; including 'internal' factors, such as the stage in the life cycle, or the physiological status of a mussel, and external ones, such as pH or oxygen levels. There is an urgent need for more critical investigation of this topic.

Despite the above, some conclusions are valid;

- Juvenile mussels and glochidia are often more susceptible than adults to poor water conditions.
- Interstitial water chemistry is of crucial importance to juvenile mussels but only one study has been carried out on the requirements of juvenile freshwater pearl mussels and apparently none for other species.
- From general studies in Germany and Scotland, it has been possible to assemble a list of water quality objectives, allowing *M. margaritifera* to survive and reproduce, however, these are neither certain nor comprehensive.
- It appears that there are no similar objectives for other Unionidae.
- For *M. margaritifera* it is known that unnaturally high levels of nutrients, conductivity, nitrates, phosphates, BOD, metals and some pesticides are detrimental, as well as unnaturally high and low pH.
- It is also known that metals are toxic to many other mussels and that the decreasing order of toxicity is Cu>Cd>Zn and Ni. Cu is especially toxic to Mollusca.
- Eutrophication is widely regarded as very damaging to mussel populations but few studies have quantified this problem.
- Biocides have frequently been shown to be toxic to mussels of all species, but with great variation in the level of toxicity in relation to the species of mussel concerned, life cycle stage, physiological status, and water quality variation.
- Careful and realistic studies are urgently needed to set water quality objectives for mussels and to quantify the field effects of potentially toxic factors.

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