A literature review of freshwater mussel survey methods and techniques used in deep, turbid environments

Rebecca M. L. Dolson, Kelly A. McNichols-O'Rourke and Todd J. Morris

Ontario and Prairie Region
Fisheries and Oceans Canada
867 Lakeshore Road
Burlington, ON L7S 1A1

2023

Canadian Technical Report of
Fisheries and Aquatic Sciences 3505

Fisheries and Oceans Canada | Pêches et Océans Canada

Canada

Canadian Technical Report of Fisheries and Aquatic Sciences

Technical reports contain scientific and technical information that contributes to existing knowledge but which is not normally appropriate for primary literature. Technical reports are directed primarily toward a worldwide audience and have an international distribution. No restriction is placed on subject matter and the series reflects the broad interests and policies of Fisheries and Oceans Canada, namely, fisheries and aquatic sciences.

Technical reports may be cited as full publications. The correct citation appears above the abstract of each report. Each report is abstracted in the data base *Aquatic Sciences and Fisheries Abstracts*.

Technical reports are produced regionally but are numbered nationally. Requests for individual reports will be filled by the issuing establishment listed on the front cover and title page.

Numbers 1-456 in this series were issued as Technical Reports of the Fisheries Research Board of Canada. Numbers 457-714 were issued as Department of the Environment, Fisheries and Marine Service, Research and Development Directorate Technical Reports. Numbers 715-924 were issued as Department of Fisheries and Environment, Fisheries and Marine Service Technical Reports. The current series name was changed with report number 925.

Rapport technique canadien des sciences halieutiques et aquatiques

Les rapports techniques contiennent des renseignements scientifiques et techniques qui constituent une contribution aux connaissances actuelles, mais qui ne sont pas normalement appropriés pour la publication dans un journal scientifique. Les rapports techniques sont destinés essentiellement à un public international et ils sont distribués à cet échelon. II n'y a aucune restriction quant au sujet; de fait, la série reflète la vaste gamme des intérêts et des politiques de Pêches et Océans Canada, c'est-à-dire les sciences halieutiques et aquatiques.

Les rapports techniques peuvent être cités comme des publications à part entière. Le titre exact figure au-dessus du résumé de chaque rapport. Les rapports techniques sont résumés dans la base de données *Résumés des sciences aquatiques et halieutiques.*

Les rapports techniques sont produits à l'échelon régional, mais numérotés à l'échelon national. Les demandes de rapports seront satisfaites par l'établissement auteur dont le nom figure sur la couverture et la page du titre.

Les numéros 1 à 456 de cette série ont été publiés à titre de Rapports techniques de l'Office des recherches sur les pêcheries du Canada. Les numéros 457 à 714 sont parus à titre de Rapports techniques de la Direction générale de la recherche et du développement, Service des pêches et de la mer, ministère de l'Environnement. Les numéros 715 à 924 ont été publiés à titre de Rapports techniques du Service des pêches et de la mer, ministère des Pêches et de l'Environnement. Le nom actuel de la série a été établi lors de la parution du numéro 925.

Canadian Technical Report
of Fisheries and Aquatic Sciences 3505

2023

A literature review of freshwater mussel survey methods and techniques used in deep, turbid environments

Rebecca M. L. Dolson, Kelly A. McNichols-O'Rourke and Todd J. Morris

Fisheries and Oceans Canada
Great Lakes Laboratory for Fisheries and Aquatic Sciences
867 Lakeshore Rd., P.O. Box 5050
Burlington, ON
L7S 1A1
Email: Todd.Morris@dfo-mpo.gc.ca

Cat. No. Fs97-6/3505E-PDF ISBN 978-0-660-45210-4 ISSN 1488-5379

Correct citation for this publication:

Dolson, R. M. L., McNichols-O'Rourke, K. A., and Morris, T. J. 2023. A literature review of freshwater mussel survey methods and techniques used in deep, turbid environments. Can. Tech. Rep. Fish. Aquat. Sci. 3505: v + 70 p.

TABLE OF CONTENTS

ABSTRACT

Dolson, R. M. L., McNichols-O'Rourke, K. A., and Morris, T. J. 2023. A literature review of freshwater mussel survey methods and techniques used in deep, turbid environments. Can. Tech. Rep. Fish. Aquat. Sci. 3505: v + 70 p.

Under the *Species at Risk Act*, Fisheries and Oceans Canada is responsible for the management of aquatic species at risk including freshwater mussels. Adaptive and responsive species management requires robust survey methods to answer management questions. To date, most of the survey method guidelines to monitor freshwater mussels in Canada have been developed for wadeable streams. However, there is a need to develop deep-water survey guidelines and protocols to ensure adequate protection for mussel species found in deep, turbid environments. This report summarizes the results from a literature review of freshwater mussel (Bivalvia: Unionidae) sampling methods and techniques used in deep, turbid, or high-flow environments. In total, 110 sources from primary and grey literature were reviewed. Reviewed methods and techniques included autonomous underwater vehicle, brail, benthic grab, environmental DNA (eDNA), electrofishing, SCUBA and surface-supplied air, skimmer dredge, sonar, and suction dredge. Diving (SCUBA or surface-supplied air) with quadrat excavation was the most frequently used and recommended survey technique to quantitatively assess the status of freshwater mussels in deep water; however, turbidity and high flow can limit its effectiveness. Brail and the skimmer dredge are recommended only as reconnaissance survey tools. Novel techniques to detect freshwater mussels (e.g., autonomous underwater vehicles, eDNA, electrofishing, and sonar) are promising and can aid in species detection and occupied habitat delineation but cannot sufficiently address population demographic or density monitoring requirements of freshwater mussels at this time.

RÉSUMÉ

Dolson, R. M. L., McNichols-O'Rourke, K. A., and Morris, T. J. 2023. A literature review of freshwater mussel survey methods and techniques used in deep, turbid environments. Can. Tech. Rep. Fish. Aquat. Sci. 3505: v + 70 p.

En vertu de la *Loi sur les espèces en péril*, Pêches et Océans Canada a la responsabilité de la gestion des espèces aquatiques en péril, notamment la moule d'eau douce. La gestion adaptative et réactive des espèces requiert des méthodes de relevé rigoureuses qui permettent de répondre aux questions de gestion. Jusqu'à présent, la plupart des lignes directrices sur les méthodes de relevé visant à surveiller la moule d'eau douce au Canada ont été élaborées pour les cours d'eau que l'on peut traverser à gué. Cependant, il s'avère nécessaire d'élaborer des lignes directrices et des protocoles pour les relevés en eaux profondes afin d'assurer une protection adéquate des espèces de moules qui se trouvent dans des milieux profonds et turbides. Le présent rapport résume les résultats d'une analyse documentaire des méthodes et des techniques d'échantillonnage de la moule d'eau douce (*Bivalvia: Unionidae*) utilisées dans les milieux profonds, turbides ou à haut débit. Au total, 110 sources issues de la documentation principale et de la documentation parallèle ont été examinées. Parmi les méthodes et techniques examinées, citons le véhicule sous-marin autonome, l'épuisette, la benne, le prélèvement d'ADN environnemental (ADNe), la pêche à l'électricité, la plongée autonome et en narghilé, la drague à benne preneuse, le sonar et la drague suceuse. La plongée (autonome ou en narghilé) avec excavation de quadrats a été la technique de relevé la plus fréquemment utilisée et recommandée pour évaluer quantitativement la situation des moules d'eau douce en eau profonde; toutefois, la turbidité et le débit élevé peuvent limiter son efficacité. L'épuisette et la drague à benne preneuse ne sont recommandées que comme outils de relevé de reconnaissance. Les nouvelles techniques de détection des moules d'eau douce (p. ex. les véhicules sous-marins autonomes, l'ADNe, la pêche à l'électricité et le sonar) sont prometteuses, et peuvent contribuer à la détection des espèces et à la délimitation des habitats occupés. Cependant, elles ne peuvent pas répondre adéquatement aux besoins de surveillance de la démographie ou de la densité des populations de moules d'eau douce pour le moment.

INTRODUCTION

Freshwater mussels (Bivalvia: Unionidae) are benthic-dwelling, sedentary organisms that are found in a variety of aquatic habitats throughout Canada. They are also one of the most imperilled taxa in North America (Strayer et al. 2004); 20 out of 55 species have been assessed by the Committee on the Status of Species at Risk in Canada (COSEWIC) as being at risk (special concern, threatened, endangered, or extirpated). The introduction of invasive species (particularly dreissenid mussels), changes in the distribution and abundance of fish hosts, and habitat destruction and degradation have all contributed to freshwater mussel decline and extirpation (Hart et al. 2016).

Fisheries and Oceans Canada is the federal authority responsible for the management of aquatic species at risk (SAR) under Canada's *Species at Risk Act* (SARA). To effectively manage and protect aquatic SAR, managers must understand their population status, distribution, and abundance in the environment. Monitoring programs can be used to provide this information. Effective monitoring programs use sampling methods that maximize information collection and the ability to detect a change in the population, while minimizing costs.

There are multiple synthesis papers and government endorsed guidance documents that outline tested, effective, and efficient methods and techniques for sampling freshwater mussel populations in North America (Isom and Gooch 1986; Dunn 1999; Strayer and Smith 2003; Roghair et al. 2005; Duncan 2008; Mackie et al. 2008; Smith et al. 2011; Rider et al. 2013; Cummings et al. 2016; Hart et al. 2016; Ontario Ministry of Natural Resources and Forestry (OMNRF) 2018; Reid et al. 2018; Sanchez and Schwalb 2021). These documents provide guidance on study design, field survey design, sampling techniques, and mussel processing techniques: all of which can be modified based on the study question or monitoring requirement (e.g., monitoring SAR). Most of the guidelines have been developed for wadeable streams and often rely on being able to visually or physically (tactile) observe the mussel at the sediment surface while wading or snorkeling. Similarly, quantitative estimates of mussel density and population demographics have traditionally been obtained by removing a known amount of substrate to a depth of 10–15 cm (Dunn 1999; Metcalfe-Smith et al. 2007) by hand or with a scoop, while wading, snorkeling, or SCUBA diving in relatively shallow water (<1.5 m) and counting the number of mussels observed.

Several mussel SAR including Mapleleaf (*Quadrula quadrula*), Eastern Pondmussel (*Sagittunio nasutus*), Lilliput (*Toxolasma parvum*), and Hickorynut (*Obovaria olivaria*) can be found in deep (>1.5 m) water habitats that often have poor visibility and/or high flows which makes sampling and monitoring of these populations challenging with traditional sampling methods (T. J. Morris, Fisheries and Oceans Canada, Burlington, Ontario, pers. comm., 2018). Additionally, mussel SAR populations generally exhibit low density (0.02–0.74 mussels/m^2; Reid and Morris 2017; McNichols-O'Rourke et al. 2018), patchy and clustered distribution, and are only seasonally

available for capture when water temperatures, water level, and flow conditions are favorable (Hart et al. 2016).

Further, as a result of their low density and patchy distribution, Strayer and Smith (2003) suggested that survey designs using only quantitative sampling methods (e.g., quadrat excavation) will have low detection probabilities for rare mussel populations and that the low number of animals caught per sample results in uninformative summaries because of high sample variance. Reid and Morris (2017) demonstrated similar results based on a standardized monitoring program that is currently used to assess freshwater mussel populations in wadeable streams in Ontario. The authors found that the survey design is suitable for providing accurate and precise estimates of total (all species included) density, but generally not for individual SAR.

The purpose of this report is to inform Fisheries and Oceans Canada's development of long-term monitoring programs for mussel SAR in non-wadeable environments. The objectives are to: 1) summarize the available literature on mussel sampling methods and techniques that addressed deep, turbid environments, 2) identify studies that compared method efficiency, and 3) prepare an annotated bibliography that can be used as a reference to inform sampling program development.

METHODS

In order to assess potential sampling methods and techniques for use with freshwater mussels in deep, turbid environments, peer-reviewed primary research papers, grey literature, and conference abstracts were evaluated (Figure 1). The literature review focused on examples of freshwater mussel sampling techniques that: 1) involved work in deep and/or turbid lentic or lotic environments, 2) used novel techniques to sample mussels in any habitat type, and 3) compared the efficiency of multiple sampling methods or techniques. This report provides a summary of the information including an overview of the most common methods and techniques used in deep, turbid environments as well as the benefits and limitations of each method or technique. An annotated bibliography accompanies this report.

A literature search was conducted from January to March 2018, and an update was completed in March 2022 to include new studies (i.e., 2018 to 2021). Literature was discovered via online search engines (i.e., Google Scholar), publication databases (i.e., United States Geological Survey Publications Warehouse, United States Department of Agriculture [PubAg], and the Canadian Science Advisory Secretariat publications archive), and specialty websites (e.g., ResearchGate). Occasionally, published grey literature was obtained from society pages. Key search terms consisted of freshwater mussel plus one of the following: "brail," "skimmer dredge," "SCUBA," "deep," "turbid," "eDNA," "and sonar." Additional independent search terms included "unionid sampling method" and "mussel sampling protocol". Initial search results were screened by title and were moved to the abstract screening stage if "mussel" or "unionid" and any additional key search term was identified.

The scope of this review was broad and strict criteria for inclusion in the review was deliberately avoided. However, to be evaluated in-depth the paper needed to meet

the following three criteria at the abstract screening stage: 1) the appearance of work in a deep or turbid environment, 2) be related to freshwater mussels or marine mussels and clams, and 3) appear to contain information or a description of the sampling methods employed. An unquantified number of abstracts were reviewed in 2018, and 158 were reviewed in 2022 (i.e., for papers published between 2018 and 2021). Abstract screening resulted in an in-depth review of 69 papers published prior to 2018, and 41 published between 2018 and 2021 that met the above three criteria.

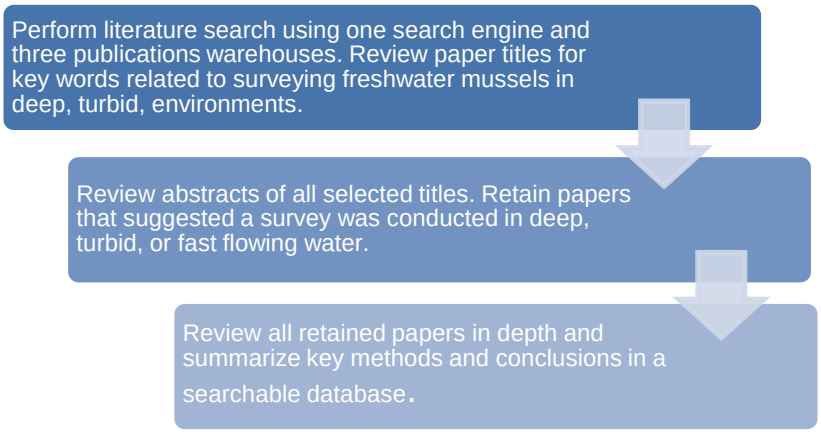

Figure 1. Flowchart of methods to perform the literature search and synthesis of sampling methods for freshwater mussels in deep, turbid, and fast-flowing environments.

Individual authors were contacted when a paper suggested a possible collection method was used in a deep or turbid environment but was not expressly explained in the paper (e.g., collection method was not the objective of the paper). Select authors (N = 8) were also contacted to discuss limitations of a particular method if it was not outlined in the paper.

SYNTHESIS OF SELECTED LITERATURE

A total of 110 sources were reviewed in detail. The majority of sources adequately reported sampling methods and locations. Nearly all sources reported whether sampling methods were qualitative or quantitative and most made this distinction based on whether or not sediment excavations were conducted using quadrats. The majority of sources utilized a combination of qualitative and quantitative methods to increase sampling efficiency, reduce cost, and sample multiple habitat types within the study site. Upon in-depth review, 39 (27 in 2018 and 12 in 2022) of the 110 sources did not meet the search criteria (e.g., were not in > 2 m water depth, turbid, or high-flow environment) or did not provide insight into techniques that are effective in

both shallow and deep environments. These papers are not included in the synthesis below but are described in the annotated bibliography.

Most papers described studies that occurred in the United States of America (73 papers) or Canada (20 papers; Figure 2).

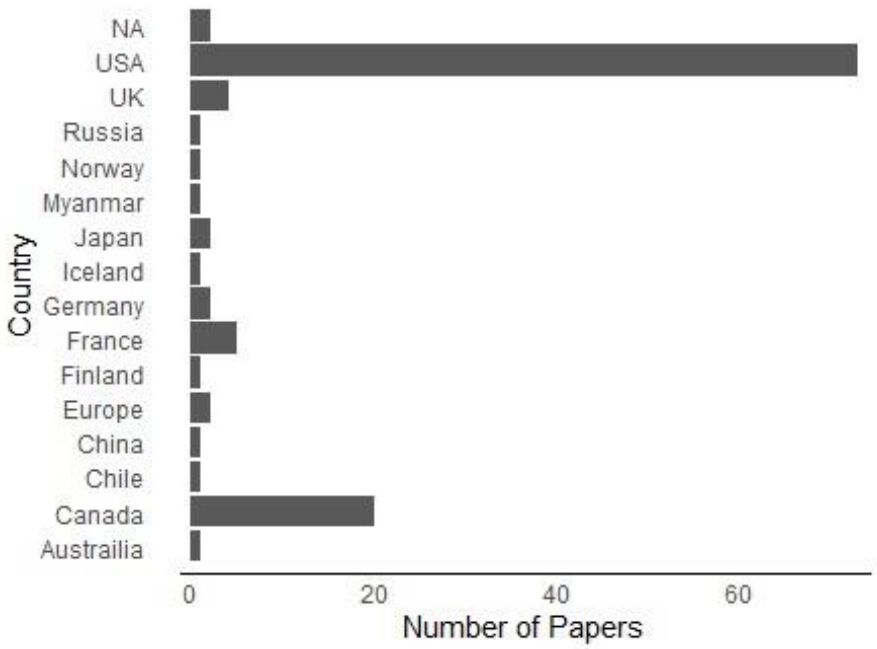

Figure 2. Global distribution of the sources reviewed for this technical report.

Most of the sources considered the efficiency and effectiveness of the chosen study design but did not address the efficiency or effectiveness of the sampling method or technique used. For example, a study which employed both snorkeling and SCUBA was likely to assume search efficiency among the techniques were equal.

Fourteen sources provided a synthesis or review of standardized sampling protocols for freshwater mussel populations with an emphasis on: study design, sampling technique, and statistical approaches to data analysis. However, these studies almost exclusively focused on shallow, wadeable, and high-visibility environments.

No source provided an assessment or evaluation of all available methods and techniques for sampling in deep, turbid environments. However, 18 sources did assess one or more techniques that have been used in deep, turbid environments.

The sections below summarize the findings of this literature review with respect to available information on the efficiency and effectiveness of sampling techniques for monitoring freshwater mussels in deep, or turbid, or high-velocity environments. Table 1 provides an overview of key findings for each sampling technique. All literature sources

originally compiled (110) are summarized in the annotated bibliography, but only the most relevant (69) sources are summarized below.

AUTONOMOUS UNDERWATER VEHICLE

Autonomous Underwater Vehicles (AUVs) can be large or small units that are towed through the water by a boat and record information through on-board photographic and acoustic technology. Typical on-board technology includes GPS, Doppler imaging, high-resolution cameras, and sonar. The data collected from the on-board technology can be used to assess mesobenthic habitat and map habitat types within a sampled area. One source was reviewed that employed AUV technology.

Singh (2015) used an AUV fitted with photographic and acoustic units to assess its effectiveness as a survey tool for population assessments of the Iceland Scallop (*Chlamys islandica*). This study was conducted in deep (< 3 m) marine environments with moderate flow and high visibility. Singh (2015) reports that the use of AUV is likely limited to shallow depths (< 2 m) when relying on photographic evidence because even high-resolution camera photos become distorted beyond 2 m. High-velocity environments would likely cause both sonar and photographic results to have low resolution. The effectiveness of AUV in turbid environments or habitats with large cobble and boulder substrate is anticipated to be low (Singh 2015). Additionally, AUV would not allow for subsurface estimation of mussel abundance and therefore should be considered qualitative. This study was the only source reviewed that used AUV as a method to survey for clams and the author is unaware of a similar study being conducted for freshwater mussels.

Benefits: coverage of large spatial areas, reduced cost and risk compared to SCUBA diving, integrated spatial GPS location for mussel bed delineation and animal location, and habitat and species count data collected simultaneously.

Limitations: upfront costs, decreased effectiveness in turbid conditions, limited ability to determine clam presence if the animal is not fully exposed (laterally) on sediment surface, poor photo quality in depths > 2 m, and subsurface surveying is not conducted which will bias results to large, exposed animals.

Table 1. Broad overview of key freshwater mussel survey methods used in deep, turbid, and fast-flow environments.

Method	Overview	Benefits	Limitations	Number of Sources Reviewed[1]
SCUBA and surface-supplied air diving	- Refer to the use of a self-contained underwater breathing apparatus (SCUBA) or to surface-supplied air diving - Most common technique used to sample freshwater mussels in deep water	- Can be used with qualitative or quantitative sampling design - Limited bias against small mussels (when quadrats are excavated) - Not depth-limited - Dive lights can be used to improve efficiency in turbid environments	- High-velocity sites may limit ability to excavate quadrats and jeopardize diver safety - Expensive and time-consuming - Specialized training and certification is required - A greater number of support crew are required	30
Environmental DNA (eDNA)	- Refers to the traces of genetic material an animal or plant sheds into the environment - eDNA is used to detect species presence in aquatic environments by assessing water samples for the presence of trace eDNA material	- Non-invasive - Method is relatively quick and inexpensive (if species-specific primer exist) - Cover large spatial area quickly - Depth is not a limiting factors - Evidence of positive detection of extremely rare animals in some environmental conditions	- No consensus on utility as a quantitative tool - Costly (if species-specific primer does not exist) - No consensus on the influence of transportation of eDNA from animal to point of collection (i.e., distance) - Unknown rate of false positive based on old, resuspended eDNA - some evidence that extremely rare species may go undetected without significant water filtration - Decreased effectiveness in waters with high turbidity and pH	15

[1] Total number of papers included in detailed synthesis. More papers on each method topic may be available in the annotated bibliography.

Table 1. (Continued) Broad overview of key freshwater mussel survey methods used in deep, turbid, and fast-flow environments.

Method	Overview	Benefits	Limitations	Number of Sources Reviewed[1]
Brail	- The brail (bar, lines, hooks) is towed behind a boat and dragged along the bottom of the river - Generally used as a reconnaissance (qualitative) tool	- Inexpensive to build and operate - Used as a primary survey to determine mussel bed/aggregation location - Cover large spatial area quickly - Density of mussels at surface may be calculated if efficiency is known	- Bias associated with hook size, substrate type, mussel size - Efficiency is typically not known and has been demonstrated to vary between mussel species - Inefficient in cobble/boulder - Mortality is expected (e.g., thin-shelled species)	10
Skimmer Dredge	- Benthic dredge that is towed behind a boat - Device consists of a pair of runners on which an angled blade and ramp bar are mounted - Extracts mussels from the sediment, and the animal slides up the ramp bar and into a trailing mesh bag	- Coverage of large spatial areas - Utilize tow time or length survey design - Some substrate is excavated - Captures smaller mussels than handpicking by divers - Not limited by site depth or turbidity	- Captures significantly fewer animals than handpicking by divers - Ineffective in all but unconsolidated substrate - May not be effective in extremely high flow - Capture efficiency is not consistent across species - May cause mortality (e.g., thin-shelled species)	5

[1] Total number of papers included in detailed synthesis. More papers on each method topic may be available in the annotated bibliography.

Table 1. (Continued) Broad overview of key freshwater mussel survey methods used in deep, turbid, and fast-flow environments.

Method	Overview	Benefits	Limitations	Number of Sources Reviewed[1]
Sonar	- The process of emitting sound waves through water and measuring aspects of the reflected wave	- Useful for large scale reconnaissance surveys to detect mussels and their habitat in large rivers - Non-invasive - Imaging could be used to count individuals exposed on substrate surface - Relatively inexpensive - Can be used in high-flow conditions	- Increasing turbidity and depth reduce utility of inexpensive side-scan sonar devices - Not suitable for use in certain substrates (e.g., pebble, cobble, or silt) - Cannot inform density or population demographic estimates - Effectiveness among mussel species is unknown	4
Benthic Grab	- Any point sediment sampling unit such as an Eckman Grab, a PONAR grab, or an undefined point sample benthic “dredge”	- Substrate excavation allows for quantitative estimates - Useful when depth or flow (for larger sampling units) limit diving - Not biased against small animals	- Not suitable for certain habitats (e.g., high vegetation, cobble and boulder substrate, high velocity) - Biased against large mussels - Time-consuming when covering a large spatial area	4
Autonomous underwater vehicle (AUV)	- Record information through on-board photographic and acoustic technology - Used to assess mesobenthic habitat	- Coverage of large spatial areas - Habitat and species count data collected simultaneously	- Up-front costs - Decreased effectiveness in turbid conditions - poor photo quality in depths > 2 m - Bias results towards large, exposed animals	1

[1] Total number of papers included in detailed synthesis. More papers on each method topic may be available in the annotated bibliography.

Table 1. (Continued) Broad overview of key freshwater mussel survey methods used in deep, turbid, and fast-flow environments.

Method	Overview	Benefits	Limitations	Number of Sources Reviewed[1]
Electrofishing	- Collect and inspect known host fishes during known mussel encystement life stage	- Excavation of sediment is not required to demonstrate recruitment - Non-invasive method if the fish do not need to be sacrificed - Useful in moderate depth, flow, and turbid environments - Reduced costs - Possibly useful in low-density environments	- Unknown effectiveness across mussel species, brooding strategies, or fish host - Has only been assessed on large fish hosts - All effectiveness caveats related to electrofishing apply	1
Suction Dredge	- A tool that is used in hydraulic gold mining in fresh water - Composed of a high-pressure water pump that mobilizes and vacuums sediment into an intake pipe, which moves the sediment to a sorting bag or holding mechanism	- Surface and subsurface mussels are collected - Supports quantitative estimates of population demographics and density - Consistent sampling across sites - Useful in a variety of visibility scenarios	- May not be suitable for certain habitats (e.g., consolidated or large substrates) - May be less effective in high-flow environments - Large mussels must be hand-collected by an accompanying diver - Destructive to the benthic environment	1

[1] Total number of papers included in detailed synthesis. More papers on each method topic may be available in the annotated bibliography.

BRAIL

A brail (or brail bar) was traditionally used by commercial freshwater mussel harvesters to capture mussels in deep water (Thiel 1981). The brail is made of three components including a bar, lines or chains that extend behind the bar, and a series of hooks that are attached to the line or chain. The brail is towed behind a boat (typically in the downstream direction) and dragged along the bottom of the river. As the brail encounters mussels exposed at the sediment surface, the mussel closes its valves on a hook and is extracted. The brail is hauled for a period of time or set distance and then brought to the surface to remove captured mussels.

Ten sources were reviewed that utilized a brail to survey for freshwater mussels, but several other studies mentioned the use of brail. Most sources (Kovalak et al. 1986; Miller et al. 1989; Dunn 1999; Sietman et al. 2001; Strayer and Smith 2003; Rider et al. 2013), suggest that the brail is only useful as a reconnaissance (qualitative) tool to determine the location of mussel beds where the environment is not suitable for traditional sampling, and that other quantitative methods are required to determine population density and demographics. However, Isom and Gooch (1986) suggested that the brail could be used for quantitative sampling when deficiencies are accounted for during data analysis, and Reid and LeBaron (2019) noted that brail can be used to complement existing survey methods assessing mussel presence and relative abundance.

A brail can be designed with various dimensions (e.g., length of chains, number of hooks, length of bar) and include wheels to facilitate movement along the sediment bottom. Because no standard brail design exists, the general effectiveness of the method is not known and will differ for each individual brail system (Isom and Gooch 1986), and capture efficiency is not consistent across species (Kovalak et al. 1986). However, the brail is biased against smaller animals (Isom and Gooch 1986; Kovalak et al. 1986; Dunn 1999; Strayer and Smith 2003).

None of the sources recommend a standard number of brail hauls (either time or distance) that would inform sampling sufficiency (either presence/absence or for population estimates) for freshwater mussels.

The literature suggests that a brail would be most effective in soft sediment (clay, sand and small gravel, but not silt), with clear or only slightly turbid water, in areas of limited vegetation, and when towed at speeds between 1 and 1.5 km/hr (Thiel 1981; Isom and Gooch 1986; Kovalak et al. 1986; Dunn 1999; Schueler and Robson 2018). No source reported a maximum water velocity or depth limit at which the brail would become ineffective. In the sources reviewed, the brail was deployed in zero to moderate (1.7 km/hr) flow, in depths ranging from 0.8 m to > 6 m, and in waters with varying degrees of visibility. It is unclear how brail effectiveness changes with mussel density, but Thiel (1981) reported that catch-per-unit-effort of the brail was correlated with abundance (estimated from SCUBA surveys). Schueler and Robson (2018) noted that the use of sonar aided in avoiding snags along the river bottom and reduced brail entanglement.

Brail capture efficiency for freshwater mussels has been compared to other methods, most commonly to SCUBA. Thiel (1981) describes that mussel surveys were first conducted via five-minute brail runs (10-foot-wide bar with 200 dovetail hooks and beaded prongs) and then the transect was resurveyed by SCUBA divers using visual and tactile methods. Thiel (1981) reported that the brail catch efficiency was 0.7% of the available population. Similarly, Thiel (1981) reported that the brail was only 3.6% as effective as SCUBA diving at capturing mussels based on a previous study. Recent sampling of the South Nation River in Ontario with a brail did not capture any unionids but did capture a few Zebra Mussels (*Dreissena polymorpha*) (Schueler and Robson 2018). Sietman et al. (2001) used a brail for reconnaissance sampling to determine mussel bed location in a turbid river (depth not reported). The authors conducted 85 brail runs of varying transect length. Surface-air-supplied divers were also employed to survey (visually and tactile) areas where the brail results suggested mussels may be present in larger quantities. Sietman et al. (2001) found that only 15.3% of brail runs resulted in the collection of more than one mussel and that the majority of animals were collected by diving. The estimated density of mussels in aggregated locations in this study was high (>19 mussels/m^2). This suggests that where mussels are found in low densities a brail may be inefficient and have low detection probability.

In the fall of 2017, Fisheries and Oceans Canada deployed a wheeled brail with 18 uneven lines, and six, four-pronged hooks downstream of Cockshutt Bridge on the Grand River in Ontario (T. J. Morris, Fisheries and Oceans Canada, Burlington, Ontario, pers. comm., 2018). Seven trail runs were completed towing the brail between 1.5 and 2 km/hr: six runs towing the brail in the downstream direction and one in the upstream direction. The habitat largely consisted of sand and the brail was run in depths between 1 and 2 m. Between zero and nine animals were caught per tow, and animals were only captured when vegetation was sparse. Similarly, Reid and Le Baron (2019) surveyed 51 sites in the Grand River, Ontario using a brail towed behind a boat. Each site was sampled along five, 50 m transects. This study captured large, sculpted mussel species and more individuals were captured in the summer than in the fall.

Of the three studies that compared the effectiveness of brail to SCUBA, all recommended SCUBA over brail as necessary when population density and demographics are desired (Thiel 1981; Isom and Gooch 1986; Kovalak et al. 1986). Dunn (1999) found that the skimmer dredge (see section 3.6) was more reliable than the brail for confirming mussel presence in a large river. Reid and Le Baron (2019) suggest that brailing can be used to complement existing survey methods to determine mussel presence.

Benefits: reduced cost and safety risk while operating in deep and turbid environments; ability to cover a large spatial area relatively quickly; no reported limitation on depth or velocity use; can be used as a primary survey to determine mussel bed/aggregation location; inexpensive to build and operate; can capture a large number of mussels; and, if efficiency is known, density of mussels at the surface may be calculated.

Limitations: biases associated with hook size and type, substrate conditions, turbidity, mussel size, vegetation presence, and water temperature; efficiency is not

known and has been demonstrated to vary between mussel species (largely based on size and shell type); inefficient in cobble and boulder environments; and, some mortality is expected during collection, especially for thin-shelled individuals.

BENTHIC GRAB

A benthic grab refers to any point sediment sampling unit such as an Eckman Grab, a PONAR grab, or an undefined point sample benthic “dredge.” Different sampling units are not likely to have the same effectiveness but given the scarcity of data, the grab types were pooled together for this review.

Of the sources reviewed four employed benthic grabs to sample freshwater mussels (Hanson et al. 1988; Bolotov et al. 2017; Karatayev et al. 2018; and Keretz et al. 2021). Several sources discussed benthic grabs to varying degrees within summaries of freshwater mussel sampling guidelines (Strayer and Smith 2003; Duncan 2008; Cummings et al. 2016; Rider et al. 2013).

Bolotov et al. (2017) used an unidentified hand dredge to collect freshwater mussels in depths of up to 3 m, but mostly in water less than 1 m and during low flow. This report did not discuss the efficiency of the dredge or any limitations. Hanson et al. (1988) quantitatively sampled a small, unproductive lake to determine the presence and abundance of freshwater mussels. The authors compared the effectiveness of mussel collection by SCUBA divers using 0.5 m by 0.5 m quadrats at select depths (1, 3, 5, 7, and 9 m) along transects to benthic grab samples taken at the same depths at a subset of locations along the transects. The benthic grab was an Eckman dredge and once the sample was taken it was washed on shore through a 6 mm sieve. Hanson et al. (1998) found that dredges were more effective than SCUBA divers for finding mussels < 30 mm in length and recommended that sediment removal accompany any SCUBA survey if population demographics are required. However, the authors noted that small dredges are often biased against large mussels. Mussel density in this study averaged 14.9 mussels/m^2.

Karatayev et al. (2018) used PONAR grabs and PONAR grabs with video imagery to assess *Dreissena* mussel presence and density in Lake Michigan. The authors also compared the PONAR grab to a benthic sled with a mounted camera. Mussel surveys were conducted in water 6.7 to 205 m in depth. The benthic sled was towed across sites for approximately 500 m. In each depth zone surveyed, the average density and biomass of *Dreissena* estimated from PONAR grabs did not differ significantly compared to the PONAR+video or benthic sled+video techniques. However, the video transect sampling using the benthic sled covered more than two orders of magnitude more area than the PONAR grab alone. The authors noted that the camera mount was not used successfully in Lake Erie due to its turbid condition and bottom dominated by macrophytes. The authors also noted that PONAR was still required to determine size-frequency distribution of mussels as well as to aid in species identification.

Keretz et al. (2021) utilized replicate PONAR grabs at 56 sites to determine the presence of extant unionid mussels in areas of historical Zebra Mussel invasion in the Detroit River basin, USA. Sites were selected randomly, chosen from historical survey locations, or based on known characteristics that may support unionid mussels. Three replicate PONAR grabs were collected at each site, one each from the bow, centre, and stern of the boat. Grab material was sieved at the surface and mussels were identified and counted. Density estimates were generated from PONAR data. Additionally at each site, SCUBA was employed for one person-hour using a timed search approach and visual and tactile techniques to collect live and dead mussels. The efficiency of the PONAR versus SCUBA was not reported.

Rider et al. (2013) recommends that if excavation is not possible with diving, then benthic grabs can be used to supplement sampling at a subset of transects in areas of high mussel density. Duncan (2008) recommends that when a complete inventory of an area is required a dredge may be used to collect sediment samples at designated intervals within a grid pattern or along transects.

None of the sources discussed how depth, water velocity, or turbidity would impact the effectiveness of the benthic grab. However, given the abundant use of benthic grabs for sampling other aspects of the mesobenthic community it is likely that some estimates of grab effectiveness are available for other species and for specific habitat types. It is unlikely that small benthic grabs would be effective at sampling specific points on a transect in high velocity environments.

Benefits: substrate excavation allowing for quantitative estimates, useful when depth or flow (for larger sampling units) limit diving, and is not biased against small animals. May be paired with video imagery for greater spatial coverage.

Limitations: not suitable for use in high-vegetation habitats or cobble and boulder substrate; biased against large mussels; may not be useful in high velocity environments; and is time consuming when covering a large spatial area.

ENVIRONMENTAL DNA (eDNA)

Environmental DNA (eDNA) refers to the traces of genetic material an animal or plant sheds into the environment. eDNA is used to detect species presence in aquatic environments by assessing water samples for the presence of trace eDNA material. Increasingly, eDNA is being used to detect the presence of rare or cryptic species including freshwater mussel SAR (Currier et al. 2018).

Collection of eDNA is non-invasive, relatively inexpensive, and can be used to determine the presence of multiple, cryptic species (Currier et al. 2018). The quantity of eDNA in the environment may be correlated with the density or abundance of the source population (Hanfling et al. 2016; Rice et al. 2018; Stoeckle et al. 2021) which may have implications for the detection rare species. Detection of extremely rare species, with low abundance, has met with variable success (e.g., Currier et al. 2018; Gasparini et al. 2019; LeBlanc et al. 2021). eDNA can be applied to detect single species or multiple species (metabarcoding) depending on the gene sequence used to

develop the primers. Surveys can be timed to match the spawning period of freshwater mussels to increase the rate of eDNA detection (e.g., spring, summer, or fall) (Wacker et al. 2019).

However, increasing turbidity can limit eDNA detection regardless of mussel population size (Stoeckle et al. 2021), and slow or impede the water filtration process while collecting eDNA (Prié et al. 2021). Depth is not believed to be a limiting factor for eDNA detection; however, water samples collected from the substrate had higher detectability than those from the surface or middle of the water column (Amberg et al. 2019; Lor et al. 2020). Imperfect detection of present species can occur when using eDNA as a result of assay methods, water sample collection methods, or environmental variables (LeBlanc et al. 2021; Lor et al. 2020; Stoeckle et al. 2021).

The effectiveness of eDNA detection in flowing water depends on a number of factors including transport, retention, and resuspension (Shogren et al. 2017). Discharge and stream flow can influence eDNA detectability (Gasparini et al. 2019; Curtis et al. 2020). In situ water properties including temperature and pH can inhibit eDNA assays and limit detection (Schmidt et al. 2021). The literature is inconclusive with respect to eDNA detectability downstream of a known mussel population (Klymus et al. 2020). Three sources report eDNA detectability several km downstream of a known population (Sansom and Sassoubre 2017; Wacker et al. 2019; Stoeckle et al. 2021,), whereas two sources failed to detect eDNA from known populations between 10 and 500 m downstream (Gasparini et al. 2019; Lor et al. 2020). It is also reported that eDNA detection is highest 10 to 100 m downstream of a known population as compared to immediately downstream of a mussel bed (Stoeckle et al. 2021). eDNA survey success may also be influenced by the gene selected for collection and amplification (COI, ND1, 16S; Mauvisseau et al. 2019; Klymus et al. 2020; Prié et al. 2021). However, a review of eDNA assay methodology is beyond the scope of this report.

Numerous recent (2018 to 2021) studies evaluate the utility and effectiveness of eDNA surveys for detecting and quantifying freshwater mussels. However, only 15 studies are discussed in the synthesis below because they speak to the most relevant aspects of eDNA as a survey method for sampling freshwater mussels or compare eDNA to traditional methods. All eDNA literature sources reviewed are summarized in the annotated bibliography.

Amberg et al. (2019) evaluated the effectiveness of eDNA survey methods to detect Zebra Mussels in lakes in Minnesota, USA. The authors collected eDNA from water samples at the surface, mid-water column, and at the sediment surface along transects from the shoreline to 6 m depth (1, 2, 4, and 6 m). eDNA detection was highest in water samples collected from the sediment surface over soft sediments. The study relied on SCUBA divers excavating 0.25 m^2 quadrats to estimate Zebra Mussel density.

Curtis et al. (2020) evaluated the influence of stream flow on freshwater mussel eDNA concentrations and detectability in two streams as well as seasonal differences in eight streams in Illinois, USA. Depth of the streams is not reported but flow is described as high, with high turbidity and low to high density of mussels. eDNA detection decreased with increasing flow and floods resulted in false negatives (non-detects when

the species was present). The authors suggest eDNA detection declines with increasing flow due to dilution. A correlation between mussel density and eDNA detection was observed where low-density sites (15 mussels/m^2) required three times the number of water samples compared to high density (> 85 mussels/m^2) sites.

Coghlan et al. (2021) developed a multispecies eDNA assay to support a metabarcoding survey to detect freshwater mussels in eight southern Ontario waterbodies. eDNA detection results were compared to known mussel community composition collected using traditional survey methods in stream and wetland habitat. Metabarcoding was successful at detecting species in 24 known and novel locations, but with imperfect detection (>80% of known species). The survey method was particularly useful in detecting novel species occurrences where quadrat excavation had not previously been completed.

Currier et al. (2018) collected water samples from long-term mussel SAR monitoring locations in a wadeable river in southern Ontario. The authors found positive eDNA detections for target species in all sites that had positively detected mussel SAR using quadrat sampling. The authors also demonstrated that eDNA concentrations were positively correlated with mussel densities (estimated via quadrat surveys).

Gasparini et al. (2019) developed species-specific primer assays to detect eDNA of a freshwater mussel species at risk in the Grand River, Ontario. Mussels were collected from wadeable portions (<1.5 m) of the river using visual and tactile methods. Water samples were also collected. Following primer development, water samples were assessed for eDNA presence. The study also evaluated the distance eDNA could be detected downstream at 0, 10, 50, and 100 m, in moderate flow, based on the presence of 1 or 10 mussels placed in a cage. When only 1 mussel was present in the cage eDNA detection was positive at 0 m and no further. When 10 mussels were present in the cage eDNA was detected at 10 m, but no further downstream. Discharge of 1,632-2,332 L/s rapidly diluted eDNA and reduced detectability beyond 10 m downstream from a low-density population (i.e., caged mussels).

Although the following studies did not focus on freshwater mussels, they were included in the literature review as they discussed the ability of eDNA to assess species abundance. Hanfling et al. (2016) evaluated how eDNA, in combination with site occupancy modelling, compared to traditional (gill net) estimates of fish abundance in a small lake in the UK. This study aimed to answer an important question: can eDNA accurately estimate abundance. Hanfling et al. (2016) found that the rank abundance estimates of fish species established from gill netting surveys were consistently correlated with eDNA abundance data. However, the authors note that the eDNA results cannot inform estimates of population demographics or individual condition. Similarly, Rice et al. (2018) demonstrated that eDNA accurately determined the presence of an endemic crayfish in a large river; however, the results do not reflect the abundance of the species. Rice et al. (2018) suggests that the ability of eDNA to accurately infer abundance of an organism may be limited in lotic systems due to the influence of downstream transport of eDNA.

Klymus et al. (2020) developed a multispecies eDNA assay to evaluate the utility of metabarcoding for freshwater mussels in the Clinch River, USA. Imperfect detection

was observed at three sites where eDNA detected 42%, 58%, and 54% of known species richness. The authors suggest low detection was based on inadequate amount of filtered water collected at each site; however, up to 16 samples were collected per site. Downstream sampling locations yielded greater eDNA concentration than upstream locations and it is suggested that eDNA accumulates throughout a watershed (given increasing inputs, or more aggregations of mussels moving downstream).

LeBlanc et al. (2021) evaluated the ability of eDNA to detect populations of a species at risk mussel in the Miramichi watershed in New Brunswick, Canada. Fifty-six sites were chosen based on past positive findings of the mussel using traditional survey methods. Positive eDNA detections were observed at 16 of 56 sites. Even in cases where the mussel of interest was visually observed at a site, eDNA detections, if positive, were often below the lower detection limit of the assay.

Lor et al. (2020) evaluated the use of eDNA to detect a rare, cryptic mussel species in the St. Croix and Mississippi Rivers, USA. The mussel is typically found under rocks limiting the utility of traditional survey methods. eDNA detectability in wadeable portions (<1.5 m) of both rivers was low (20.2% and 0.6%, respectively) but collections in spring and from bottom (e.g., taken just above the riverbed) yielded higher eDNA concentrations. eDNA detections declined only after 500 m in bottom water samples. The higher rate of discharge in the Mississippi was suggested to be responsible for the extremely low eDNA detection rates.

Mauvisseau et al. (2019) utilized a mesocosm experiment to evaluate the repeatability and accuracy of eDNA assays to detect the Freshwater Pearl Mussel (*Margaritifera margaritifera*) using two genes, COI and 16S. The COI gene had a lower limit of detection suggesting it may be more useful in field applications for rare species.

Prié et al. (2021) developed a metabarcoding eDNA approach to assess the status of freshwater mussel biodiversity in three European countries. The 16S gene was used for multispecies primer development. Survey locations included multiple habitat types including small, wadeable streams, standing water, and fast-flowing rivers over a variety of substrate types with varying levels of pH. eDNA species detection results were compared to known mussel distributions across three scales: the country level based on known mussel distributions, at two intensively sampled rivers in France (each with > 50 sites spanning several kms), and at 15 small survey sites (~ 200 m sites). Historical and contemporary mussel species presence was collected using a variety of techniques (dredging, SCUBA transects, wadeable timed-search approach). eDNA detected up to 90% of known species across the three spatial scales. However, at sites with higher turbidity positive eDNA detection was lower due to the influence of humic substances and sediment in the water collection process.

Sansom and Sassoubre (2017) used a mesocosm experiment to determine the rate of freshwater mussel eDNA contribution to the environment, and how long it persists (rate of decay). The authors found that using modelled flow rates of 0.09 m/s, with mussel density of 0.1 mussels/m^2, eDNA is likely to persist up to 36.7 km downstream.

Schmidt et al. (2021) developed a single species eDNA primer to detect a rare mussel species in the Lynches River basin of North and South Carolina. Water samples were collected from 116 sites across stream orders of 1 to 5 at sites where the endangered mussel was known to occur. No positive eDNA detections were observed at any of the extant mussel sites and almost all eDNA assays were inhibited during analysis. The authors suggest that 100% assay inhibition occurs when pH is < 5.5 and that visual and tactile methods are more effective than eDNA in low pH environments.

Stoeckle et al. (2021) evaluated the relationship between freshwater mussel abundance, stream discharge, sampling distance, and eDNA detectability and quantity for a common mussel species in the Danube River, Germany. eDNA was positively detected up to 3 km downstream of known mussel aggregations and eDNA detectability increased with increasing mussel population size. However, eDNA detectability was negatively correlated with turbidity, independent of population size. The authors found that eDNA quantity was highest 100 m downstream of known aggregations. At sites with low turbidity (1.1 NTU) and small mussel aggregations (500 individuals) eDNA detection was 95.2%.

Wacker et al. (2019) surveyed a small, wadeable stream (depth < 0.5 m; flow 0.02-0.04 m/s) in Norway to evaluate the relationship between the spatial distribution of mussels and eDNA concentrations in a lotic system. The authors surveyed eDNA concentrations above and downstream (1700 m) of small and large mussel aggregations and across seasons (spring and summer). The authors found complete eDNA detection at sites downstream (1700 m) of large mussel aggregations. However, eDNA detection was low downstream of small aggregations (13%). Mussel species present in the surveyed stream are summer spawners and eDNA detection was higher in summer compared to spring.

eDNA is a novel technique that has demonstrated utility as a reconnaissance tool to detect common, and in some cases rare, species under a variety of habitat conditions (e.g., deep, fast-flowing). Not all environmental conditions are suitable for eDNA surveys (e.g., high turbidity, low pH). Further, eDNA is unable to assess population structure (e.g., size distribution, recruitment) and may not provide fine-scale resolution to determine habitat associations, especially in lotic environments.

eDNA is novel technique and may be useful in a variety of conditions, but many questions related to its effective and efficient application as a reconnaissance survey tool, and more so, to inform density estimates, remain to be answered (Currier et al. 2018, Hanfling et al. 2016, Rice et al. 2008, Shogren et al. 2017).

It is important to note that the field of eDNA, both individual species and metabarcoding, is rapidly evolving and methods are constantly changing.

Benefits: non-invasive; if species-specific primers exist the method is relatively quick and inexpensive; large areas can be surveyed relatively quickly; depth is not a limiting factor; and evidence of positive detection of extremely rare animals in some environmental conditions.

Limitations: no consensus as a quantitative tool; costly if species-specific primers do not exist; no consensus on the influence of transportation (via flow) on

distance from animal to point of eDNA collection; some evidence that extremely rare species may go undetected without significant water filtration; unknown rate of false positive based on old, resuspended eDNA; and, decreased effectiveness in waters with high turbidity and pH.

ELECTROFISHING

The freshwater mussel life cycle includes a brief, obligatory stage as a parasite. Larval mussels (glochidia) parasitize a host, usually a fish, before transforming and dropping off as juvenile mussels. When the host is a fish species, glochidia are most often found attached to the gills; however, some species can attach to the body and fins of a fish.

Salonen and Taskinen (2017) exploited the parasitoid phase of the mussel life cycle to determine the presence of the Freshwater Pearl Mussel in rivers throughout Finland. The Freshwater Pearl mussel's known fish hosts are salmonids: Atlantic Salmon (*Salmo salar*) and Brown Trout (*Salmo trutta*). The Freshwater Pearl Mussel is a long-term brooder and parasitizes the fish host for up to a year. Near the time of transformation glochidia are approximately 400-500 µm in size and visible without magnification on the gills of their hosts. Salonen and Taskinen (2017) performed haphazard backpack electrofishing surveys on rivers in Finland to determine the presence of the Freshwater Pearl Mussel by visually examining the gills of captured Brown Trout. They compared in-situ estimates of glochidial presence and rate of infestation on wild Brown Trout to laboratory-raised and -infested Brown Trout. The results suggested that visual inspection of the gills of an anesthetized Brown Trout in the field can accurately determine the presence of the Freshwater Pearl Mussel. Field trials had accuracy that was similar to microscopic inspection in the lab. The rivers surveyed were wadeable, clear, and had low to moderate flow at the time of sampling.

No other study was available to assess the validity of using electrofishing for other species of mussels, or mussels with different host species. However, Holliman et al. (2007) demonstrated that that both mussels and their glochidia have high survivorship after exposure to current during electrofishing events. Presumably, determining the presence of mussels based on inspection of fish host gills would be ineffective for mussels that are short-term brooders or whose glochidia do not reach a visible size. Additionally, Salonen and Taskinen (2017) studied a mussel species that is known to use a limited number of fish hosts and in a location where mussel diversity is extremely low. Any glochidia observed on the gills of Atlantic Salmon or Brown Trout could be reasonably assumed to be the Freshwater Pearl Mussel. Salonen and Taskinen (2017) reported successful identification of glochidia and therefore mussel presence in extremely low-density environments.

Salonen and Taskinen (2017) used backpack electrofishing in wadeable streams; it is feasible that boat electrofishing could be used to capture fish hosts in deep, turbid, or high-velocity environments. It is beyond the scope of this report to summarize the efficiency of boat electrofishing for all possible fish host species and in different habitat types, but this would certainly need to be considered before determining if this method

would be useful. Further, many of the traditional non-lethal sampling methods for fish (summarized in Bonar et al. 2009) could be used to capture potential fish hosts and evaluate gill structures for the presence of glochidia; however, a discussion of fish sampling methods is beyond the scope of this report.

However, where deep, turbid, or velocity barriers restrict the use of quantitative methods to determine mussel population recruitment (e.g., sediment excavation), and where the fish host is known, inspecting the gills of known fish hosts may be a viable method to demonstrate population recruitment (at least to the glochidial life stage) or the presence of a species. This method would not be considered quantitative, and no information exists to suggest infestation rate is proportional to adult mussel abundance and therefore the method would also not be considered qualitative. The method would be most suited for exploratory surveys to detect mussel presence.

Benefits: excavation of sediment is not required to demonstrate recruitment; non-invasive method because the fish do not need to be sacrificed; useful in moderate depth, flow, and turbid environments; reduced costs; and possibly useful in low density environments.

Limitations: unknown effectiveness across mussel species, brooding strategies, or fish host; has only been assessed on large fish hosts; and all effectiveness caveats related to electrofishing apply.

SCUBA AND SURFACE SUPPLIED AIR DIVING

In water that is too deep to wade or effectively snorkel, typically > 1.5 m, diving can be used to complete freshwater mussel surveys. Diving can refer to the use of a self-contained underwater breathing apparatus (SCUBA) or to surface-supplied air diving. There is little difference between these two methods other than that SCUBA requires a diver to more frequently return to the shore or a boat to exchange air tanks, and licencing/training requirements. Diving is the most common technique used to sample freshwater mussels in deep water.

Unless the objective of a study is to haphazardly detect mussels, diving must be used in conjunction with a defined survey design to sample freshwater mussels. This section summarizes the available literature across various survey designs that have been carried out via divers, with a focus on those that have been used in deep, turbid waters.

Thirty freshwater mussel surveys that used divers were discovered during this literature search. Several of the studies employed SCUBA divers when sites (or portions of sites) were not wadeable but did not employ specific methods or techniques for use in deep water or discuss differences in capture efficiencies between methods or techniques. When the survey site was not previously known (e.g., not a long-term monitoring site, construction site, or whole lake study), a reconnaissance survey tool such as mapping of commercial harvest areas, sonar, brail, or skimmer dredge was typically used to delineate the survey site based on specific habitat conditions or estimated mussel density (Thiel 1981; Miller et al. 1989; Sietman et al. 2001; Christian

and Harris 2005; Smit and Kaeser 2016; Kaeser et al. 2019) prior to diver surveys. When the survey site was delineated using existing habitat mapping or as defined by a point of interest in the watershed (e.g., construction site or entire lake), studies tended to employ timed-search or transect sampling methods and allowed divers to capture animals visually or by handpicking (Cvancara 1972; Isom and Gooch 1986; Kovalak et al.1986; Hanson et al. 1988; Miller and Payne 1993; Smith et al. 2001; Meador et al. 2011; Galbraith 2012; Biodrawversity LLC 2015; Prié et al. 2017; Kriege 2018; Randklev et al. 2018; Stoeckl et al. 2019; Wegscheider et al. 2019; Keretz et al. 2021). Several studies used divers to undertake quadrat sampling following reconnaissance or qualitative surveys; however, a limited number of studies undertook (or recommended) quadrat excavation (Miller and Payne 1993; Smith et al. 2001; Christian and Harris 2005; Meador et al. 2011; McAlpine and Sollows 2014; Smit and Kaeser 2016; Hornbach et al. 2018; Karatayev et al. 2018; Boon et al. 2019; Reed et al. 2019).

A combination of survey methods is commonly used at deep-water sites (Hornbach et al., 2018; Kriege 2018; Boon et al. 2019; Reed et al. 2019). Typically, several transects are established perpendicular to the flow of the site which are searched using a timed-search approach with visual and tactile techniques along the entire length of the transect, or within defined quadrats along the route. Quadrats may or may not be excavated depending on the objective of the study. Only one source recommended a standardized number of transects and quadrats per site to meet defined detectability limits (WVDNRWRS 2020).

McAlpine and Sollows (2014) report a survey method that utilized a combined quadrat/sieve apparatus to undertake quadrat sampling at a site without additional qualitative sampling. The authors sampled rivers in New Brunswick, Canada, at a variety of sites with varying turbidity (clear to murky), depth (> 1 to 5 m), substrate type (sand to cobble), and flow velocity (0 to 0.28 m/s). The quadrat sieve was a 0.25 m^2 frame with an attached screened (5 mm) stage. The quadrat sieve is used by laying the quadrat on the sediment surface oriented with the sieve at the downstream end. The diver, visually and by hand, surveys the quadrat placing any animals found in a coloured mesh bag. The quadrat is then excavated by hand or by metal scoop to a depth of 15 cm. The sediment material is put onto the sieve, and any current washes away fine material. Animals collected in the sieve are placed in a differently coloured mesh bag. McAlpine and Sollows (2014) report capturing mussels as small as 11.5 mm during excavation. The authors also report that when conditions were favorable (e.g., fine-medium sand with little vegetation, low current, moderate visibility, and mussel density of 0-8 per square m^2), quadrats could be visually searched and excavated in, on average, seven minutes. The quadrat sieve apparatus could presumably be used with adaptive, cluster, or systematic sampling survey designs.

The Ontario Ministry of Natural Resources and Forestry (2018) suggests that where surveys are required to determine the presence or absence of freshwater mussels in a coastal wetland, a timed search with 12 random starts or a half-hectare timed search survey can be employed using SCUBA. The survey type chosen is based on wetland size and whether there is a priori knowledge of mussel presence at a site. However, where habitat conditions reduce search efficiency, increased sampling effort

is required to confidently assess species absence. Increasing depth, flow, and turbidity are all believed to lower search efficiency.

Boon et al. (2019) develops a standard method approach to detect and quantify the Freshwater Pearl Mussel in European rivers. When sites are deep (> 1.5 m) the authors recommend preliminary SCUBA surveys to delineate the survey area of interest followed by a transect with quadrat survey to estimate population size and demographics. The authors note that surveys are most effective in low-flow, high-visibility conditions.

Christian and Harris (2005) report that it took 94 person-days to sample 68 river kilometers in habitats with effectively zero (< 0.01 m) visibility and where 0.3–2.0% of mussel beds were searched with quadrats. They also found that to obtain 80% confidence in density estimates at high density mussel beds (>10 mussels/m^2) roughly 18–63, 1 m^2 quadrats would need to be excavated. The authors recommend that a tiered approach be used to efficiently sample large rivers where habitat or historical information informs where to place the survey reach, followed by a preliminary qualitative search by divers (transects). Finally, based on estimated density of mussel beds during the preliminary survey (low, medium, or high), a second qualitative survey (low density) or quantitative quadrat surveys with excavation should be conducted (Christian and Harris 2005).

Hornbach et al. (2018) surveyed nine sites in the St. Croix River, USA to assess the long-term status of freshwater mussel populations. Long-term monitoring sites were visited between five to nine times from 1991 to 2011. At deep-water sites SCUBA divers were used to excavate quadrats within 10 sampling arrays per site. Each sampling array was 2 m by 5 m and 10 quadrats were excavated within each array. Excavated material was sieved at the surface to detect mussels. The study did not compare traditional survey methods used in shallow sites to those of SCUBA, but did report that even when 100+ quadrats were excavated at a site the estimate of juvenile density was likely an under-estimate.

Kriege (2018) assessed freshwater mussel community composition and diversity in the Greenup Pool of the Ohio River, USA. Survey site selection was informed using historical sampling records. Sites surveyed in this study utilized SCUBA to detect mussels along transects established perpendicular to the flow of the river. The transects were 100 m in length, spaced 100 m apart. The author placed six transects at each survey site and each transect was divided into 10, 10 m^2 cells that were visually searched. The transects were marked using lines and anchors and divers moved from deep (~ 7 m) to shallow habitat during the survey during high visibility (> 0.5 m) conditions.

Reed et al. (2019) used SCUBA divers to assess the freshwater mussel community in the Buffalo River basin of Tennessee, USA. Preliminary surveys and museum records were used to inform the selection of sites for quantitative mussel sampling. SCUBA divers were employed at sites up to 2 m in depth and surveyed quadrats placed long transects established perpendicular to the river flow. Transects were spaced 4 m apart and 100 quadrats were excavated per site. Water clarity and

flow were not reported. Qualitative surveys detected 13 more species than quantitative sampling among all sites.

WVDNRWRS (2020) developed a standardized survey method to determine freshwater mussel presence/absence in support of development proposal reviews undertaken by state regulators. The report recommends transect surveys to be completed by SCUBA in deep-water sites. Transect surveys must contain at least 500 m of transect within a site, with a minimum of five transects, and visual/tactile searches should be employed along and between transects. A minimum search effort of 1 minute per m^2 is required along transects. Transects should be spaced up to 20 m apart. Timed searches are also required in “mussel concentration areas” where visual/tactile searches are performed in 10-minute increments until no new species are found in six consecutive samples.

In the flowing waters of Canada where mussels are usually found, it is unlikely that depth would limit the utility of diving as a survey technique because commercial divers are licenced up to at least 30 m. However, increasing turbidity will limit a diver’s ability to discover mussels by visual or tactile techniques, although a dive light (headlamp) can remedy this in most circumstances (D. McAlpine, New Brunswick Museum, Saint John, New Brunswick, pers. comm., 2018).

In high velocity environments, Prié et al. (2017) noted that divers could be secured via a climbing harness and ropes established along transects. However, quadrat excavation in high velocity environments was found to be time-consuming and costly (Meador et al. 2011; although see McAlpine and Sollows 2014).

Timed, area-based, or transect surveys by divers using visual and tactile techniques have been shown to be more effective than brail, skimmer dredge, or benthic grabs for capturing animals at the sediment surface (Thiel 1981; Isom and Gooch 1986; Hanson et al. 1988; Miller et al. 1989; Cawley 1993; Sietman et al. 2001). However, the skimmer dredge and benthic grabs consistently capture more small individuals (Hanson et al. 1988; Miller et al. 1989). Diving is more suitable to a range of sediment substrates (e.g., cobble, vegetation, consolidated sediment) where brail, skimmer dredge, or benthic grabs have limited utility.

Smith et al. (2001) recommends that in non-wadeable environments diving be used in a combined qualitative/quantitative survey design to assess mussel presence and density. The authors recommend divers undertake a qualitative survey either using transects or timed search, followed by a subsample of sites (as determine by double sampling or systematic sampling with random starts) using quadrats (including excavation).

Sanchez and Schwalb (2021) compared mussel detectability among timed-search, transect, and adaptive cluster sampling methods in wadeable streams, and their findings may be relevant to deep-water sites that have high visibility. The authors note that timed searches captured more large and sculpted species whereas adaptive cluster sampling (quadrats with excavation) captured a higher proportion of smaller species. Transect sampling methods had the lowest effort per person-hour, but adaptive cluster

sampling detected more individuals when patchiness was high, density was high or moderate, and in soft substrates.

The ability of divers to detect freshwater mussels is related to mussel density at a site, but an appropriate survey design can be used to improve diver efficiency.

Benefits: can be used with qualitative or quantitative sampling design in most habitat conditions; when quadrats are excavated there is limited bias against small mussels; not depth-limited; and dive lights can be used to improve efficiency in turbid environments.

Limitations: high-velocity sites may limit ability to excavate quadrats and jeopardize diver safety; diving is expensive and time-consuming if multiple sites must be sampled; few sites can be sampled per day; specialized training and certification is required; and a greater number of support crew are required to assist divers.

SKIMMER DREDGE

A skimmer dredge is a benthic dredge that is towed behind a boat. The device consists of a pair of runners on which an angled blade and ramp bar are mounted (Miller et al. 1989). The blade of the skimmer dredge extracts mussels from the sediment and the animal slides up the ramp bar and into a trailing mesh bag. The height of the angled blade can be adjusted to dredge more or less sediment. Additionally, hydraulic jets can be fitted to the skimmer dredge to mobilize the sediment and facilitate mussel collection (Miller et al. 1989).

Sampling with the skimmer dredge is usually conducted along a fixed transect (time or length) and is towed in the upstream direction.

Of the literature reviewed, one source evaluated the use of a skimmer dredge to sample freshwater mussels (Miller et al. 1989), one source reported a towed dredge from historical sampling in Europe (Prié et al. 2017), one source (Lui et al. 2020) discussed the use of a homemade rake dredge (60 cm wide opening) towed over 50 m transects, and two sources discuss towed dredges in their review of freshwater mussel sampling methods (Dunn 1999; Strayer and Smith 2003).

Miller et al. (1989) evaluated the effectiveness of the skimmer dredge in a large, deep (> 1.5 m) river. The authors focused on the skimmer dredge's ability to assess mussel density and species composition and compared its efficiency against handpicking by SCUBA divers. In the study the skimmer dredge was towed along 30 m transects and dredged up to 6 cm of the sediment; nine tows were completed in four hours. Divers followed behind the skimmer dredge collecting mussels that were missed. Miller et al. (1989) reported that the skimmer dredge collected significantly more small mussels than divers, but it collected only 62% of all individuals. Additionally, the skimmer dredge did not successfully collect mussels that were buried more than 6 cm in the sediment and caused 10% mortality of thin-shelled individuals. Also, the skimmer dredge was not equally efficient across species. The authors note, anecdotally, that the skimmer dredge is more cumbersome than a brail, but more efficient. Finally, the study

notes that the skimmer dredge is not suitable in cobble, boulder, or armoured gravel substrates or in areas with a significant number of potential snags. Miller et al. (1989) recommend the skimmer dredge to assess species richness, diversity, and relative abundance during exploratory surveys.

Prié et al. (2017) summarizes historical and contemporary sampling of the Giant Freshwater Pearl Mussel (*Margaritifera auricularia*) in Europe. Collection and monitoring of this species can be challenging because it is often found in the downstream sections of large, turbid, deep, high-velocity rivers. Prié et al. (2017) discuss the use of a "dredger" that was the only method viable to sample the Seine and Eure rivers. These rivers are deep (>6 m sections) and turbid (riverbed not visible). The dredger was towed on transects between 8 and 50 m long, and collected sediment was sorted on the boat. While it is not clear if the dredger was identical to a skimmer dredge, it is similar in principle if not design. Prié et al. (2017) suggest that transect and quadrat sampling via SCUBA are more efficient sampling methods for the Giant Freshwater Pearl Mussel than a towed dredger.

Liu et al. (2020) used a "homemade rake" to collect freshwater mussels from the Yangtze River, China, when depth exceeded 2 m. The Yangtze is a large, turbid river with variable substrate. The rake was towed behind a boat at uniform speeds along 50 m transects. It is unclear how many sites were surveyed using the rake. The rake had a 60 cm opening and dredged material was sorted at the surface to detect freshwater mussels. The efficiency and effectiveness of the rake is not discussed or compared to traditional survey methods that were employed in shallow (< 1.5 m) water.

Dunn (1999) notes that the skimmer dredge is preferred to the brail for confirming mussel presence in a large river, and that the skimmer dredge can reduce the cost of sampling (compared to diving) when sampling a large area. The skimmer dredge is effective in unconsolidated substrate, whereas its effectiveness decreases with an increase in substrate size and consolidation (Dunn 1999). Strayer and Smith (2003) suggest that a rolling dredge would have similar limitations as a brail.

No quantitative information is available on how the effectiveness of a skimmer dredge changes with changes in environmental conditions (e.g., flow) or mussel density.

Benefits: can be used to sample a large area relatively quickly; can utilize tow time or length survey design; some substrate is excavated; captures smaller mussels than handpicking by divers; and is not limited by site depth or turbidity.

Limitations: captures significantly fewer animals than handpicking by divers; ineffective in all but unconsolidated substrate; may not be effective in extremely high flow; capture efficiency is not consistent across species; and causes mortality in thin-shelled species.

SONAR

The use of sonar (the process of emitting sound waves through water and measuring aspects of the reflected wave) technology to detect mussels has increased

dramatically in the last decade. Traditional methods include single-beam sonar with down imaging or side imaging (side-scan) that interpret reflected waves to give a detailed picture of the substrate below the surface of the water.

Four sources were discovered that evaluated the utility of sonar to detect mussel beds and individual mussels in freshwater habitats.

Kaeser et al. (2019) evaluated the use of side-scan sonar to define freshwater mussel habitat associations in the Apalachicola River, USA. The Apalachicola River is a large, turbid river. A Hummingbird 1198c side-imaging (SI) system was used to collect mesohabitat information from the riverbed and assign bottom-type classifications during spring high flows. Habitat occupancy was verified via SCUBA in situ mussel surveys. Two surveys were required to capture the necessary sonar images. SCUBA divers used cables to delineate six random sampling points in each of the five mesohabitat types per survey area defined by sonar imagery. Divers were tethered to a dive block for safety in fast-flowing conditions. The delineated site area (5 to 10 m^2) was excavated to 10 cm and sorted at the surface to detect mussels. Combined mussel habitat occupancy (sonar) and abundance data (quantitative surveys) were used to estimate the spatial distribution and abundance of a rare mussel across the entire river system. In situ mussel surveys took ~ 1,900 hours to complete (from 2012 to 2017).

Powers et al. (2015) investigated the ability of an inexpensive (~ $2000 USD) side-scan sonar unit mounted on a canoe to identify freshwater mussel beds in a large river. The authors report that a priori reference images of exposed mussels on sand sediment are useful for interpreting field sonar images. The study was conducted during both spring freshet and base flow conditions, in less than 2 m depth, and where turbidity was 20 NTU. Powers et al. (2015) found that surveys during the spring freshet were more effective for locating mussel beds than during base flow conditions, and that mussels exposed on, or partially buried in, sand and clay were easily identified. However, drawbacks included a lack of species identification; inability to observe mussels not fully exposed on the sediment surface; increasing depth significantly limited ability to detect mussel beds; and mussels in pebble, cobble or silt substrates were impossible to detect. Powers et al. (2015) suggest that a more powerful sonar unit would improve image quality at depths greater than 2 m. The authors conclude that side-scan sonar detection of mussel beds is an effective tool for preliminary mussel surveys in depths of 1 to 2 m. However, Powers et al. (2015) did not report the density of mussels in the area where the test was conducted, and it is not known how mussel density would impact the effectiveness of this method.

Smit and Kaeser (2016) evaluated the ability of a low-cost, side-scan sonar unit to map mussel habitat and identify flow refugia in order to predict mussel occurrence in a large, turbid river. Side-scan sonar was used to determine habitat conditions during high- and low-flow events and develop a mesohabitat classification scheme based on substrate type and location in the river channel. The mesohabitat scheme was used to develop a stratified mussel survey. Field surveys were conducted via visual or tactile quadrat sampling in each of the mesohabitat types. Depending on depth, snorkeling or SCUBA was used to survey the quadrat. Visual and tactile searchers were followed by excavation of the sediment to 10 cm. Sampling depths ranged from 0.6 to 4.3 m. This

study did not report the density of mussels in the sample area and the side-scan sonar was not used to actively discover mussels, but rather potential habitat.

White et al. (2019) used a Ping DSP 450 sonar instrument to collect sonar imagery of substrate type in the Congaree River, South Carolina, USA. The surveys were completed during high flow in the spring and fall. Sediment was also collected for grain-size analysis. Habitat classifications were developed based on the sonar data and habitat maps were created. The maps were overlain with existing freshwater mussel presence and abundance data to associate freshwater mussel presence with habitat types in the river. Mussels were more frequently associated with shallow depths (1–3 m) and small riffles. The authors note that sonar calibration and habitat classification can be challenging, especially with respect to bathymetric roughness.

Benefits: useful for large scale reconnaissance surveys to detect mussels and their habitat in large rivers; non-invasive imaging could be used to count individuals exposed on substrate surface; relatively inexpensive; and can be used in high-flow conditions.

Limitations: increasing turbidity and depth reduce utility of inexpensive side-scan sonar devices; not suitable for use in pebble, cobble, or silt habitats; cannot inform density or population demographic estimates; and difference in effectiveness among mussel species and sizes is not known.

SUCTION DREDGE

A suction dredge is a tool that is used in hydraulic gold mining in fresh water. Variations of a suction dredge have also been infrequently used to sample benthos in freshwater habitats. The dredge is composed of a high-pressure water pump that mobilizes and vacuums sediment into an intake pipe, which moves the sediment to a sorting bag or holding mechanism. The pump and sorting bag are usually transported on board a boat, and the intake pipe is moved along the river bottom to sampling points. Benthic organisms, including mussels, may be collected by the suction dredge if they can pass into and through the intake pipe.

One source was reviewed that used a suction dredge to collect freshwater mussels (Haag and Warren 2007). The study was conducted in an impounded and regulated portion of a large river. Haag and Warren (2007) do not discuss why they elected to use a suction dredge for mussel collection; however, all sites in the impoundment were 3 m or greater in depth. The authors used a systematic sampling array approach based on grid cells (4 km^2) to determine sampling points. At each site two replicate subsamples of the substrate were taken using the suction dredge — one off each side of the boat at the centroid of the grid cell. Each sample consisted of 2.5 m^2 of excavated substrate to a depth of 15 cm using the gas-powered suction dredge. The dredge was operated and put in position by an accompanying SCUBA diver. The intake pipe was limited to mussels less than 80 mm in size. Mussels that would not fit in the intake pipe were handpicked by the diver. Vacuumed sediment was sieved on shore to collect mussels. Haag and Warren (2007) reported that the flow during sampling was

0.02-0.16 m/s, and that mussel density was estimated as low (< 2 mussels/m^2) and high (> 10 mussels/m^2). Substrate in the impoundment consisted largely of silt, silt-sand, clean sand, or sand-gravel (Haag and Warren 2007).

Haag and Warren (2007) did not report the proportion of mussels not collected by the suction dredge, and did not discuss the influence of flow, substrate, or mussel species and density on the effectiveness of the suction dredge to collect mussels.

Benefits: surface and subsurface mussels are collected; supports quantitative estimates of population demographics and density; consistent sampling across sites; and useful in a variety of visibility scenarios.

Limitations: likely not suitable for use in consolidated or large particle substrate; likely less effective in high-flow environments; large mussels must be hand-collected by an accompanying diver; and destructive to the benthic environment.

CONCLUSION

One hundred and ten sources were reviewed in this report to highlight the benefits and limitations of methods used to survey freshwater mussels in deep, turbid, and fast-flowing environments. The most frequently used and recommended technique for use in deep water was diving (SCUBA or surface-supplied air). However, turbidity and high flow can reduce the effectiveness of mussel surveys conducted by divers. Therefore, under these conditions a modified quadrat sieve apparatus has the potential to improve excavation effectiveness by divers. Other techniques were found to be useful for preliminary surveys (e.g., brail, skimmer dredge); however, the effectiveness of these often depended on the habitat type and diving would still be required to collect population demographics or to estimate densities. Finally, novel techniques, including autonomous underwater vehicles, eDNA, electrofishing, and sonar, were reviewed showing some positive aspects but the effectiveness of each of these methods was variable and most were unable to provide quantitative information on demographics or densities.

This information will be used to inform the development of a guidance document for turbid, high flow, deep-water mussel habitat in Canada. Based on this review, diving is the most effective means of mussel surveys in deep-water habitat. However, a combination of techniques may be useful depending on the objectives of the survey.

ACKNOWLEDGEMENTS

Funding for this contract was provided by the Species at Risk program. Thanks to Scott Reid and Jessica Epp-Martindale for reviewing earlier drafts of this technical report.

REFERENCES

Amberg, J.J., Merkes, C.M., Stott, W., Rees, C.B., and Erickson, R., A. 2019. Environmental DNA as a tool to help inform zebra mussel, *Dreissena polymorpha*, management in inland lakes. Manag. Biol. Invasions. **10**(1): 96–110.

Biodrawversity LLC. 2015. Freshwater mussel survey in the lamprey river. Prepared for Lamprey River Advisory Committee. Available from www.lampreyriver.org/UploadedFiles/Files/LRAC_Mussel_Report_Redacted_2015.pdf [accessed January 2018].

Bonar, S. A., Hubert, W.A., and Willis, D.W. (*Editors*). 2009. Standard methods for sampling North American freshwater fishes. American Fisheries Society, Bethesda, Maryland.

Bolotov, I.N., Vikhrev, I.V., Kondakov, A.V., Konopleva, E.S., Gofarov, M.Y., Aksenova, O.V., and Tumpeesuwan, S. 2017. New taxa of freshwater mussels (Unionidae) from a species-rich but overlooked evolutionary hotspot in Southeast Asia. Sci. Rep. **7**: 11573.

Boon, R., Cooksley, S., Geist, J., and Killeen, I. 2019. Developing a standard approach for monitoring freshwater pearl mussel (*Margaritifera margaritifera*) populations in European rivers. Aquat. Conserv. Mar. Freshw. Ecosyst. **29**(8).

Christian, A.D., and Harris, J.L. 2005. Development and assessment of a sampling design for mussel assemblages in large streams. Am. Midl. Nat. **158**: 284–292.

Cawley, E., 1993. Sampling adequacy in population studies of freshwater mussels. *In* Conservation and management of freshwater mussels. Proceedings of an Upper Mississippi River Conservation Committee (UMRCC) symposium, 14 October 1992, St. Louis, Missouri. *Edited by* K.S. Cummings, A.C. Buchanan, and L.M. Koch. Upper Mississippi River Conservation Committee, Rock Island, IL. pp. 168–172.

Coghlan, S.A., Currier, C.A., Freeland, J., Morris, T., and Wilson, C.C. 2021. Community eDNA metabarcoding as a detection tool for documenting freshwater mussel (Unionidae) species assemblages. Environmental DNA. **3**: 1172–1191.

Cummings, D.S., Jones, H.A., and Lopes-Lima, M. 2016. Rapid bioassessment methods for freshwater molluscs. *In* Core Standardized Methods for Rapid Biological Field Assessment, Chapter: Rapid Bioassessment Methods for Freshwater Molluscs. *Edited by* T. H. Larsen. Conservation International. pp: 185–207.

Currier, C.A., Morris, T.J., Wilson, C.C., and Freeland, J. 2018. Validation of environmental DNA (eDNA) as a detection tool for at-risk freshwater pearly mussel species (Bivalvia: Unionidae). Aquat. Conserv. Mar. Freshw. Ecosyst. **1**: 14.

Curtis, A.N., Tiemann, J.S., Douglass, S.A., Davis, M.A., and Larson, E.R. 2020. High stream flows dilute environmental DNA (eDNA) concentrations and reduce detectability. Divers. Distrib. **27**: 1918–1931.

Cvancara, A., M. 1972. Lake mussel distribution as determined with scuba. 1972. Ecology. **53**(1): 154–157.

Duncan, N. 2008. Survey protocol for aquatic mollusk species: preliminary inventory and presence/absence sampling, Version 3.1. Interagency Special Status/Sensitive Species Program. U.S. Department of Interior, Bureau of Land Management, Oregon/Washington and U.S. Department of Agriculture, Forest Service, Region 6. pp: 52. Available from www.blm.gov/or/plans/surveyandmanage/files/10-mollusks_v3-1.pdf [accessed January 2018].

Dunn, H. 1999. Development of strategies for sampling freshwater mussels (Bivalvia: Unionidae). *In* Proceedings of the First Freshwater Mollusk Conservation Society Symposium 1999. pp. 161–167.

Galbraith, H., S. 2012. Phase 1 Freshwater mussel survey and comparison to historical surveys at the Pond Eddy Bridge, Delaware River, New York and Pennsylvania. U.S. Geological Survey Open-File Report. **1224**: 17.

Gasparini, L., Crookes, S., Prosser, R., and Hanner, R. 2019. Detection of freshwater mussels (Unionidae) using environmental DNA in riverine systems. Environmental DNA. **2**: 321–329.

Hagg, W.R., and Warren, M.L. 2007. Freshwater mussel assemblage structure in a regulated river in the Lower Mississippi River Alluvial Basin, USA. Aquat. Conserv. Mar. Freshw. Ecosyst. **17**: 25–36.

Hanfling, B., Handley, L.L., Read, D.S., Hahn, C., Li, J., Nichols, P., Blackman, R.C., Oliver, A., and Winfield, I.J. 2016. Environmental DNA metabarcoding of lake fish communities reflects long-term data from established survey methods. Mol. Ecol. **25**: 3101–3119.

Hanson, J.M., Mackay, W.C., and Prepas, E.E. 1988. Population size, growth, and production of a unionid clam, *Anodonta grandis simpsoniana*, in a small, deep Boreal Forest lake in central Alberta. Can. J. Zool. **66**: 247–253.

Hart, M., Randklev, C., Dickson, J., Ford, N., Hernandez, B., and Schwalb, A. 2016. A literature review of freshwater mussel survey and relocation guidelines. Project Number 0-6865. Final report submitted to Texas Department of Transportation.

Holliman, F.M., Kwak, T.J., Cope, W.G. and Levine, J.F. 2007. Exposure of unionid mussels to electric current: assessing risks associated with electrofishing. Trans. Am. Fish. Soc. **136**: 1593–1606.

Hornbach, D.J., Allen, D.C., Hove, M.C., and MacGregor, K.R. 2018. Long-term decline of native freshwater mussel assemblages in a federally protected river. Freshw. Biol. **63**: 243–263.

Isom, B.G., and Gooch, C. 1986. Rationale and sampling designs for freshwater mussels Unionidae in streams, large rivers, impoundments, and lakes. *In* Rationale for Sampling and Interpretation of Ecological Data in the Assessment of Freshwater Ecosystems. *Edited by* B.G. Isom. Special Technical Publication 894. American Society for Testing and Materials, Philadelphia. pp 46–59.

Karatayev, A.Y. Mehler, K., Burlakova, L.E., Hinchey, E.K., and Warren, G.J. 2018. Benthic video image analysis facilitates monitoring of *Dreissena* populations across spatial scales. J. Great Lakes Res. **44**(4): 629–638.

Kaeser, A.J., Smit, R., and Gangloff, M. 2019. Mapping and modeling the distribution, abundance, and habitat associations of the endangered fat threeridge in the Apalachicola River system. J. Fish Wild. Manag. 10(2): 653.

Keretz, S.S., Woolnough, D.A., Morris, T.J., Roseman, E.F., Elgin, A.K., and Zanatta. D. 2021. Limited co- existence of native unionids and invasive dreissenid mussels more than 30 y post dreissenid invasion in a large river system. Am. Midl. Nat. **186**: 157–175.

Klymus, K.E., Richter, C.A., Thompson, N., Hinck, J.E., and Jones, W.W. 2020. Metabarcoding assays for the detection of freshwater mussels (Unionida) with environmental DNA. Environmental DNA **3**(1): 231–247.

Kriege, M.D. 2018. Freshwater mussels of the Greenup Navigational Pool, Ohio River, with a comparison to fish host communities. Marshall University Theses, Dissertations and Capstones Digital Scholar.

Kovalak, W.P., Dennis., D.S., and Bates., J.M. 1986. Sampling effort required to find rare species of freshwater mussels. *In* Rationale for Sampling and Interpretation of Ecological Data in the Assessment of Freshwater Ecosystems. *Edited by* B.G. Isom. Special Technical Publication 894. American Society for Testing and Materials, Philadelphia, pp. 34-45.

LeBlanc, F., Steeves, R., Belliveau, V., Akaishi, F., and Gagné, N. 2021. Detecting the Brook Floater, a freshwater mussel species at risk, using environmental DNA. Aquat. Conserv. Mar. Freshw. Ecosyst. **31**: 1233–1244.

Lor, Y., Schreier, T.M., Waller, D.L., and Merkes, C.M. 2020. Using environmental DNA (eDNA) to detect the endangered Spectaclecase Mussel (*Margaritifera monodonta*). Freshw. Sci. **39**(4): 837–847.

Liu, X., Lopes-Lima, M., Xue, T., Zhou, Y., Li, K., Xu, Y., Qin, J., Ouyang, S., and Wu, X. 2020. Changes and drivers of freshwater mussel diversity patterns in the middle and lower Yangtze River Basin, China. Glob. Ecol. Cons. **22** (2020) e00998.

Mackie, G., Morris, T.J., and Ming, D. 2008. Protocol for the detection and relocation of freshwater mussel species at risk in Ontario-Great Lakes Area (OGLA). Can. Manuscr. Rep. Fish. Aquat. Sci. **2790**: vi + 50 p.

Mauvisseau, Q., Burian, A., Gibson, C., Brys, R., Ramsey, A., and Sweet, M. 2019. Influence of accuracy, repeatability and detection probability in the reliability of species-specific eDNA based approaches. Sci. Rep. **9**: 580.

McAlpine, D., F., and Sollows, M., C. 2014. A Quadrat-Sieve System for Sampling Freshwater Mussels Using SCUBA. Northeast. Nat. **21**: N1–N4

McNichols-O'Rourke, K.A., Meilutis, A., and Morris, T J. 2018. The Lost Mussels of Pelee Island. Program Schedule. *In* Proceedings of the 2017 Canadian Freshwater Mollusc Research Meeting: November 8–9, 2017, Burlington, Ontario. Can. *Edited by* T. J. Morris, K. A. McNichols-O'Rourke, and S. M. Reid. Can. Tech. Rep. Fish. Aquat. Sci. **3246**: viii + 26 p.

Meador, J.R., Peterson, J.T., and Wisniewski, J.M. 2011. An evaluation of the factors influencing freshwater mussel capture probability, survival, and temporary emigration in a large lowland river. J. N. Am. Benthol. Soc. **30**(2): 507–521.

Metcalfe-Smith, J.L., McGoldrick, D.J., Zanatta, D.T., and Grapentine, L.C. 2007. Development of a monitoring program for tracking the recovery of endangered freshwater mussels in the Sydenham River, Ontario. Water Science and Technology Directorate, Environment Canada, Burlington, Ontario. 07-510: 61 p.

Miller, A.C., and Payne, B.S. 1993. Qualitative versus quantitative sampling to evaluate population and community characteristics at a large-river mussel bed. The Am. Midl. Nat. **130**(1): 133–145.

Miller, A.C., Whiting, R., and Wilcox, D.B. 1989. An evaluation of a skimmer dredge for collecting freshwater mussels. J. Freshw. Ecol. **5**(2): 151–154.

Ontario Ministry of Natural Resources and Forestry (OMNRF). 2018. Survey protocol for species at risk unionid mussels in wetlands in Ontario. Species Conservation Policy Branch. Peterborough, Ontario. ii + 30 pp. Available from https://files.ontario.ca/survey_protocol_for_sar_wetland_mussel_species_2018_.pdf [accessed January 2018].

Powers, J., Brewer, S.K., Long J.M., and Campbell, T. 2015. Evaluating the use of side-scan sonar for detecting freshwater mussel beds in turbid river environments. Hydrobiologia. **743**(1): 127–137.

Prié, V., Soler, J., Araujo, R., Cucherat, X., Philippe, L., Patry, N., Adam, B., Legrand, N., Jugé, P., Richard, N., and Wantzen, K.M. 2017. Challenging exploration of troubled waters: a decade of surveys of the giant freshwater pearl mussel *Margaritifera auricularia* in Europe. Hydrobiologia. **810**(1): 157–175.

Prié, V., Valentini, A., Lopes-Lima, M., Froufe, E., Rocle, M., Poulet, N., Taberlet, P., and Dejean, T. 2021. Environmental DNA metabarcoding for freshwater bivalves biodiversity assessment: methods and results for the Western Palearctic (European sub-region). Hydrobiologia. **848**(1): 2931–2950.

Randklev, C.R., Miller, R., Hart, M., Morton, J., Johnson, N.A., Skow, K., Inoue, K., Tsakiris, E.T., Oetker, S., Smith, R., Robertson, C., and Lopez, R. 2018. A semi-arid river in distress: Contributing factors and recovery solutions for three

imperiled freshwater mussels (Family Unionidae) endemic to the Rio Grande basin in North America. Sci. Total Environ. **631–632**: 733–744.

Reed, M.P., Dinkins, G.R, and Ahlstedt, S.A. 2019. Freshwater mussels (Bivalvia: Margaritiferidae and Unionidae) of the Buffalo River drainage, Tennessee. Southeastern Nat. **18**(2): 346–372.

Reid, S. and LeBaron, A. 2019. Lower Grand River freshwater mussels: Results from brail sampling of non-wadeable habitats. *In* Morris, T.J., McNichols-O'Rourke, K. A., and Reid, S.M. (*Editors*). 2020. Proceedings of the 2019 Canadian Freshwater Mollusc Research Meeting: December 3–4, 2019, Burlington, Ontario. Can. Tech. Rep. Fish. Aquat. Sci. **3352**: viii + 34 p.

Reid, S.M., LeBaron, A., and Morris, T.J. 2018. Can adaptive cluster sampling improve Ontario mussel species at risk monitoring? Can. Manuscr. Rep. Fish. Aquat. Sci. **3152**: iv +16p.

Reid, S.M. and Morris, T.J. 2017. Tracking the recovery of freshwater mussel diversity in Ontario rivers: evaluation of a quadrat-based monitoring protocol. Diversity. **9**: 1–17.

Rice, C.J., Larson, E.R., and Taylor, C.A. 2018. Environmental DNA detects a rare large river crayfish but with little relation to local abundance. Freshw. Biol. **63**(5): 443–455.

Rider, T., Wilcut, L., Barash, S., and Robiou, G. 2013. Technical support document for conducting and reviewing freshwater mussel occurrence surveys for the development of site-specific water quality criteria for ammonia. U.S. Environmental Protection Agency Office of Water. https://goo.gl/nbczzF [accessed January 2018].

Roghair, C.N., Nuckols, D.R., and Haag, W.R. 2005. Establishment of a monitoring program for freshwater mussels in the Chattooga River, SC and GA. USDA Forest Service Report. Blacksburg, VA. Available from www.srs.fs.usda.gov/catt/pdf/sc/2005_sc_catt_report.pdf [accessed January 2018].

Salonen, J., and Taskinen, J. 2017. Electrofishing as a new method to search for unknown populations of the endangered freshwater pearl mussel *Margaritifera margaritifera*. Aquat. Conserv. Mar. Freshw. Ecosys. **27**(1): 115–127.

Sanchez, B., and Schwalb, A.N. 2021. Detectability affects the performance of survey methods: a comparison of sampling methods of freshwater mussels in Central Texas. Hydrobiologia **848**(12–13): 2919–2929.

Sansom, B.J., and Sassoubre, L.M. 2017. Environmental DNA (eDNA) shedding and decay rates to model freshwater mussel eDNA transport in a river. J. Environ. Sci. Technol. **5**(24): 14244–14253.

Schmidt, B.C., Spear, S.F., Tomi, A., and Bodinof Jachowski., C.M. 2021. Evaluating the efficacy of environmental DNA (eDNA) to detect an endangered freshwater mussel *Lasmigona decorata* (Bivalvia: Unionidae). Freshw. Sci. **40**(2): 354–367.

Schueler, F., and Robson, R. 2018. Brailing the Plantagenet Reach: Not catching anything where there may not be anything to catch. *In* Proceedings of the 2017 Canadian Freshwater Mollusc Research Meeting: November 8–9, 2017, Burlington, Ontario. Can. *Edited by* T. J. Morris, K. A. McNichols-O'Rourke, and S. M. Reid. Can. Tech. Rep. Fish. Aquat. Sci. **3246**: viii + 26 p.

Shogren, A.J., Tank, J.L., Andruszkiewicz, E., Olds, B., Mahon, A.R., Jerde, C.L., and Bolster, D. 2017. Controls on eDNA movement in streams: Transport, Retention, and Resuspension. Sci. Rep. **7**: 5065.

Sietman, B.E., Whitne, S.D., Kelner, D.E., Blodgett, K.D., and Dunn, H.L. 2001. Post-extirpation recovery of the freshwater mussel (bivalvia: unionidae) fauna in the upper Illinois River. J. Freshw. Ecol. **16**(2): 273–281.

Singh, W. 2015. Towards efficient benthic survey design with the use of Autonomous Underwater Vehicles. PhD Thesis. School of Engineering and Natural Sciences, Faculty of Physical Sciences. University of Iceland. Available from https://skemman.is/bitstream/1946/22561/1/PhD_Thesis_WarshaSingh.pdf [accessed January 2018].

Smit, R., and Kaeser, A. 2016. Defining freshwater mussel mesohabitat associations in an alluvial, Coastal Plain river. Freshw. Sci. **35**(4): 1276–1290.

Smith, D.R., Villella, R.F., and Lemari, D.P. 2001. Survey protocol for assessment of endangered freshwater mussels in the Allegheny River, Pennsylvania. Freshw. Sci. **20**(1): 118–132.

Smith, D.R., Rogala, J.T., Gray, B.R., Zigler, S.J., and Newton, T.J. 2011. Evaluation of single and two-stage adaptive sampling designs for estimate of density and abundance of freshwater mussel sin a large river. River Res. Appl. **27**(1): 121–133.

Strayer, D.L., and Smith, D.R. 2003. A guide to sampling freshwater mussel populations. American Fisheries Society Monograph 8. Bethesda, Maryland.

Strayer, D.L., Downing, J.A., Haag, W.R., King, T.L., Layzer, J.B., Newton, T.J., and Nichols, S., J. 2004. Changing perspectives on pearly mussels, North America's most imperiled animals. Bio.Sci. **54**: 429–439.

Stoeckle, B.C., Beggel, S., Kuehn, R., and Geist, J. 2021. Influence of stream characteristics and population size on downstream transport of freshwater mollusk environmental DNA. Freshw. Sci. **40**(1): 191–201.

Stoeckl, K., Denic, M., and Geist, J. 2019. Conservation status of two endangered freshwater mussel species in Bavaria, Germany: Habitat quality, threats, and implications for conservation management. Aquat. Cons: Mar. Freshw. Ecosyst. **30**: 647–661.

Thiel, P. 1981. A survey of unionid mussels in the upper Mississippi River. Technical Bulletin No. 124. Wisconsin Department of Natural Resources. Madison, Wisconsin.

Wacker, S., Fossøy, F., Mejdell Larsen, B., Brandsegg, H., Sivertsgård, R., and Karlsson, S. 2019. Downstream transport and seasonal variation in freshwater pearl mussel (*Margaritifera margaritifera*) eDNA concentration. Environmental DNA. **1**: 64–73.

Wegscheider, B., MacLean, H., Linnansaari, T., and Curry, R., A. 2019. Freshwater mussel abundance and species composition downstream of a large hydroelectric generating station. Hydrobiologia. **836**(1): 207–218.

White, S., Koehane, I., Woodford, E., and Burstein, J. 2019. Congaree River Mussel Habitat Sonar Survey. Final report submitted to USFWS and to Congaree National Park.

WVDNRWRS (West Virginia Division of Natural Resources Wildlife Resources Section). 2020. West Virginia Mussel Survey Protocols. Available from https://wvdnr.gov/wp-content/uploads/2021/07/2020-WV-Mussel-Survey-Protocols.pdf [accessed March 2022].

ANNOTATED BIBLIOGRAPHY

1. Ahlstedt, S.A., Jones, J.W., and Walker, C. 2017. Current status of freshwater mussel populations in the Clinch River at the Appalachia Power Company's Clinch River Steam Plant, Russel County, Virginia (Clinch River Miles 268.3-264.2). Malacol. Rev. **45–46**: 213–225.

   Surveyed shallow water areas of Clinch River 30+ years after a pollution event. Upstream and downstream ends of mussel beds were delineated based on visual inspection of the substrate for what is suitable to mussels (e.g., search area limited to suitable gravel substrate and excluded bedrock or soft sediments), water depth, flow velocity and absence of mussels. The study used a systematic sampling design by placing quadrats (0.25 m^2) along a transect line. Quadrats and transects were spaced evenly across shoal area of site. All 0.25 m^2 quadrats were excavated to approximately 20 cm depth and sieved. Qualitative sampling with timed searches was used to supplement spatial coverage and where mussels were expected to occur at low densities to find rare species.

2. Aldridge, D., Fayle, T., and Jackson, N. 2007. Freshwater mussel abundance predicts biodiversity in UK lowland rivers. Aquat. Cons. Mar. Freshw. Ecosyst. **17**(6): 554–564.

   Study examined if mussels can be used for rapid biodiversity assessment given "no expert knowledge" is required for identification as compared to aquatic insects. Thirty sites were assessed and the rivers ranged in width from 3 to 50 m and maximum depths of 0.5 to 4 m. Rivers were lowland rivers characteristic of UK. Rivers had sluggish summer flow, predominantly silt sediment, and macrophyte cover. Mussels surveyed at 30 sites using 0.5 X 0.5 m quadrats. Preliminary surveys used to estimate sampling sufficiency (# of quadrats). Fifteen randomly placed quadrats were hand searched within 1-3 m from the bank in 4 rivers. Quadrats were subdivided into 25 sectors and systematically searched by tactile means due to low visibility. No information on how depths >1.5 m were sampled.

3. Amberg, J.J., Merkes, C.M., Stott, W., Rees, C.B., and Erickson, R.A. 2019. Environmental DNA as a tool to help inform zebra mussel, *Dreissena polymorpha*, management in inland lakes. Manag. Biol. Invasions. **10**(1): 96–110.

   The authors evaluated the use of eDNA methods to detect Zebra Mussels in lakes in Minnesota, USA. The surveys were conducted in the fall and spring in lakes that had been either recently invaded and or had a long history of Zebra Mussel presence. The study used the COI gene for eDNA assays. Water samples were collected at the water surface, mid water column, and at the sediment surface using a Van Dorn water sampler. The samples were taken

along four transects moving from shore to the middle of the lake across habitat types. The maximum sampling depth was 6 m. SCUBA divers were used to complete quadrat surveys to estimate the density of Zebra Mussels at a site. eDNA detection was highest in bottom water samples and over softer substrates.

4. Baisley, K.L., and Bredin, K. 2009. Freshwater mussel survey for the Miramichi River Watershed. Miramichi River Environmental Assessment Committee. Available from http://mreac.org/wp-content/uploads/2015/01/Mussel_Report_MREAC09.pdf [accessed January 2018].

   Freshwater mussel surveys were conducted on the Miramichi River in New Brunswick to inform species distribution and presence in the river. Mussel surveys were completed by teams of 2–3 people searching a site for four person-hours. Each team member used a glass-bottom viewing bucket and searched different sections of the survey site. Shoreline searches for dead shells were also conducted. Waters deeper than 1.2 meters were not surveyed, excluding larger, deeper tributaries from the analysis.

5. Bales, S.A., Price, A.L., and Shasteen, D.K. 2012. Freshwater mussels of the Rock River. INHS Technical Report 2012 (17). Available from https://www.dnr.illinois.gov/conservation/iwap/documents/mussel%20basin%20surveys/rock_mussels.pdf [accessed January 2018].

   The objective of this study was to obtain systematically collected freshwater mussel distribution and abundance information from 33 sites in Illinois. Surveyors searched visually for mussels (e.g., trails, siphons, exposed shell) during ideal water conditions. Surveys consisted of a timed search of 4 person hours. The survey sites included all habitat types present including riffles, pools, slack water, and areas of differing substrates. No information was provided on sampling in waters deeper than 1.5 m.

6. Biodrawversity LLC. 2015. Freshwater mussel survey in the Lamprey River. Prepared for Lamprey River Advisory Committee. Available from www.lampreyriver.org/UploadedFiles/Files/LRAC_Mussel_Report_Redacted_2015.pdf [accessed January 2018].

   A qualitative mussel survey was conducted to assess the presence and relative abundance of mussels in the Lamprey River. Surveys were adaptive and surveyors spent more time in suitable Brook Floater habitat. Snorkeling and SCUBA were used, according to the site depth with SCUBA being used in deeper water. The report did not provide information on the time or area searched or if transects were used to limit the search area. A semi-quantitative survey was conducted at another site on the same river: a complete visual survey (snorkel) of the site was completed by establishing transects 5–10 m

apart within the 400 m reach. Six quadrats were placed evenly along each transect and visually searched; quadrats were not excavated. Water depth at all sites was less than 1 m.

7. Bolotov, I.N., Vikhrev, I.V., Kondakov, A.V., Konopleva, E.S., Gofarov, M.Y., Aksenova, O.V., and Tumpeesuwan, S. 2017. New taxa of freshwater mussels (Unionidae) from a species-rich but overlooked evolutionary hotspot in Southeast Asia. Sci. Rep. **7**: 11573.

   Freshwater mussels were sampled to determine the level of endemism in rivers in Myanmar. Collection sites were extremely turbid. Survey design and collection methods are not described in the paper; however, the author provided the following information when contacted. “We did not use any special sampling approach and collected mussels by hand or by dredge…and our collection localities were not deep, up to 3 m, but mostly 0.5–1 m during the dry period.” (I. N. Bolotov, Northern Arctic Federal University, Arkhangelsk, Russian Federation, 2018, personal communication).

8. Boon, R., Cooksley, S., Geist, J., and Killeen, I. 2019. Developing a standard approach for monitoring freshwater pearl mussel (*Margaritifera margaritifera*) populations in European rivers. Aquat. Conserv. Mar. Freshw. Ecosyst **29**(8).

   The standard method development proposed techniques to survey for the freshwater pearl mussel in Europe. Preliminary surveys of potential sites are recommended followed by baseline surveys for detection and to estimate population size and recruitment. The methods recommended include wading and the use of viewing boxes or SCUBA for preliminary surveys and at sites where mussels are present, evaluating population size based using a transect approach. Quadrat excavation was recommended along the transect route. Surveys should be completed in low flow, high visibility conditions.

9. Braun, C.L., Stevens, C.L., Echo-Hawk, P.D., Johnson, N., and Moring, J.B. 2014. Abundance of host fish and frequency of glochidial parasitism in fish assessed in field and laboratory settings and frequency of juvenile mussels or glochidia recovered from hatchery-held fish, Central and Southeastern Texas. Scientific Investigations Report 2014-5217. USGS.

   This study evaluated the relationship between fish host abundance and frequency of parasitism by endemic freshwater mussel species in Texas. Mussels were collected from all habitats within the sampling reach, following a qualitative random timed search approach and utilizing wading, snorkeling, and viewing boxes to detect animals (Strayer and Smith 2003).

10. Brown, K.M., and Banks, PD. 2001. The conservation of unionid mussels in Louisiana rivers: diversity, assemblage composition and substrate use. Aquat. Cons. Mar. Freshw. Ecosyst. **11**(3): 189–198.

Qualitative freshwater mussel surveys were conducted in southeastern Louisiana, USA focusing on areas with limited mussel community information. Timed searches were used to increase the likelihood of encountering a rare species. Sampling sites consisted of ~ 2 km reaches and all habitat types were surveyed. At each site, two surveyors spent 45 minutes visually searching the reach using snorkeling equipment. The authors do not report site depth or turbidity levels, but sampling occurred during low flow.

11. Cawley, E., 1993. Sampling Adequacy in population studies of freshwater mussels. *In* K.S. Cummings, A.C. Buchanan, and L.M. Koch (*Editors*). Conservation and Management of Freshwater Mussels. Proceedings of an Upper Mississippi River Conservation Committee (UMRCC) Symposium, 14 October 1992, St. Louis, Missouri. Upper Mississippi River Conservation Committee, Rock Island, IL. pp: 168–172.

Historical sampling of freshwater mussels on the Mississippi River, USA was compared to inform sample size sufficiency of future surveys. The authors report that handpicking by divers was the predominant sampling method, followed by brail surveys. The study generalized that divers collected more animals compared to brailing given the time expended, and that multiple small sampling sites with subsamples along transects was more efficient than random searches by divers. Habitat variables associated with historical sampling were not provided (e.g., depth, turbidity, and flow).

12. Christian, A.D., and Harris, J.L. 2005. Development and assessment of a sampling design for mussel assemblages in large streams. Am. Midl. Nat. **158**: 284–292.

The objective of this study was to propose a quantitative survey method for sampling freshwater mussels in a large river and to determine the effectiveness of the method. Survey sites ranged in depth from 2 to 10 m, with an average depth of 5 m, and where visibility was effectively zero (<0.01 m). Questionnaires were sent to commercial mussel harvesters to determine locations of historical mussel abundance. Interview responses were combined with available habitat information to delineate the survey reach. Preliminary surveys of candidate sites were conducted along three transects, searched by a surface-supplied air diver. Based on the preliminary assessment of mussel density the site was classified as low, medium, or high density. Low-density mussel beds were qualitatively searched, while medium- and high-density mussel beds were searched using 1 m^2 quadrats. Quadrats were excavated but excavation depth was not reported. At a survey reach, between 0.3 and 2% of mussel beds were surveyed with

quadrats. The authors report that it took 94 person-days to sample 68 river kilometres with both qualitative and quantitative methods.

13. Crail, T.D., Krebs, R.A., and Zanatta, D.T. 2011. Unionid mussels from nearshore zones of Lake Erie. J. Great Lakes Res. **37**(1): 199–202.

    The objective was to determine the native freshwater mussel composition of potential dreissenid refugia in the western basin of Lake Erie. The authors relied on natural seiche of the large lake to expose and search shoreline areas that would normally be non-wadeable. Timed searches were conducted along the shoreline in addition to sampling 4 by 100 m^2 quadrats. Quadrats were not excavated. Sites were searched visually and by tactile means. The mean density of freshwater mussels, estimated only from mussels collected at the sediment surface, was 0.09 mussels/m^2.

14. Coghlan, S.A., Currier, C.A., Freeland, J., Morris, T., and Wilson, C.C. 2021. Community eDNA metabarcoding as a detection tool for documenting freshwater mussel (Unionidae) species assemblages. Environmental DNA. **3**: 1172–1191.

    The authors undertook a metabarcoding study using eDNA primers to determine the presence of mussel species in southern Ontario rivers. The results were compared against known mussel populations that have been extensively sampled using traditional methods in wadeable streams (<1.5 m). The authors found that eDNA detection was influenced by water temperature, pH, and turbidity. Metabarcoding allowed for the detection of multiple species in known and novel locations, especially those that had not been sampled with quadrat excavation.

15. Cummings, D.S., Jones, H.A., and Lopes-Lima, M. 2016. Rapid bioassessment methods for freshwater molluscs. *In* Core Standardized Methods for Rapid Biological Field Assessment, Chapter: Rapid Bioassessment Methods for Freshwater Molluscs. *Edited by* T. H. Larsen. Conservation International. pp: 185–207.

    This report presents a synthesis of freshwater mussel sampling protocols in support of developing a rapid biological assessment survey. The synthesis focused on wadeable streams. Qualitative surveys are outlined as including dip net sweeps, visual/tactile search, and use of a brail. Quantitative surveys are outlined as including dredging, benthic grabs, quadrats (with excavation), and line transects distributed over a defined area. Semi-quantitative surveys included visual and tactile techniques of quadrats or over a fixed area. Stratified random sampling in combination with a preliminary reconnaissance survey is recommended for spatially aggregated populations. The report states that at low densities (< 1 mussel/m^2) at least 100 x 0.25 m^2 quadrats would be required to

achieve 25% precision of density estimates. Diving with transect sampling and the use of dredges are reported as important for sampling mussels in deep-water habitats.

16. Currier, C.A., Morris, T.J., Wilson, C.C., and Freeland, J. 2018. Validation of environmental DNA (eDNA) as a detection tool for at-risk freshwater pearly mussel species (Bivalvia: Unionidae). Aquat. Cons. Mar. Freshw. Ecosyst. **1**:14.

    Currier et al. collected water samples from long-term monitoring locations in a wadeable river in southern Ontario, in order to validate species-specific markers for four freshwater mussel species at risk. The authors found positive eDNA detections for target species in all sites that had positively detected mussel SAR using quadrat sampling. eDNA was able to detect target species at densities as low as 0.03 mussels/m^2. The authors also demonstrated that eDNA concentrations were positively correlated with mussel densities (estimated via quadrat surveys). Resuspended sediment (and therefore mobilized eDNA) was suspected to cause higher than expected eDNA copy estimates given mussel densities.

17. Curtis, A.N., Tiemann, J.S., Douglass, S.A., Davis, M.A., and Larson, E.R. 2020. High stream flows dilute environmental DNA (eDNA) concentrations and reduce detectability. Divers. Distrib. **27**: 1918–1931.

    This paper evaluated the influence of stream flow on freshwater mussel eDNA concentrations and detectability in two streams in Illinois, USA. The authors further compared eDNA detection between summer and fall seasons in eight streams. Study sites had variable flow (from low to high among sites) and density of mussel aggregations (low to high). Turbidity was high at all sites. High flow was found to decrease eDNA concentrations and floods resulted in false negative detections. The authors suggest that eDNA is diluted during high-flow events. For summer spawning species, eDNA detections were higher in summer than fall. Sampled streams have high conductivity, low pH, high TDS, and turbidity. Density of mussels affected eDNA detection: eDNA detection required three water samples at low densities (<15 individuals/m^2) and one water sample at high densities (>85 individuals/m^2).

18. Cvancara, A.M. 1972. Lake mussel distribution as determined with scuba. Ecology. **53-1**: 154–157.

    The objective of this study was to characterize the mussel community of Long Lake in Minnesota, USA. The lake was clear with high visibility and limited flow. The entire lakebed was searched using 20 m by 1.7 m belts placed and staked on the lakebed by divers. Divers searched for mussels using visual and tactile methods at six stations along the transect. The transects were placed parallel to the shoreline along depth contours. The authors found that mussel density

decreased with increasing depth in the lake and no mussels were found below 9 m. Mussel density decreased with increasing amounts of vegetation.

19. Cyr, H. 2020 Site exposure, substrate, depth, and the thermocline affect the growth of native unionid mussels in a stratified lake. Freshw. Sci. **39**(4): 773–790.

    The author collected mussels from shallow, littoral (~ 4 m), and deep (6 - 7 m) sites in the south arm of Lake Opeongo, Ontario. Surveys were completed in 2006 and 2007. SCUBA was used to collect mussels in > 2 m depth using transects, quadrats, and haphazard collection. The study did not evaluate differences in sampling technique.

20. Daniel, W.M., and Brown, K.M. 2014. The role of life history and behavior in explaining unionid mussel distributions. Hydrobiologia. **734**(1): 57–68.

    The authors evaluated whether life history and behavioural traits of freshwater mussels explained the longitudinal pattern of species distribution within watersheds. Semi-quantitative timed-search methods were used to collect mussels: two snorkelers spent 45 minutes surveying a 300 m long sample reach. All sites were < 2 m deep, and were relatively clear, but with varying velocity. The results suggest that shell shape, thickness, and individual movement patterns play a role in mussel distribution in rivers.

21. Daraio, J.A., Weber, L.J., Zigler, S.J., Newton, T.J., and Nestler J.M. 2012. Simulated effects of host fish distribution on juvenile unionid mussel dispersal in a large river. River Res. Appl. **28**(5): 594–608.

    This paper simulated juvenile freshwater mussel dispersal in the Mississippi River, following glochidial transformation. A three-dimensional model was used and no live mussels were collected for this study.

22. Davis, E.A., David, A.T., Norgaard, K.M., Parker, T.H., McKay, K., Tennant, C., Soto, T., Rowe, K., and Reed, R. 2013. Distribution and Abundance of Freshwater Mussels in the mid Klamath Subbasin, California. Northwest Sci. **87**(3): 189–206.

    The Klamath River in California, USA was surveyed to determine the distribution and abundance of freshwater mussels. Given the scarcity of existing information, the river was divided into classified channel units based on flow and substrate characteristics (size/type) and 82, 50 m sites were surveyed. Sites were searched visually by snorkeling parallel to the shoreline, avoiding swift water and the mid-channel of the river. No excavation was completed. Flow at sites was moderate to low. Site depth and turbidity was not reported, but visibility was assumed to be high for visual surveys.

23. Duncan, N. 2006. Mussel survey techniques and results of 2006 surveys in the Umpqua Basin, Douglas County, OR. Roseburg District Bureau of Land

Management, Roseburg. Available from www.fs.fed.us/r6/sfpnw/issssp/documents3/inv-rpt-ibi-mussel-survey-2006.pdf [accessed January 2018].

The South Umpqua River was chosen as a potential site for long term freshwater mussel population monitoring. This report summarizes the survey results of an attempt to find at least two mussel beds at which to establish the monitoring sites. Sites were assessed visually for the presence or absence of mussels. A team of three snorkelers each surveyed 1/3 of the available habitat, moving in the downstream direction using a flotation device. The survey sites were shallow (< 3 m), had low turbidity, and low flow. Four populations were found during the survey that contained more than 20 individuals.

24. Duncan, N. 2008. Survey protocol for aquatic mollusk species: Preliminary inventory and presence/absence sampling, Version 3.1. Interagency Special Status/Sensitive Species Program. U.S. Department of Interior, Bureau of Land Management, Oregon/Washington and U.S. Department of Agriculture, Forest Service, Region 6. pp: 52. Available from www.blm.gov/or/plans/surveyandmanage/files/10-mollusks_v3-1.pdf [accessed January 2018].

This report recommends standardized survey methods to determine the presence or absence of freshwater mussels in a variety of habitats. The recommendations are categorized based on study objectives. The author states that deep-water habitats will likely require snorkeling or SCUBA equipment, depending on visibility and depth. The report recommends at least a 10-minute visual survey of a lake bottom area of 10 m^2, but that time spent surveying will increase as lakebed complexity increases. The author also recommends that when excavation is required, a dredge can be used to collect samples using a grid pattern or at designated intervals along a transect. It is suggested that transects with marked intervals placed parallel to the shoreline and spaced evenly along the lakebed may be the most efficient survey design for divers.

25. Dunn, H. 1999. Development of strategies for sampling freshwater mussels (Bivalvia: Unionidae). *In* Proceedings of the First Freshwater Mollusk Conservation Society Symposium. pp: 161–167.

This paper presents a summary of freshwater mussel sampling methods. It outlines that the method chosen should be based on the study objectives. The summary briefly mentions sampling in deep water, including large rivers. In these environments, a brail is recommended for reconnaissance surveys, but the report lists the equipment's numerous limitations based on hook type and size, water temperature, substrate size and type, and turbidity. The report states that survey by brail should not be considered even semi-quantitative. The report recommends a skimmer dredge to determine mussel presence at a site and notes it is effective in unconsolidated substrate. When quantitative estimates are required from deep-water habitats, the report recommends 0.25 m^2 quadrats be

surveyed to a substrate depth of 10–15 cm. Where the substrate is large or coarse, or where turbidity is very high, excavated material should be taken to the surface in a 20 L bucket and sieved on shore.

26. Dycus, J.C., Wisniewski, J.M., and Peterson, J.T. 2015. The effects of flow and stream characteristics on the variation in freshwater mussel growth in a Southeast US river basin. Freshw. Biol. **60**: 395–409.

    This study evaluates methods to age individual mussels based on shell sectioning. It does not provide information on how mussels were collected from the field, and it appears they were collected from shallow water.

27. Farrington, J.W., Tripp, B.W., Tanabe, S.S., Annamalai, S., Josa, L., Wade, T.L., and Knap, A.H. 2016. Edward D. Goldberg's proposal of "the Mussel Watch": Reflections after 40 years. Mar. Poll. Bull. **110**: 501–510.

    The authors provide a summary of the "Mussel Watch" program which is focused on the collection of mussels to monitor for environmental contaminants and toxicity levels. Mussels were collected primarily from marine environments. Collection methods are not described in the report.

28. Galbraith, H.S. 2012. Phase 1 Freshwater mussel survey and comparison to historical surveys at the Pond Eddy Bridge, Delaware River, New York and Pennsylvania. U.S. Geological Survey Open-File Report. 2012-1224, 17 p.

    To assess the abundance of a federally listed mussel species at risk, the Delaware River was surveyed in New York and Pennsylvania, USA. The study also evaluated how survey methods performed over time at long term monitoring sites. Qualitative sampling methods were used to efficiently estimate species richness and diversity, while acknowledging that visual searches are biased towards larger, sculptured, or surface-dwelling individuals. Timed searches were conducted within a 200 m reach using 25 m x 25 m grid cells. Each cell was surveyed for two person-hours using visual or tactile techniques and employing snorkeling or SCUBA equipment. The reach was less than 10 m deep, with moderate flow. Turbidity was not reported. Catch-per-unit-effort of mussels was determined to be between 0.05 and 31 mussels/person-hour.

29. Gangloff, M.M., and Feminella, J.W. 2007. Stream channel geomorphology influences mussel abundance in southern Appalachian streams, U.S.A. Freshw. Biol. **52**(1): 64–74.

    This report examined the influence of hydraulic parameters and stream geomorphology on mussel abundance and distribution across an entire catchment. The authors assessed mussel abundance, diversity, and distribution at 24 sites in eight rivers between May and October. Crews used visual and tactile techniques to perform a timed search of a 50 m stream reach at each site.

Surveys were conducted during base flow conditions, with clear water and minimal depth.

30. Garlapati, D., Charankumar, B., Ramu K., Madeswaran, P., and Ramana Murthy, M., V. 2019. A review on the applications and recent advances in environmental DNA (eDNA) metagenomics. Rev. Environ. Sci. Biotechnol. **18**: 389–411.

    This review paper focused on the application of eDNA in all environments and upon inspection was not relevant to freshwater mussels specifically. However, important points are raised regarding consideration of eDNA results in light of the transport, retention, and resuspension of eDNA in flowing waters.

31. Gasparini, L., Crookes, S., Prosser, R., and Hanner, R. 2019. Detection of freshwater mussels (Unionidae) using environmental DNA in riverine systems. Environmental DNA. **2**: 321–329.

    This study developed and tested eDNA primer assays for the Wavy-Rayed Lampmussel (*Lampsilis fasciola*), a species at risk in the Grand River, Ontario. Timed searches using visual and tactile techniques were used to collect mussels at sites < 1.5 m in depth. Flow and turbidity are not reported. Water samples were also taken at the same time (in 2013). Additionally, collected mussels were placed in cages at a known location and at different densities (N = 1 to N = 10). Water samples for eDNA were collected at 0, 10, 50, and 100 m downstream of the cages and assessed for eDNA detectability. When only one mussel was present in the cage eDNA detection was positive only at 0 m and no further. When 10 mussels were present in the cage eDNA was still not detected further than 10 m downstream.

32. Hagg, W.R., and Warren, M.L. 2007. Freshwater mussel assemblage structure in a regulated river in the Lower Mississippi River Alluvial Basin, USA. Aquat. Cons. Mar. Freshw. Ecosyst. **17**: 25–36.

    The study reports on the composition and abundance of freshwater mussels in an impounded and regulated portion of a large river in Mississippi, USA. The authors did not discuss why they elected to use a suction dredge for mussel collection; however, all sites in the impoundment were 3 m or greater in depth. The authors utilized a systematic sampling array approach based on grid cells (4 km^2) to determine sampling points. At each site two replicate subsamples of the substrate were taken using the suction dredge — one off each side of the boat at the centroid of the grid cell. Each sample consisted of 2.5 m^2 of excavated substrate to a depth of 15 cm using the gas-powered suction dredge. The dredge was operated and put in position by an accompanying SCUBA diver. The intake pipe was limited to mussels less than 80 mm in size. Mussels that would not fit in the intake pipe were handpicked by the diver. Vacuumed sediment was sieved on shore to collect mussels. The authors reported that the flow during sampling was 0.02-0.16m/s, and that mussel density was estimated as low (< 2

mussels/m^2) and high (> 10 mussels/m^2). Substrate in the impoundment consisted largely of silt, silt-sand, clean sand, or sand-gravel.

33. Hanfling, B., Handley, L.L., Read, D.S., Hahn, C., Li, J., Nichols, P., Blackman, R., C., Oliver, A., and Winfield, I., J. 2016. Environmental DNA metabarcoding of lake fish communities reflects long-term data from established survey methods. Mol. Ecol. **25**: 3101–3119.

    The authors evaluated how eDNA, in combination with site occupancy modelling, compared to traditional (gill-net) estimates of fish abundance in a small lake in the UK. This study aimed to answer the important question: can eDNA accurately estimate abundance. Water samples were collected from various depths in the lake and eDNA copy estimates for all species were estimated. The study reports that the rank abundance estimates of fish species established from gill-netting surveys was consistently correlated with eDNA abundance data. The eDNA results also suggested a higher diversity in the lake than has been observed with recent gill-netting data. However, the authors note that the eDNA results cannot inform estimates of population demographics or individual condition. This study was included in the literature review because it is one of the few eDNA studies currently available that discusses the ability of eDNA to assess species abundance.

34. Hanson, J.M., Mackay, W.C., and Prepas, E.E. 1988. Population size, growth, and production of a unionid clam, *Anodonta grandis simpsoniana*, in a small, deep boreal forest lake in central Alberta. Can. J. Zoo. **66**: 247–253.

    This study quantitatively sampled a small, unproductive lake to determine the presence and abundance of freshwater mussels. The authors compared the efficiency of mussel collection by SCUBA divers using 0.5 m by 0.5 m quadrats at select depths (1, 3, 5, 7, and 9 m) along transects, to benthic grab samples taken at the same depths at a subset of the transects. The benthic grab was an Eckman dredge, and once the sample was taken it was washed on shore through a 6 mm sieve. No mussels were found in the 9 m samples. The authors found that compared to the dredge, divers were unable to effectively find mussels smaller than 30 mm in length and recommended that sediment removal accompany any SCUBA survey if population demographics are required. The authors note however that small dredges are often biased against large mussels. Mussel density in this study averaged 14.9 mussels/m^2 (but was less in the sublittoral zone, > 6 m). Turbidity and flow conditions during the survey were not reported.

35. Hart, M., Randklev, C., Dickson, J., Ford, N., Hernandez, B., and Schwalb, A. 2016. A literature review of Freshwater Mussel Survey and Relocation Guidelines. Final report submitted to Texas Department of Transportation. Project Number 0-6865.

In order to provide a consistent and standardized approach for the survey and relocation of freshwater mussels as a result of instream works, the authors summarize the available literature and provide a review of sampling guidelines and methods. The report focuses on methods and techniques to survey for freshwater mussels in wadeable streams. To undertake presence/absence surveys in non-wadeable habitats the authors recommend SCUBA divers search a fixed area using transects aligned parallel to the current and spaced at 10 m intervals across the channel. After the fixed-area search, a timed search of high-density mussel areas is conducted with effort of at least 0.6 minute/m^2. Both surveys were completed using visual and tactile techniques.

36. Hart, M.A., Haag, W.R., Bringolf, R., Stoeckel, J., A. 2018. Novel technique to identify large river host fish for freshwater mussel propagation and conservation. Aquac. **9**: 10–17.

    This report examined habitat associations of three genera of freshwater mussels in the western United States. Specifically, the authors evaluated how hydrogeomorphic structure influenced mussel occurrence and density. The study utilized a stratified random sampling design and sites were chosen based on habitat variables. Snorkel surveys were conducted moving in a downstream direction at each site and snorkelers were 2 m apart. Visual and tactile techniques were used. Variation among samples was estimated by undertaking 14 repeat snorkel surveys. This survey took place in a large stream with depths < 1.5 m, discharge of < 1.5 m^3/s, and where mussel density was 0.3-3.6 mussels/m^2. The authors report that the hierarchical structure of the river influences the spatial patterns of freshwater mussels.

37. Hegeman, E.E., Miller, S.W., and Mock, K.E. 2014. Modeling freshwater mussel distribution in relation to biotic and abiotic habitat variables at multiple spatial scales. Can. J. Fish. Aquat. Sci. **71**(10): 1483–1497.

    The objective of this report was to assess scale-specific habitat associations of three freshwater mussel genre found in the Middle Fork John Day River in Oregon, USA. The authors used a multiscale random forest modelling approach to define functional habitat parameters. Mussel density and occurrence was characterized based with respect to habitat variables within the sampling (reach) unit. Mussel occurrence and density was estimated using a random sampling design. Mussels were collected using a fixed-area visual survey method. Teams of two snorkelers, spaced 2 m apart, swam upstream visually searching for mussels. When the river reach was > 4 m wide multiple swim passes were undertaken. Wading and viewing buckets were used in depths < 10 cm. Mussel density was calculated as the linear density (mussels per meter length of the channel). Variation among density estimates was calculated based on 14 repeat surveys (completed within 1 to 6 weeks after the original survey). Site characteristics of this survey included: depth of < 1.5 m, discharge of < 1.5 m^3/s, and estimated mussel density of 0.3-3.6 mussels/m^2.

38. Hopkins, D., Joly, T.L., Sykes, H., Waniandy, A., Grant, J., Gallagher, L., Hansen, L., Wall, K., Fortna, P., and Bailey, M. 2019. “Learning Together”: Braiding Indigenous and Western Knowledge Systems to Understand Freshwater Mussel Health in the Lower Athabasca Region of Alberta, Canada. J. Ethnobiol. **39**(2): 315–336.

A community-based participatory approach to surveying freshwater mussels in the Athabasca River, Alberta, Canada. Blending traditional and western science to create a picture of past mussel distribution using land-based interviews and site tours with elders. Sites selected for survey were assessed using a timed-search method in shallow water with viewing boxes and tactile techniques.

39. Hornbach, D.J., Allen, D.C., Hove, M.C., and MacGregor, K.R. 2018. Long-term decline of native freshwater mussel assemblages in a federally protected river. Freshw. Biol. **63**: 243–263.

Mussel surveys were completed in the St. Croix River at nine sites within the mainstem of the river. Quadrat excavation (0.25 m^2) was used at each site. At eight sites 100 quadrats were excavated, and at one site 150 quadrats were excavated. Sites were chosen based on the known presence of large mussel beds. From 1991–2011 each site was visited from five to nine times during summer low flow. Ten sampling arrays were established at each site to delineate the sampling area (2 m by 5m) and 10 quadrats were searched in each array. Deep site surveys were limited to the nearshore area of the river margins. When sites were > 2 m, SCUBA divers excavated the quadrats and placed material in a bucket which was sieved at the surface. Depth, flow, and turbidity of survey sites are not reported. The authors noted that even with 100+ quadrats per site the estimate of juvenile mussel density is likely low and inaccurate.

40. Hornbach, D.J., Hove, M.C., MacGregor, K.G., Kozarek, J.L., Sietman, B.E., and Davis, M. 2019. A comparison of freshwater mussel assemblages along a land-use gradient in Minnesota. Aquat. Cons. **29**(11): 1826–1838.

This study evaluated a change in mussel assemblage over time in four rivers in Minnesota, USA. Sites were chosen based on those surveyed in the 1990s and 2000s. Between 7 and 13 of historically sampled sites were revisited in 2015 within the four rivers. Semi-quantitative visual and tactile methods were used with snorkeling or SCUBA, and CPUE was estimated using person-hours. A subset of sites was surveyed in 2017 using quantitative methods (quadrat with excavation). Mussel abundance was found to decrease with increasing sediment load between sites, but no within-site decline was observed. The authors note that the time-search method captured more species than the quantitative methods, and that density estimates varied between the methods. However, no explanation is given for the differences.

41. Isom, B.G., and Gooch, C. 1986. Rationale and sampling designs for freshwater mussels Unionidae in streams, large rivers, impoundments, and lakes. *In* Rationale for Sampling and Interpretation of Ecological Data in the Assessment of Freshwater Ecosystems. *Edited by* B.G. Isom. Special Technical Publication 894. American Society for Testing and Materials, Philadelphia. pp: 46–59.

This historical report provides a summary of quantitative freshwater sampling methods in large rivers, lakes, and impoundments, and offers a case study using a brail in the Cumberland River. The author describes the attributes of a quantitative sample as including "collections made from a single, well-defined population; must include all variation in the population; and, variation must occur in the sample with the same relative frequency they have in the whole population." In order, the author recommends the following methods for surveying in deep water: SCUBA with quadrats, brail, and SCUBA with line transects. The author also notes that SCUBA with line transects assumes divers can adequately see or feel the area to be surveyed and that comparing brail results across studies and rivers will be difficult given differences in brail specification. The author suggests that brail can be used to quantitatively assess mussel density and abundance, if deficiencies and limitations are addressed during data analysis. The case study compared the results from a freshwater mussel survey using SCUBA with quadrats sampled at random sites along transects to brail hauls (50 m in length). Three hauls were taken from bank to bank. At the site depths were > 6 m, and turbidity was not reported. Mussel density was estimated to be 9 mussels/m^2. The author notes that the population estimates from large rivers will arise from systematic sampling collected by SCUBA divers sampling quadrats.

42. Johnson, J.A., Wisniewski, J.M., Fritts, A.K., and Bringolf, R.B. 2012. Host identification and glochidia morphology of freshwater mussels from the Altamaha River Basin. Southeastern Nat. **11**(4): 733–746.

This study reports the results of fish host experiments for six species of freshwater mussel species from the Altamaha River in Georgia, USA. Gravid females were collected using SCUBA, snorkeling equipment, and visual and tactile techniques. The study did not report whether a defined survey collection method was used (e.g., timed search). Environmental characteristics of the site were not provided.

43. Kaeser, A.J., Smit, R., and Gangloff, M. 2019. Mapping and modeling the distribution, abundance, and habitat associations of the endangered fat threeridge in the Apalachicola River System. J. Fish Wild. Manag. **10**(2): 653.

This study utilized side-scan sonar to map the distribution of mussel mesohabitat and paired the assessment with quantitative in situ surveys to define habitat associations in the Apalachicola River. The river is a large, fast-flowing (156–348

m3/s), and turbid (reported as high turbidity) river. Side-scan sonar data was collected with a Hummingbird 1198c side-imaging (SI) system during periods of high river discharge. Nearly the entire river was surveyed to assess bottom habitat type. In situ surveys were chosen in areas that matched the 10 mesohabitat types defined by the side-scan sonar: hard bottom, smooth bank attached, man-made structure, point bar, point/shallow ban, inner recirculation zone, outer recirculation zone, pool/outer bend, mid channel, and rip rap. At each survey site two metal cables were used to delineate the survey area which was assessed using tactile methods by SCUBA divers. Two divers searched each site and were tethered to a dive block for safety. Divers excavated roughly 5 to 10 m^2 areas (to 10 cm depth) in each plot. Using larger plots allowed for lower detection limit for rare species. The in situ mussel surveys required ~ 1,900 person-hours to complete and were aided by the side-scan survey to focus on key habitats.

44. Karatayev, A., Burlakova, L.E., Miller, T.D., Perrelli, M.F. 2018. Reconstructing historical range and population size of an endangered mollusc: long-term decline of *Popenaias popeii* in the Rio Grande, Texas. Hydrobiologia. **810**(1): 333–349.

    Survey of long-term decline of *P.popeii* in the Rio Grande, Texas. The authors surveyed 250 sites in four rivers of the Rio Grande watershed. The study used visual and tactile surveys with snorkeling and SCUBA where depth was >2 m during a reconnaissance survey. Where mussel density was > 0.1 mussel/m^2, then a quantitative search was performed. Of the 15 sites surveyed in the Rio Grande, 6 were randomly selected for quantitative surveys using quadrats (between 3 to 15 per site). The authors do not report mean site depth, flow, or turbidity.

45. Karatayev, A.Y. Mehler, K., Burlakova, L.E., Hinchey, E.K., and Warren, G.J. 2018. Benthic video image analysis facilitates monitoring of *Dreissena* populations across spatial scales. J. Great Lakes Res. **44**(4): 629–638.

    The study evaluates the use of remote sensing using videography paired with traditional sampling to detect and quantify Zebra Mussels in Lake Michigan. Three gear configurations were used to detect and quantify Zebra Mussels in deep-water lake habitat: a traditional PONAR grab, video images from a GoPro mounted to the PONAR, and video images from a GoPro mounted on a benthic sled (mounted 60 cm off the bottom). Video images from the PONAR mount were compared to estimates from the processed PONAR samples. The benthic sled was towed for approximately 500 m which resulted in ~ 37.7 images per metre. Several challenges were found during image collection: insufficient light, camera angle being off, sled cable causing resuspension of sediments, and interference from macrophyte cover. The survey depth ranged from 6.7 to 205 m. The authors also trialled the method in Lake Erie but found that despite the shallow depth, turbidity and macrophyte cover inhibited image collection. The authors report that the ability to detect a 20% change in density of mussels (at 90% confidence) was lower with the PONAR alone than the PONAR+GoPro setup, and lower than the

sled+camera setup. The authors suggest that precision of density estimates can be increased by incorporating image capture and that the PONAR samples were essential to estimate size-frequency and differentiate between species.

46. Keretz, S.S., Woolnough, D.A., Morris, T.J., Roseman, E.F., Elgin, A.K., and Zanatta. D. 2021. Limited Co- existence of native unionids and invasive dreissenid mussels more than 30 y post dreissenid invasion in a large river system. Am. Mid. Natural. **186**: 157–175.

    This study aimed to assess the status of freshwater unionids in the Detroit River, USA. A random, stratified approach was used to select sites through the Detroit River basin. The dredged commercial shipping channel was removed from possibly site selection. Three site-types were used: randomly selected, sites that were historically sampled, and sites with potential to find extant mussel populations. Sites with strong currents were not surveyed for safety reasons and average flow at surveyed sites was 0.3 m/s. Site depth was estimated using a marked rope attached to a PONAR grab (mean site depth was not reported). The PONAR was used to collect six samples per site (grab area = 0.23 m^2). At each site, samples were taken from the bow, centre, and stern of the boat. In addition to the PONAR grab, one person-hour of timed search effort using SCUBA was employed. Two divers used visual and tactile techniques. The divers divided the site into six, 10-minute search areas. If rare or live mussels were encountered, an additional 10-minute search effort was employed.

47. Klymus, K.E., Richter, C.A., Thompson, N., Hinck, J.E., and Jones, W.W. 2020. Metabarcoding assays for the detection of freshwater mussels (Unionida) with environmental DNA. Environmental DNA **3**(1): 231–247.

    The authors evaluated the utility of eDNA metabarcoding for unionids in the Clinch River, located in the southeastern United States. They tested assays using the COI and ND1 genes. They compared the success of eDNA detection using both genes at six sites. Site characteristics are not reported except for discharge which is reported as 59 m^3/s. At three of the sites eDNA detected 42%, 58%, and 54% of total known species richness, and suggest that filtering more water at each site would have resulted in a higher number of detections. Up to 16 water samples were taken at each site from the middle of the river. The study concluded that metabarcoding is useful for evaluating the presence of some mussel species and prioritizing streams for in situ sampling surveys. Downstream survey sites had greater detection rates than upstream survey sites (theorized due to increasing DNA content downstream). ND1 gene had consistent detection rates regardless of position in the river (up or downstream), unlike COI which showed a marked increase in detections downstream.

48. Kovalak, W.P., Dennis., D.S., and Bates., J.M. 1986. Sampling effort required to find rare species of freshwater mussels. *In* Rationale for Sampling and Interpretation of Ecological Data in the Assessment of Freshwater Ecosystems. *Edited by* B.G. Isom.

Special Technical Publication 894. American Society for Testing and Materials, Philadelphia, pp. 34-45.

This report summarizes an evaluation of three methods used to collect freshwater mussels and the sampling effort required to find rare species. The three methods evaluated were: visual survey of wadeable 1 m^2 quadrats, brail hauls in 3–4 m cobble-sand sites, and visual survey of 0.8-1.8 m sites using SCUBA. Flow and turbidity conditions during sampling are not reported. The author suggests that brail results can be converted into density estimates if the efficiency of the brail unit is known. The results of this study suggest that efficiency of the brail is not consistent across species based on shell length and the author notes the brail is biased against smaller animals. The author recommends two-stage sampling for quantitative estimates where qualitative sampling is undertaken by brail or visual surveys, followed by quadrat sampling that is stratified by habitat containing rare species.

49. Krause, C., and Roghair, C. 2014. Initial implementation of a long-term freshwater mussel monitoring program for the Chattooga River. United States Department of Agriculture Forest Service Southern Research Station. Center for Aquatic Technology Transfer. Blacksburg, VA.

The objectives of this study were to assess a site in the Chattooga River for baseline population estimates of freshwater mussels and compare the current distribution of animals to historical records, and to inform a long-term monitoring sampling approach. Environmental characteristics (turbidity, flow) were not reported, but the site was wadeable. Systematic random sampling with three random starts was used to sample 0.25 m^2 quadrats visually. Total sampling effort was estimated as 2.5% of the wadeable area of the site.

50. Krebs, R.A., Prescott, T.J., Clapham, W.B., and Klarer, D.M. 2018. Freshwater mussel assemblages at the lotic-lentic interface along Lake Erie. Am. Malacol. Bull. **36**(1): 31–41.

The goals of this study were to assess freshwater mussel species richness in comparison to watershed size, land use, water chemistry, and turbidity in tributaries of wide, shallow embayments in Lake Erie. Twelve small streams were surveyed along with two embayments in 2010 and 2012. Embayments were < 2 m deep. High turbidity and low flow were observed at most sites and substrates were fine to mixed. A timed-search method was used with visual and tactile techniques for two person-hours at each site.

51. Kriege, M.D. 2018. Freshwater mussels of the Greenup Navigational Pool, Ohio River, with a comparison to fish host communities. Marshall University Theses, Dissertations and Capstones Digital Scholar.

The author evaluated the mussel community composition and diversity in Greenup Pool of the Ohio River, USA. The author utilized past sampling records to inform sampling location: brailing and midden pile assessments were used in the 1970s; brailing, diving, and transects were used in the 1980s; transects were used in the 1990s and early 2000s; “cells” were surveyed in 2012; and transects were surveyed from 2008–2016. In 2017, the author employed SCUBA and a transect method to survey the pool as outlined in the West Virginia Mussel Survey Protocol. This protocol calls for 100 m transects to be placed perpendicular to the river flow. The author placed six transects at each site spaced 100 m apart. The transects were divided into 10 “cells” that were each 10 m^2. The transects were marked by lines and anchors and divers surveyed from deep to shallow habitat. Visual surveys were performed and only when visibility conditions were > 0.5m. Average pool depth was 7.9 m.

52. Lamand, F., and Beisel, J., N. 2014. Comparison of visual observation and excavation to quantify density of the endangered bivalve *Unio crassus* in rivers of north-eastern France. Knowl. Manag. Aquat. Ecosys. **413**: 11.

    In order to assess trends in freshwater mussel populations, visual and quadrat surveys in a wadeable stream were compared. The site was < 1.5 m deep, had low turbidity, and was sampled during base flow conditions. Mussel density at the site was estimated as 6 mussels/m^2. Visual surveys of a 50 m^2 area were conducted and quadrats were sampled from within the same area. Quadrats were excavated to 10 cm. The authors report that for every 10 animals excavated from a quadrat, on average, 1 was observed visually.

53. LeBlanc, F., Steeves, R., Belliveau, V., Akaishi, F., and Gagné, N. 2021. Detecting the brook floater, a freshwater mussel species at risk, using environmental DNA. Aquatic Conserv: Mar Freshw Ecosyst. **31**(6): 1233–1244.

    Study evaluated the ability of eDNA to detect populations of Brook Floater (*Alasmidonta varicosa*) (a species at risk) in watersheds of New Brunswick. Traditional methods do not detect Brook Floater well due to its burrowing behaviour. Water samples were collected in 2017 and 2018. The study sites included rivers and tributaries of the Miramichi River and other watersheds in NB. Site characteristics are not provided. Fifty-six sites were surveyed for eDNA and positive detections occurred at 16 sites. Sites were chosen based on past positive findings of Brook Floater using traditional methods. Most assays where positive eDNA detection occurred were below the theoretical minimum detection limit, even when Brook Floater were visibly observed during water collection.

54. Liu, X., Lopes-Lima, M., Xue, T., Zhou, Y., Li, K., Xu, Y., Qin, J., Ouyang, S., and Wu, X. 2020. Changes and drivers of freshwater mussel diversity patterns in the middle and lower Yangtze River Basin, China. Glob. Ecol. Cons. **22** (2020) e00998.

This study surveyed the Yangtze River in China to assess how the diversity patterns of mussels have changed over time, assess the influence of climate change in mussel distribution, and provide conservation measures for freshwater mussels. The authors used the results from historical mussel surveys to inform current site selection, as well as interviewed local fishers to determine mussel bed location. When water depth was > 2 m mussels were sampled using a homemade rake (60 cm wide) that was pulled over 50 m transects. Sampling depths ranged from 0.9 to 25 m in turbid and clear conditions. The area dredged by the rake was calculated as the width of the rake multiped by the length of the transect. Visual and tactile timed searches were employed in shallow water for 0.5–2 person hours. In poor visibility hand rakes were used. The Yangtze River is deep and turbid with varying substrate types. The paper does not discuss the effectiveness of the methods or possible non-detections.

55. Lor, Y., Schreier, T.M., Waller, D.L., and Merkes, C.M. 2020. Using environmental DNA (eDNA) to detect the endangered Spectaclecase Mussel (*Margaritifera monodonta*). Freshw. Sci. **39**(4): 837–847.

    The study evaluated the presence of the Spectaclecase Mussel (*Margaritifera monodonta*) in the St. Croix and Mississippi River in the USA using eDNA. The mussel is typically found under rocks and boulders making its detection with traditional sampling methods difficult. eDNA detection rates were low at both sites (30.2% in the St. Croix River and 0.6% in the Mississippi River). Higher eDNA detections occurred in the spring during the period of larval release and bottom water samples were more successful than surface water samples and there was no appreciable decline in detections moving downstream until 500 m (in the St. Croix). However, all detection rates were below the theoretical minimum detection limit. The Mississippi has a higher discharge rate than the St. Croix and the authors suspect the lower detection rate in the Mississippi River is due to flow and discharge rate. Depth, flow, and turbidity parameters are not reported for the surveyed sites.

56. Makhrov, A.A. 2010. Distribution of the Freshwater Pearl mussel in Russia. *In* Conservation of freshwater pearl mussel *Margaritifera margaritifera* populations in Northern Europe. *Edited by* E. P. Ieshko, and T. Lindholm. Proceedings of the International Workshop. pp: 108.

    This report summarizes the historical commercial sampling of the freshwater pearl mussel. Sampling details (e.g., efficiency, environmental conditions, etc.) with respect to deep-water sampling are not provided.

57. Mauvisseau, Q., Burian, A., Gibson, C., Brys, R., Ramsey, A., and Sweet, M. 2019. Influence of accuracy, repeatability and detection probability in the reliability of species-specific eDNA based approaches. Sci. Rept. **9**: 580.

This paper evaluated the repeatability and accuracy of existing eDNA assays for the freshwater pearl mussel at two commonly used genes, COI and 16s, in a mesocosm experiment. Both genes were specific to the target species, but the limit of detection was lower for the COI gene. The authors found that the variability between natural replicates of eDNA samples influence the number of replicates required for reliable species detection and quantification in the field.

58. McAlpine, D.F., and Sollows, M.C. 2014. A quadrat-sieve system for sampling freshwater mussels using SCUBA. Northeastern Nat. **21**: N1–N4.

    This report presents a survey method that utilizes a combined quadrat/sieve apparatus to undertake quadrat sampling at large river sites using SCUBA. Here, the authors sampled rivers in New Brunswick, Canada, at a variety of sites with varying turbidity (clear to murky), depth (> 1 to 5 m), substrate type (sand to cobble), and flow velocity (0 to 0.28 m/s). The quadrat sieve apparatus developed by the authors is a 0.25 m^2 frame with an attached screened (5 mm) stage. The quadrat sieve is used by laying the quadrat on the sediment surface oriented with the sieve at the downstream end. The diver, visually and by hand, surveys the quadrat placing any animals found in a coloured mesh bag. The quadrat is then excavated by hand or by metal scoop to a depth of 15 cm. The sediment material is put onto the sieve, and any current washes away fine material. Animals collected in the sieve are placed in a differently coloured mesh bag. The authors report capturing mussels as small as 11.5 mm. The authors also report that when conditions were favorable (e.g., fine-medium sand with little vegetation, low current, moderate visibility, and mussel density of 0–8 per square m^2), quadrats could be visually searched and excavated in, on average, seven minutes. The quadrat sieve apparatus could presumably be used with adaptive, cluster or systematic sampling survey designs.

59. Meador, J.R., Peterson, J.T., and Wisniewski, J.M. 2011. An evaluation of the factors influencing freshwater mussel capture probability, survival, and temporary emigration in a large lowland river. J. North Am. Benthol. Soc. **30**(2): 507–521.

    To estimate population characteristics of freshwater mussels in a larger river the authors employed a Robust Design, a mark-recapture method. This method estimates population demographics and capture probabilities. Several habitats were sampled within a site with varying environmental characteristics: slack water, pool, and swiftwater with depth ranging between 0.6 and 2 m. Flow was low to moderate (0–0.53 m/s) and turbidity was low. Density was estimated as > 5 mussels/m^2. SCUBA equipment was used in waters > 1.5 m. All samples were surveyed using tactile techniques and the sediment was probed up to 5 cm. The mark-recapture design utilized primary and secondary sampling units (time) in an effort to reduce the burden and cost of sampling large rivers while maintaining high estimate precision. Sampling units consisted of nine 10 x 1 m randomly placed transects set perpendicular to the river flow within a 300 m^2 survey area.

Excavation of quadrats was initially attempted but was abandoned due to time and difficulty.

60. Metcalfe-Smith, J.L., Di Maio, J., Staton, S.K., and Mackie, G. 2000. Effect of sampling effort on the efficiency of the timed search method for sampling freshwater mussel communities. J. North Am. Benthol. Soc. 2000. **19**(4): 725.

    This report evaluates the effect of sampling effort on timed-search sampling efficiency to detect rare species and the number of freshwater mussel species found at a site. Timed searches at 28 sites in five rivers were compared at intervals of increasing sampling effort. Sampling effort was estimated as person-hours of searching. All sites were wadeable, with low flow and low to moderate turbidity. Visual surveys were conducted at each site, unless turbidity was < 15 cm when tactile techniques were used. All habitats were surveyed at a site, but more time was spent in preferred mussel habitat. The authors report that 29% of the rare species encountered were detected in the last 1.5 hr interval of a 4.5 person-hour period.

61. Miller, A.C., and Payne, B.S. 1993. Qualitative versus quantitative sampling to evaluate population and community characteristics at a large-river mussel bed. Am. Midl. Nat. **130**(1): 133–145.

    The freshwater mussel population of the Ohio River, downstream of a coal power plant, was assessed to obtain baseline community data including demographics and demography. The study site was deep (> 1.5 m) and had moderate to fast flow and substrate of predominantly gravel and sand (70/25%). Visibility at the substrate was < 15 cm. Qualitative surveys were completed in a fixed area using tactile techniques and SCUBA equipment. Quadrat surveys (0.25 m^2) were then conducted. Sediment was excavated by divers and sent to the surface in a 20 L bucket and the material was sieved on shore. The authors reported that the qualitative survey demonstrated bias towards large, sculpted animals compared to the quantitative samples, but that both methods tended not to detect the rarest species. Mussel density was estimated between 4.4 to >1000 mussels/m^2.

62. Miller, A.C., Whiting, R., and Wilcox, D.B. 1989. An evaluation of a skimmer dredge for collecting freshwater mussels. Freshw. Ecol. **5**(2): 151–154.

    This study evaluated the effectiveness of the skimmer dredge in a large, deep (> 1.5 m) river. Flow and turbidity were not reported. The authors focused on the skimmer dredge's ability to assess mussel density and species composition and compared its efficiency against handpicking by SCUBA divers. In the study the skimmer dredge was towed along 30 m transects and dredge up to 6 cm of the sediment; nine tows were completed in four hours. Divers followed behind the skimmer dredge collecting mussels that were missed. The authors report that the skimmer dredge collected significantly more small mussels than divers, but that it collected only 62% of all individuals. Additionally, the skimmer dredge did not

successfully collect mussels that were buried more than 6 cm in the sediment and caused mortality in 10% of collected thin-shelled individuals. Also, the skimmer dredge was not equally efficient across species. The authors also note, anecdotally, that while the skimmer dredge is more cumbersome than a brail, it is more efficient. Finally, the study notes that the skimmer dredge is not suitable in cobble, boulder, or armoured gravel substrates or in areas with a significant number of potential snags. The authors recommend the skimmer dredge to assess species richness, diversity, and relative abundance during exploratory surveys.

63. Moore, A., and Machial, L. 2007. Freshwater mussel surveys (target species *Gonidea angulata*) in the Okanagan and Kootenay regions, summer 2007. B.C. Conservation Corps Invertebrates at Risk Crew. Internal Working Report. Available from http://a100.gov.bc.ca/appsdata/acat/documents/r17349/CB-PE07-35407_Report_1259884060822_6eb134fb7ec55f01a27d4014691790ac0fa5a7e5edf3c215b638efd95e0d6c17.pdf [accessed January 2018].

    In the Okanagan and Kootenay regions of British Columbia freshwater mussel surveys were conducted in streams to characterize local species distributions and habitat. One- to four-person crews waded or snorkeled adjacent to each other within the site and surveyed the area following a fixed-area transect design. Visual surveys were employed. Large mussel beds were enumerated by counting the mussels within a 1 m^2 block and then estimating the total number of animals in the bed. The sites were relatively shallow (always < 3 m), with low flow and moderate mussel density.

64. Nagayama, S., Morihiro, H., and Kayaba, Y. 2016. Distribution and microhabitats of freshwater mussels in waterbodies in the terrestrialized floodplains of a lowland river. Limnology. **17**(3): 263–272.

    This study examined the freshwater mussel diversity of floodplain waterbodies of a lowland river in Japan. All of the sites were shallow (< 1 m), were characterized by very little flow, and had high turbidity. The authors used a transect-quadrat design: transects were placed within the waterbody spaced 5 m apart, and quadrats (2 x 2 m) were placed at even intervals along the transect. Tactile searches were employed in the quadrats by disturbing the sediment to 5 cm by hand when the site was shallow (< 60 cm), and using an iron winnow with a shaft (e.g., mussel scoop) when the site was deep (> 60 cm). The results show that mussel abundance was negatively related to benthic litter and had a unimodal association with water depth and mud depth.

65. Obermeyer, B., K. 1998. A comparison of quadrats versus timed snorkel searches for assessing freshwater mussels. Am. Midl. Nat. **139**(2): 331–339.

    Timed snorkel searches are compared to quadrat sampling to assess effectiveness in estimating diversity, relative abundance, species richness, size,

and recruitment. The survey was completed in wadeable (< 1 m) riffle habitat of a river in Kansas, USA. Sediment was predominately a cobble-gravel-sand mix. Flow and turbidity were not reported, but turbidity is assumed to be low to moderate because both visual and tactile collection techniques were used. Timed snorkel searches involved surveying a 100 x 10 m stream reach for on average 81 minutes. The reach was surveyed from downstream to upstream snorkeling in a zig-zag pattern. The same reach was further surveyed with 40 random 1 m^2 quadrats and quadrats were excavated to 15 cm and sieved. The author found that quadrat samples captured more species and individuals, as well as rarer species, compared to timed snorkel searches. Also, that quadrat samples detected more small and smooth-shelled individuals than the timed snorkel search. Mussel density in the study area was estimated to be 2.5 mussels/m^2

66. Olson, P.J., and Vaughn, C.C. 2020. Population genetics of a common freshwater mussel, *Amblema plicata*, in a southern U.S. river. Freshw. Moll. Biol. Cons. **23**(2): 124–133.

    Microsatellite genetic information was used to describe the genetic structure of mussel beds and estimate the effective population size of beds in Little River, Oklahoma, USA. Authors genotyped 270 mussels from nine mussel beds from the mainstem or the river and tributaries. The estimated effective population size was lower than total measured abundance. A quadrat survey with excavation was used to capture mussels for genotyping at large sites, and a timed search was used to collect 30 mussels at small sites using visual and tactile approaches and SCUBA or snorkeling.

67. Ontario Ministry of Natural Resources and Forestry (OMNRF). 2018. Survey Protocol for Species at Risk Unionid Mussels in Wetlands in Ontario. Species Conservation Policy Branch. Peterborough, Ontario. ii + 30 pp. Available from https://files.ontario.ca/survey_protocol_for_sar_wetland_mussel_species_2018_.pdf [accessed January 2018].

    This government report provides a standardized protocol to determine freshwater mussel presence or absence in wadeable and non-wadeable coastal wetlands in Ontario, Canada. The authors provide guidance on sampling timing (June to September), and ideal conditions for detecting mussels (low turbidity, limited vegetation, and relatively open water habitat). Two survey types are recommended depending on a priori knowledge of mussel presence, and the size of the wetland. A timed search with 12 random starts is recommended for large wetlands and where mussel presence is not known. A half-hectare timed search is recommended when the presence of mussels is known, or where wetlands are smaller than 5 km^2. Both survey types employ wading or snorkeling techniques using visual or tactile sampling. A mussel scoop can also be used. Either survey type could also be conducted using SCUBA equipment. The protocols offered in this report will inform species presence and absence, but will not inform density estimates, evaluate trends through time, or inform population

demographics of a freshwater mussel population. The protocol outlined in this report is likely to be most effective in shallow (< 2 m), low-flow, and high-visibility environments where search efficiency is assumed to be high. Where habitat conditions varying from the ideal, search efficiency is reduced and a greater number of repeat samples (sampling effort) is required.

68. Powers, J., Brewer, S.K., Long J.M., and Campbell, T. 2015. Evaluating the use of side-scan sonar for detecting freshwater mussel beds in turbid river environments. Hydrobiologia. **743**(1): 127–137.

    This study investigated the ability of an inexpensive (~ $2000 USD) side-scan sonar unit mounted on a canoe to identify freshwater mussel beds in a reservoir (Lake McMurtry, USA) of the large Muddy Boggy river. The authors report that reference images of exposed mussels on sand sediment are useful for interpreting field sonar images. The study was conducted during both spring freshet and base flow conditions, in less than 2 m depth, and where turbidity was 20 NTU. Surveys were conducted by capturing sonar imagery while canoeing upstream, mid-channel, at selected sites. Sonar imagery was validated in the field by divers (> 1.5 m) and snorkelers (< 1.5 m) undertaking visual and tactile surveys. The authors found that surveys during the spring freshet were more effective for locating mussel beds than during base flow conditions, and that mussels exposed on, or partially buried in, sand and clay were easily identified. Several limitations of the method include lack of species identification, inability to observe mussels not fully exposed on the sediment surface, increasing depth significantly limited ability to detect mussel beds, and mussels in pebble, cobble, or silt substrates were impossible to detect. The authors conclude that side-scan sonar detection of mussel beds is an effective tool for preliminary mussel surveys in depths of 1 to 2 m. The authors did not report the density of mussels in the area where their test was conducted and it is not known how mussel density would impact the effectiveness of this method, but it is suspected that efficiency would decrease with decreasing density.

69. Prié, V., Soler, J., Araujo, R., Cucherat, X., Philippe, L., Patry, N., Adam, B., Legrand, N., Jugé, P., Richard, N., and Wantzen, K.M. 2017. Challenging exploration of troubled waters: a decade of surveys of the giant freshwater pearl mussel *Margaritifera auricularia* in Europe. Hydrobiologia. **810**(1): 157–175.

    This report summarizes historical and contemporary sampling of the Giant Freshwater Pearl Mussel in Europe. Collection and monitoring of this species can be challenging because it is often found in the downstream sections of large, turbid, deep, high velocity rivers. The authors discuss the use of a “dredger” that was the only viable method to sample the Seine and Eure rivers. These rivers are deep (> 6 m sections) and turbid (riverbed not visible). The dredger was towed between 8 and 50 m long transects and collected sediment was sorted on the boat. While it is not clear if the dredger was identical to a skimmer dredge, it is similar in principle. The authors suggest that transect and quadrat sampling via

SCUBA are more efficient sampling methods for the Giant Freshwater Pearl Mussel than a towed dredger.

70. Prié, V., Lopes-Lima, M., Taberlet, P., Valentini, A., Poulet, N., Jean, P., Breugnot, E., Couprie, S., Jardin, G., Roset, N., Vigneron, T., Lamand, R., Gargominy, O., Rocle, M., and Dejean, T. 2020. Large-scale monitoring of freshwater bivalves: an eDNA point of view on species distribution and conservation. Authorea.

    This paper was in pre-print when reviewed and had not been through the peer review process. Data and interpretation are preliminary.

    This paper evaluates the uses of eDNA metabarcoding to detect mussel species in rivers across France. The authors suggests that eDNA can be less costly, time-consuming, and risky for surveyors than traditional sampling methods that rely on SCUBA. In this paper, 350 sites were surveyed between May and October in areas that had been previously under-sampled or where rare species were suspected. Site characteristics including depth, flow, and turbidity are not described in the paper; however, the authors note that many of the sites were located in the Rhone River which is a large, fast-flowing, deep, and turbid river that is difficult to sample. Water at each site was filtered for 30 minutes or until the capsule was complete saturated. The amount of volume varied among sites due to the level of turbidity which slows the filtration process. At low turbidity sites the volume of water filtered was estimated to be 30 L. At three sites no eDNA detections were recorded and these habitats were small, still ponds. Few traditional surveys have been completed in the Rhone. eDNA detected the first occurrence of a rare species of mussel in the Rhone. The authors note that eDNA based on mitochondrial DNA metabarcoding (multispecies detection) may not fully resolve true species boundaries among closely related groups. If metabarcoding surveys are completed with primers developed based on a single gene (e.g., 16S) the rate of haplotype diversity of the gene may influence detection results of closely related groups.

71. Prié, V., Valentini, A., Lopes-Lima, M., Froufe, E., Rocle, M., Poulet, N., Taberlet, P., and Dejean, T. 2021. Environmental DNA metabarcoding for freshwater bivalves biodiversity assessment: methods and results for the Western Palearctic (European sub-region). Hydrobiologia **848**(1): 2931–2950.

    Study evaluated a metabarcoding approach to eDNA surveys for freshwater mussels in Europe. The authors compare metabarcoding results to findings from traditional surveys. The 16S gene was used for primer development because the COI gene was not suitably conserved to develop a universal primer. Samples were collected between 2015 and 2019 from Italy, France, and Morocco. Sites in France made up most of the sampling sites. Sites covered multiple habitat types including small streams and fast-flowing rivers as well a standing water and areas of varying pH. Neither individual site nor mean site characteristics are reported (i.e., depth, turbidity, or flow). Compared eDNA results at three scales:

all of France based on existing mussel distributions; at two intensively sampled rivers (Rhone and Meuse each with > 50 sites for eDNA collection) which could assess false negatives — data were compared to existing mussel database; and at small survey areas (15 sites) of a few hundred meters. Traditional methods described include dredging and screening of sediment to look for mussels. However, small site surveys for traditional methods were later described as using transect surveys by SCUBA and a timed-search approach (2–3 person-hours). The results show that 90% of known species occurrence were detected using eDNA and the method was successful compared to traditional surveys. However, for mussels found in turbid habitats, eDNA detection is lower due to humic substances and sediment which reduces the amount of water that can be filtered for eDNA samples. The authors suggest metabarcoding is useful to detect rare species that are masked by the abundance of common species.

72. Porto-Hannes, I., McNichols-O'Rourke, K., Goguen, M., Fang, M., and Morris, T. J. 2021. Sampling protocol for the freshwater mussel *Simpsonaias ambigua* (Salamander Mussel) in Canada. Can. Tech. Rep. Fish. Aquat. Sci. **3411**: vii + 60 p.

    The study primarily delt with detecting the salamander mussel in rivers in Ontario. Recommended methods are suited to the habitat in which the mussel is found and involve "rock flipping;" however, eDNA is recommended for detecting the mussel at new locations and a tool to assist in site selection for in situ surveys. In deeper waters the authors recommend rock flipping via SCUBA. Survey sites relevant to the guidance include those that are shallow (< 1.5 m), with moderate to high flow and high turbidity.

73. Pursifull, S., Holcomb, J., Rowe, M., Williams, J.D., and Wisniewski, J.M. 2021. Status of Freshwater Mussels in the Ochlockonee River Basin of Georgia and Florida. Southeastern Nat. **20**(1): 1–19.

    The study assessed the status of mussels in the Ochlockonee River of Georgia and Florida, USA from 2006 to 2017. Historical and recent survey results collected by multiple agencies were compiled to create a historical and contemporary picture of mussel distribution and abundance in the river following anthropogenic changes. No mussel sampling was undertaken in this study. The authors report that depths greater than 1.5 m were infrequently sampled and only one SCUBA survey was completed in 2009 at a small site.

74. Randklev, C.R., Miller, R., Hart, M., Morton, J., Johnson, N.A., Skow, K., Inoue, K., Tsakiris, E.T., Oetker, S., Smith, R., Robertson, C., and Lopez, R. 2018. A semi-arid river in distress: Contributing factors and recovery solutions for three imperiled freshwater mussels (Family Unionidae) endemic to the Rio Grande basin in North America. Sci. Total Environ. **631–632**: 733–744.

    The study evaluates threats to rivers and mussels in semi-arid locations and evaluated the conservation status of three endemic mussel species in the Rio

Grande of southwester USA. The survey sites spanned four sub-watersheds and within each study reach 10 km was defined with randomly assigned survey points. Survey points were 150 m^2 and included multiple habitat types (site characteristics not provided). Timed searches were used to increase the probability of detecting rare species. Visual and tactile methods were used for four person-hours at each site, and SCUBA was used in depths > 1.5 m.

75. Reed, M.P., Dinkins, G.R, and Ahlstedt, S.A. 2019. Freshwater Mussels (Bivalvia: Margaritiferidae and Unionidae) of the Buffalo River Drainage, Tennessee. Southeastern Nat. **18**(2): 346–372.

    This study reports the results from a large mussel survey of the Buffalo River Basin in Tennessee, USA. Mussel community composition was qualitatively assessed at sites chosen based on museum records and preliminary site visits. This information was used to chose sites for quantitative surveys. SCUBA divers were used in sites up to 2 m depth (0.15 - 2 m); no sampling occurred below 2 m. Transects set perpendicular to the flow were used to guide quadrat sampling. Quadrats were placed along the transect spaced 4 m apart; 100 quadrats were surveyed at each site. Qualitative site visits captured 13 more species than quantitative surveys.

76. Reid, S.M, Kopf, V., LeBaron, A., and Morris, T.J. 2016. Remnant Freshwater Mussel Diversity in Rondeau Bay, Lake Erie. Can. Field-Nat. **130**(1): 76–81.

    Rondeau Bay, a large embayment and coastal wetland complex found on the north shore of Lake Erie, was surveyed for the presence of native freshwater mussels. Twenty-seven sites were surveyed over two years. Survey effort was designed to maximize the detection of rare species and species at risk. A timed search with random starts was employed and each site was searched for 4.5 person-hours. Surveyors either waded or floated on pool mattresses to visually, or with tactile techniques, collect mussels. Depth at all sites did not exceed 1.5 m, flow was minimal, and turbidity was low to moderate. The sediment composition of the sites was largely sand, with some gravel and silt, but the contribution of silt, clay, and organics increased in the second year of sampling. Mussel relative abundance was low at all sites.

77. Reid, S. and LeBaron, A. 2019. Lower Grand River freshwater mussels: Results from brail sampling of non-wadeable habitats. *In* Morris, T.J., McNichols-O'Rourke, K. A., and Reid, S.M. (*Editors*). 2020. Proceedings of the 2019 Canadian Freshwater Mollusc Research Meeting: December 3–4, 2019, Burlington, Ontario. Can. Tech. Rep. Fish. Aquat. Sci. **3352**: viii + 34 p.

    This abstract was presented at the 2019 Canadian Freshwater Mollusc Research Meeting and provided preliminary results of a brail trial in the Grand River, Ontario. The study aimed to assess if brailing could be used in deep, riverine environments to detect freshwater mussels. The authors towed a brail behind a

boat at 51 sites in the Grand River. At each site, 5 m by 50 m transects were surveyed. Sites were visited in the summer and fall. Summer brailing resulted in more individual mussel capture as well as more species compared to fall brailing. Mussels captured by the brail were large and highly sculpted species. The abstract did not provide information on the number of transects which did not detect mussels, nor on the habitat types in which the brail was used. The average site depth was 3.6 m.

78. Reid, S.M. LeBaron, A., and Morris, T.J. 2018. Can Adaptive Cluster Sampling Improve Ontario Mussel Species at Risk Monitoring? Can. Manuscr. Rep. Fish. Aquat. Sci. **3152**: iv +16 p.

    The study reports the results of a computer simulation to evaluate the utility of adaptive cluster sampling as a tool to detect and quantify rare freshwater mussels. Input data from heavily surveyed sites in the Sydenham River and Rawdon Creek were used. These habitats are both shallow (mean depth 0.29 m), wadeable, but may be turbid. Adaptive cluster sampling was less accurate and less efficient than simple random sampling and systematic sampling with random starts. Recommend that increasing the spatial coverage of existing systematic sampling is the best approach to improve detection probabilities. The authors suggested that the protocol does not work well for rare species and could not detect small changes (<70%) in mussel populations.

79. Rice, C.J., Larson, E.R., and Taylor, C.A. 2018. Environmental DNA detects a rare large river crayfish but with little relation to local abundance. Freshw. Biol. **63**(5): 443–455.

    The authors aimed to evaluate the effect of directional streamflow on the detection of an endemic species' eDNA in a lotic environment. Specifically, the study aimed to determine if eDNA results were comparable to results from traditional sampling methods used to assess the presence and abundance of the target species. Data were interpreted by evaluating the relationship between site-scale variables and eDNA detection probability using a detection probability and occupancy-modelling framework. The authors found that while eDNA detection was reasonably consistent (90%) with traditional sampling methods, there was a poor relationship between eDNA detection probability and target species abundance. However, the authors observed a strong risk of downstream transport of eDNA from upstream locations. The study concludes that downstream transport may limit the ability of eDNA to be used as a reliable estimator of local target species abundance. This report focused on sampling crayfish from a variety of stream habitats.

80. Rider, T., Wilcut, L., Barash, S., and Robiou, G. 2013. Technical support document for conducting and reviewing freshwater mussel occurrence surveys for the development of site-specific water quality criteria for ammonia. U.S. Environmental

Protection Agency Office of Water. Available from https://goo.gl/nbczzF [accessed January 2018].

Water quality criteria thresholds in some states and tribal areas are assessed based on the presence or absence of sensitive species, including freshwater mussels. This report provides an overview of freshwater survey methods and suggests important considerations and limitations of each method that should inform method choice when undertaking a survey to assess mussel presence or absence. Qualitative, quantitative, and semi-quantitative methods are discussed along with survey design options (e.g., systematic sampling with random starts). The benefits and limitations of snorkeling, diving, brail, and sediment grabs are discussed, as well as their ability to perform qualitative or quantitative assessments.

81. Roghair, C.N., Nuckols, D.R., and Haag, W.R. 2005. Establishment of a monitoring program for freshwater mussels in the Chattooga River, SC and GA. USDA Report. Available from www.srs.fs.usda.gov/catt/pdf/sc/2005_sc_catt_report.pdf [accessed January 2018].

A long-term freshwater mussel sampling program is presented in this report for the Chattooga River watershed. The goal of the monitoring program is to detect changes over time in the mussel population at the entire river reach level. The report provides a review of freshwater mussel sampling methods for wadeable rivers and based on that information suggests a consistent method to be employed at all future monitoring sites. The report suggests that at each site systematic sampling of three randomly located arrays with 0.25 m^2 quadrats should occur. Visual and tactile searches will precede excavation and sieving. Only wadeable portions of the site will be surveyed with a viewing bucket or by snorkeling.

82. Salonen, J., and Taskinen, J. 2017. Electrofishing as a new method to search for unknown populations of the endangered freshwater pearl mussel *Margaritifera margaritifera*. Mar. Freshw. Ecosys. **27**(1): 115–127.

The authors exploited the parasitoid phase of the mussel life cycle to determine the presence of the Freshwater Pearl Mussel (*Margaritifera margaritifera*) in rivers throughout Finland. The Freshwater Pearl Mussel's known fish hosts are salmonids: Atlantic Salmon and Brown Trout. The Freshwater Pearl Mussel is a long-term brooder and parasitizes the fish host for up to a year. Near the time of transformation glochidia are up to 400–500 µm and visible with the naked eye on the gills of their hosts. The authors performed haphazard backpack electrofishing surveys on rivers in Finland to determine the presence of the Freshwater Pearl Mussel by visually examining the gills of captured Brown Trout. They compared in-situ estimates of glochidial presence and rate of infestation on wild Brown Trout to laboratory-raised and -infested Brown Trout. The results suggested that visual inspection of the gills of an anesthetized Brown Trout in the field can

accurately determine the presence of the Freshwater Pearl Mussel. Field trials had accuracy that was similar to microscopic inspection in the lab. The rivers surveyed were wadeable, clear, and had low to moderate flow at the time of sampling.

83. Sansom, B.J., Bennett. S.J., Atkinson, J.F., and Vaughn, C.C. 2018. Long-term persistence of freshwater mussel beds in labile river channels Freshw. Biol. **63**: 1469–1481.

    The goal of this study was to determine the association between freshwater mussel persistence in a river and sediment stability. The authors evaluated two streams with historically long records of mussel sampling that are also ecologically significant: Tonawanda Creek and French Creek in New York State. French Creek is one of the most diverse in the northeast USA. Mussels in these creeks have patchy distribution and typically occur in riffle-run reaches. Survey reaches were ~ 100 m long and surveys followed a timed search method. Survey sites ranged in depth from 0.3 to 1.2 m, with discharge of 67 and 113 m^3/s in Tonawanda and French Creek, respectively. Turbidity is not reported. Each site was surveyed for one person-hour using visual and tactile techniques. At one site in each stream the visual search was augmented with the excavation of one small quadrat. The authors report that both surveyed streams demonstrated mobilization of up to 90% of the bedload during ~ 2-year high-flow events. However, mussel diversity and abundance has not changed over a 20-year period, suggesting mussels can withstand bedload movement during high-flow events.

84. Sansom, B.J., and Sassoubre, L.M. 2017. Environmental DNA (eDNA) shedding and decay rates to model freshwater mussel eDNA transport in a river. J. Environ. Sci. Technol. **5**(24): 14244–14253.

    Freshwater mussels contribute DNA to the environment (eDNA) from sloughed gametes, tissue, and cells, and from filtered excretions. This study used a mesocosm experiment to determine the rate of freshwater mussel eDNA contribution to the environment, and how long it persists (rate of decay). The authors found that in modelled flow rates of 0.09 m/s, with mussel density of 0.1 mussels/m^2, eDNA is likely to persist up to 36.7 km downstream. This study did not discuss live mussel collection or collection methods.

85. Sanchez, B., and Schwalb, A.N. 2021. Detectability affects the performance of survey methods: a comparison of sampling methods of freshwater mussels in Central Texas. Hydrobiologia. **848**(12–13): 2919–2929.

    Although this survey only considers shallow, wadeable habitats, it discusses the performance of three commonly used sampling methods that are applied in deep, turbid habitats. Timed search, transects, and adaptive cluster sampling methods are compared to assess method performance in terms of species richness, total

capture of mussels per unit search effort, species composition, and mussel size distribution. The survey was conducted in rivers in Central Texas. The survey sites were each 50 m in length and 10 m wide, ranging in depth from 0.45 to 1.7 m, with low to moderate flow (0.03–1.1 m/s). Sites were visited weekly or bi-weekly between application of survey methods. At each visit the survey method order was changed. Timed searches were completed using visual/tactile techniques and one person-hour. Transect method involved 9 by 10 m transects set perpendicular to the flow at 5 m intervals. On each transect five quadrats (50 cm by 50 cm) were spaced evenly and excavated to a depth of 10 cm. For adaptive cluster sampling, the search area was divided into non-overlapping quadrat locations. Three locations were chosen randomly to start the survey and if a mussel was found in a quadrat the adjacent quadrats were searched until a total of 45 quadrats were searched. The authors note that it is important to have a high number of random starts, relative to the total number of quadrats, in the adaptive cluster approach or the results may have high variation in computed density estimator and confidence interval. The study found that timed searches detected more, and larger, sculpted mussels than the other methods. Adaptive cluster sampling detected a higher proportion of smaller species and had a similar total search effort to the timed search method. The transect method had the lowest search effort in person-hours. Adaptive cluster sampling detected more mussels per search hour than transect method when patchiness was high, and density was high or moderate, particularly in sandy habitats. Timed search performed best for mussels per search hour but not in gravel/cobble substrates. Adaptive cluster sampling performance degraded as mussel detectability declined.

86. Schmidt, B.C., Spear, S.F., Tomi, A., and Bodinof Jachowski., C.M. 2021. Evaluating the efficacy of environmental DNA (eDNA) to detect an endangered freshwater mussel *Lasmigona decorata* (Bivalvia: Unionidae). Freshw. Sci. **40**(2): 354–367.

    The authors developed eDNA primers for the endangered species *L. decorata* to assess detection rates with respect to environmental parameters in the Lynches River Basin in North and South Carolina. The authors sampled 116 sites across stream orders 1 to 5. Two replicate 2 L water samples were collected from the stream bank at each site where mussels were known to occur. Turbidity at the sites ranged from 0.1 to 24 NTU and pH ranged from 2.9 to 7.3. The authors found no positive eDNA detections in the field and almost all samples were inhibited during analysis. The authors conclude that the probability of eDNA inhibition is 100% when pH is <5.5. For species that occur in low pH environments visual and tactile methods are more effective than eDNA.

87. Schöne, B.R., and Krause, R.A. 2016. Retrospective environmental biomonitoring of Mussel Watch expanded. Glob. Planet. Change. **144**: 228–251.

This report provides a summary of toxicological assessment to support environmental biomonitoring of marine clams. Clam sampling and collection methods are not provided.

88. Schueler, F., and Robson, R. 2018. Brailing the Plantagenet Reach: Not catching anything where there may not be anything to catch. *In* Proceedings of the 2017 Canadian Freshwater Mollusc Research Meeting: November 8–9, 2017, Burlington, Ontario. Can. *Edited by* T. J. Morris, K. A. McNichols-O'Rourke, and S. M. Reid. Can. Tech. Rep. Fish. Aquat. Sci. **3246**: viii + 26 p.

    The authors presented the preliminary results from a field trial of a brail on a medium-sized river in eastern Ontario. The objective of the field trial was to determine if brail could be used to determine unionid presence. The area of the South Nation River that was sampled had predominantly clay substrate and was thought to be heavily infested by Zebra Mussels. The brail was built based on specifications available online. The brail was towed behind a boat for approximately 1 hour on several occasions over three days. The sample reach was deep (>1.5 m) and turbid. Boat speed during brail deployment was 1.2-2.5 km/hr. The authors report that lower speeds were more effective. Also, the authors used sonar while towing the brail to avoid snags. No freshwater mussels were captured.

89. Schwalb, A.N., Morris, T.J., and Cottenie, K. 2015. Dispersal abilities of riverine freshwater mussels influence metacommunity structure. Freshw. Biol. **60**(5): 911–921.

    The authors examined contemporary and historical freshwater mussel distribution and abundance data from the Great Lakes region to determine the degree of association between freshwater mussel dispersal ability and environmental factors. The authors performed a meta-analysis of existing data and did not collect live mussels. The data used in the study was based on previous mussel collections, largely from wadeable streams.

90. Selckmann G.M., Smith Z., Cummins J., Griggs A., and C. Buchanan. 2018. Biological surveys of three Potomac River Mainstem reaches (2012–2014) with considerations for large river sampling. ICPRB Report 18-2. Interstate Commission on the Potomac River Basin. Available from https://www.potomacriver.org/wp-content/uploads/2018/12/LargeRiver_2012_2014-FInal.pdf [accessed March 2020]

    The Potomac River Basin, Maryland, was surveyed in under-represented reaches to assess the effort required to accurately determine freshwater mussel and benthic macroinvertebrate populations in a large river. The mainstem of the river in the study area has a stream order of 7 and is not wadeable in most locations. Sampling sites were chosen based on satellite images and preliminary site visits. Sampling sites were < 1 m in depth, with moderate flow. Turbidity was not reported. A 5 m by 5 m grid was overlain on satellite images of the site area

and 25 grid cells were randomly selected as survey plots. Each site was broken into four quadrants and cells were assigned to riffle-pool habitat in each quadrant equally. Within each cell a timed visual search was conducted in the 12.5 m2 area. Within each cell one quadrat was excavated (based on a random throw of a quadrat within the cell). Excavated material was scooped into a sieve box. No deep, turbid, or fast-flowing sections of the river were surveyed.

91. Sherwood, J. 2011. A survey of the freshwater mussel of the La Moine and Spoon River Basins, Illinois. MSc Thesis. Western Illinois University. www.dnr.illinois.gov/grants/Documents/WPFGrantReports/2011023W.pdf [accessed online January 2018].

    This report summarizes a Master of Science project that aimed to document the occurrence of freshwater mussels and estimate mussel diversity in La Moine and Spoon Rivers. Mussels were collected using a four-person-hour timed-search approach and using visual and tactile sampling techniques. Thirty-seven sites were surveyed. At three deep-water sites a brallie [brail] was used to collect mussels. No further details of the use of the brail were provided. A comparison of handpicking to brail collection was not provided.

92. Shogren, A.J., Tank, J.L., Andruszkiewicz, E., Olds, B., Mahon, A.R., Jerde, C.L., and Bolster, D. 2017. Controls on eDNA movement in streams: Transport, Retention, and Resuspension. Sci. Rep. **7**: 5065.

    The objective of this report is to provide general guidance for understanding how environmental factors influence eDNA movement in lotic systems. The authors provide a framework to inform how eDNA is resuspended, transported, and retained in lotic systems. Understanding transport and retention of eDNA will assist in developing protocols to estimate biomass and target species abundance. This study provides a case study based on fish in a mostly wadeable stream.

93. Sietman, B.E., Whitne, S.D., Kelner, D.E., Blodgett, K.D., and Dunn, H.L. 2001. Post-Extirpation recovery of the freshwater mussel (Bivalvia: Unionidae) fauna in the Upper Illinois River. J. Freshw. Ecol. **16**(2): 273–281.

    This report summarizes the results of a freshwater mussel survey in the Illinois River that aimed to determine if mussels have recolonized the upper river following their extirpation in the 20th century. The authors used a brail for reconnaissance sampling and to determine mussel bed location in the Illinois River, which is a turbid river (depth not reported). The authors conducted 85 brail runs of varying transect length (50 to 670 m). Surface air supplied divers were also employed to survey (visually and tactile) areas where the brail results suggest mussels may be present in larger quantities. The authors found that only 15.3% of brail runs resulted in the collection of more than one mussel and that the majority of animals were collected by diving. The estimated density of

mussels in aggregated locations in this study was high (>19 mussels/m^2). The brail had the dimensions of 157.5 cm in length, with 21 30.5 cm chains, and three four-pronged hooks per chain.

94. Singh, W. 2015. Towards efficient benthic survey design with the use of Autonomous Underwater Vehicles. PhD Thesis. School of Engineering and Natural Sciences, Faculty of Physical Sciences. University of Iceland. Available from https://skemman.is/bitstream/1946/22561/1/PhD_Thesis_WarshaSingh.pdf [accessed January 2018].

    This doctoral dissertation describes a project that examined the utility of an autonomous underwater vehicle (AUV) as a survey technique for the Iceland Scallop. The AUV was fitted with photographic and acoustic units to assess its effectiveness as a survey tool for population assessments of the Iceland Scallop. This study was conducted in deep (< 3 m), marine environments with moderate flow and high visibility using a Gavia modular AUV system. The Gavia system (with its five modules: the nose, battery, Doppler Velocity Log, velocity meter, control and propulsion module) was 2.2 m in length and weighed roughly 60 kg. Surveys with the AUV involved sampling four parallel transects 300 m long placed 50 m apart. Transect surveys were repeated five times with a random start of each initial transect within the study area. The author reports that the use of AUV is likely limited to shallow depths (< 2 m) when relying on photographic evidence as even high-resolution camera photos become distorted beyond this depth. High-velocity environments would likely cause both sonar and photographic results to have low resolution. The efficiency of AUV in turbid environments or with large cobble and boulder substrate is anticipated to be low. Additionally, the author notes that the AUV could not document the estimation of subsurface or partially buried clams.

95. Smit, R., and Kaeser, A. 2016. Defining freshwater mussel mesohabitat associations in an alluvial, Coastal Plain river. Freshw. Sci. **35**(4): 1276–1290.

    The objectives of this study were to assess freshwater mussel mesohabitat associations and persistence of mesohabitats after high flow events in the Apalachicola River. Sonar was used to determine habitat types within the river and to inform a stratified freshwater mussel sampling design. Environmental characteristics of the study site were: low to moderate flow, low turbidity, and depths ranging from 0.6 to 4.3 m in a meandering portion of the river. Mussel density was not reported. Mussel surveys were conducted at six sites in each of the five mesohabitat types defined by sonar imagery. The authors collected mussels from stratified habitat units by wading, snorkeling, and SCUBA and using visual or tactile techniques, depending on depth and visibility. At the center point of each site a 10 m^2 circular area was staked with rebar to delineate a fixed search area. Following visual or tactile search, excavation of the sediment to 10 cm occurred. The area was searched until no new mussels were found.

96. Smith, D.R., Villella, R.F., and Lemari, D.P. 2001. Survey protocol for assessment of endangered freshwater mussels in the Allegheny River, Pennsylvania. Freshw. Sci. **20**(1): 118–132.

    In order to meet the biological assessment requirements of the U.S. *Endangered Species Act* (ESA), the authors developed a standardized survey protocol for endangered freshwater mussels in the Allegheny River. The survey protocol can broadly inform freshwater mussel population status at the site level. Qualitative and quantitative sampling methods are combined to determine species presence, assess diversity, and estimate density, sex ratios, and recruitment. The protocol recommends using a statistical sampling design, double sampling, to reduce the number of quantitative samples that are required. In this protocol, quantitative samples are obtained through quadrat surveys and where a portion of quadrats are also excavated. The protocol design was tested in a wadeable (< 1.6 m depth) river with low turbidity and during low flow. This protocol was found to be effective where mussel density was low (0.1 mussels/m^2). Qualitative surveys employed a timed search within a fixed 50 m x 50 m area. The fixed area was searched by a team of four, each surveying for 60 minutes for a total of four person-hours. The area was searched by snorkeling (0.5–1 m depth) and SCUBA (1–1.5 m). Snorkeling was employed 80% of the time. The authors assumed the search efficiency of snorkelers and divers was equal. Visual and tactile techniques were used to collect mussels. Following the timed-search, systematic double sampling of the fixed area with 0.25 m^2 quadrats was completed; quadrats were systematically placed with multiple random starts. The proportion of excavated quadrats that would minimize the variance of the density estimate was calculated based on the expected percent of mussels at the substrate surface, as informed by the qualitative survey.

97. Smith, D.R., Rogala, J.T., Gray, B.R., Zigler, S.J., and Newton, T.J. 2011. Evaluation of single and two-stage adaptive sampling designs for estimate of density and abundance of freshwater mussels in a large river. River Res. Appl. **27**(1): 121–133.

    The authors performed a computer modelling exercise to determine the most effective, in terms of cost and adequate population estimates, sampling design to inform freshwater mussel population assessments. The case study used data from a large river, the Upper Mississippi River. However, no field work or assessment of physical mussel collection methods is presented in this paper. The authors assessed adaptive and non-adaptive versions of single and two-stage statistical sampling designs using simulated freshwater mussel populations that varied in their degree of spatial clustering and density. These designs were chosen because they are suitable when sampling low-density, clustered populations and can reduce the burden of sampling in a large, deep river. The authors assumed that surveys would be undertaken by SCUBA diving and using quadrats to conduct surveys. Adaptive sampling designs were found to select occupied sampling units more often than conventional sampling designs. However, where mussel population density is low (0.01 mussels/m^2) the sample

size (quadrats) required to achieve a probability of species detection of 0.9 was over 1000, regardless of the sampling design.

98. Sollows, M.C., McAlpine, D.F., and Munkittrick, K.R. 2013. Density and abundance of the Freshwater Pearl mussel, *Margaritifera margaritifera*, in the Kennebecasis River, New Brunswick and evidence of recent recruitment. Can. Field-Nat. **127**(4): 303–309.

    The density, abundance, and recruitment of the freshwater mussel, *M. margaritifera*, was assessed in the Kennebecasis River, N.B.. Average environmental characteristics of the mussel beds included: a large, wadeable river where depth = < 0.7 m, flow = < 0.28 m/s, low turbidity, and mussel density = 1.2 mussels/m^2. The survey site, as defined by the mussel bed, was determined visually while snorkeling downstream. The survey was completed by placing 60 random quadrats within the mussel bed, employing a visual search, and then excavating the quadrat to 15 cm.

99. Stirling, D.A., Boulcott, P., Scott, B.E., and Wright, P.J. 2016. Using verified species distribution models to inform the conservation of a rare marine species. Divers. Distrib. **22**(7): 808–822.

    This report summarizes the results of a species distribution modelling exercise that aimed to predict new occurrences and extent of suitable habitat for the Fan mussel (*Atrina fragilis*) in a marine protected area of Scotland. The model, based on presence-only data, was ground-truthed using quadrat-based digital still photography in areas of predicted high and low suitability. Predicted high-suitability areas were additionally surveyed with towed video cameras. This work was undertaken in a clear-water marine environment. The utility of the still photography or towed camera at depths of 50 m or more was not discussed. The target species can be semi-buried in the sediment and presumably the photography and video are able to discern these individuals.

100. Stoeckle, B.C., Beggel, S., Kuehn, R., and Geist, J. 2021. Influence of stream characteristics and population size on downstream transport of freshwater mollusk environmental DNA. Freshw. Sci. **40**(1): 191–201.

    This paper evaluated the relationship between freshwater mussel abundance, stream discharge, sampling distance, and eDNA detectability and quantity of a common mussel species in the Danube River system in Germany. The study focused on a single species detection method. eDNA samples were collected from sites with variable discharge rates (0.21–2.41 m3/s) and turbidity (1.1 to 18.6 NTU). Site depth is not reported. The authors found that eDNA of the Thick-shelled River Mussel (Unio crassus) could be detected upwards of 3 km downstream, and that eDNA detectability was positively related to population size. Further, the authors found that turbidity was negatively correlated to eDNA detectability, independently of population size. Three water samples were taken

at each site and from 50 m to 3200 m downstream. Highest rates of eDNA detection occurred 100 m downstream of a large mussel bed. Large mussel beds (~ 42,000 individuals) had a PCR detection rate of 70.3% when turbidity was measured at 14 NTU, whereas PCR detection rate was 95.2% at small mussel beds (~ 500 individuals) when turbidity was measured at 1.1 NTU.

101. Stoeckl, K., Denic, M., and Geist, J. 2019. Conservation status of two endangered freshwater mussel species in Bavaria, Germany: Habitat quality, threats, and implications for conservation management. Aquatic Conserv: Mar Freshw. Ecosyst. **30**: 647–661.

This study evaluated the status of two mussel species in Germany. Habitat quality was evaluated based on the presence of fish hosts and physical parameters. Thirty-four sites were surveyed based on known mussel locations. Sites were between 0.2–18 km long, < 1.5 m in depth, low to moderate flow (0.01–1.2 m/s), and low turbidity (4.36–9.13 NTU). A systematic sampling method using cross-channel transects was used to collect mussels and record habitat parameters. Each transect was 20 m long and spaced 80 m apart. Visual and tactile techniques were used along each transect by wading in the upstream direction or floating downstream with SCUBA or snorkeling gear.

102. Strayer, D., L., and Smith, D., R. 2003. A Guide to Sampling Freshwater Mussel Populations. American Fisheries Society Monograph 8. Bethesda, Maryland.

The authors provide a guide to sampling freshwater mussel populations focusing largely on wadeable streams. Specifically, the report is a summary of sampling methods and statistical survey designs that can be used to assess freshwater mussel occurrence at a site. The survey designs reviewed include: complete censuses, simple random sampling, informal sampling, stratified sampling systematic sampling, double sampling, two-stage designs, adaptive sampling, mark-recapture methods, regression designs, and distance sampling. The guide does not report on sampling in deep, turbid rivers. Brailing is discussed exclusively as a qualitative sampling method because it collects a small and biased sample of the mussel community. The authors also suggest that in deep water divers can be employed to scoop sediment into a bucket (along transects or from quadrats) that can be examined and sieved on shore. The authors suggest that PONAR and Eckman grabs can be used to excavate sediment from deep or turbid waters, but that PONARs are not effective in cobble substrates, and that PONAR is preferred to an Eckman unit. However, the authors note that the efficiency of PONAR sampling is not known and may underestimate aspects of the mussel population.

103. Thiel, P. 1981. A survey of unionid mussels in the upper Mississippi River. Technical Bulletin No. 124. Wisconsin Department of Natural Resources. Madison, Wisconsin.

The objective of this study was to assess the presence and absence of freshwater mussels in the Upper Mississippi River and to monitor the commercial freshwater mussel harvest. The sampling locations had variable flow and depths > 1.8 m. The substrate was predominately sand and gravel with silt. Turbidity was not reported. Mussel surveys were first conducted via 5-minute brail runs along a 100 ft transect. The brail had a 10 ft wide bar with 200 dovetail hooks and beaded prongs. Where the brail captured a mussel a 5 ft metal frame was placed around the area, and it was resurveyed by SCUBA divers using visual and tactile methods. Thus, the most productive mussel areas were resurveyed with SCUBA. The results show that that the brail catch efficiency was 0.7% of the available population. The author also reports that a brail was only 3.6% as effective as SCUBA diving at capturing mussels based on a previous study. Catch-per-unit-effort of the brail was correlated with abundance (estimated from SCUBA surveys).

104. Togaki, D., Doi, H., and Katano, I. 2018. Detection of freshwater mussels (*Sinanodonta* spp.) in artificial ponds through environmental DNA: a comparison with traditional hand collection methods. Limnology. **21**: 59–65.

This study evaluated the use of eDNA to detect freshwater mussels in ponds in Japan. A total of 24 ponds were surveyed ranging in size from 81 to 124,816 m^2 with depths from 1 to 5 m. eDNA was collected from water samples and confirmation of mussel presence was completed by hand collection using a timed-search approach. The paper does not describe how depths of >2 m were sampled. eDNA failed to detect mussels when present at one site and false positives were reported at 5 of 18 sites. However, search effort may have resulted in non-detections during the timed search. The authors report a positive relationship between the number of mussels collected by hand and the quantity of eDNA detections.

105. Wacker, S., Fossøy, F., Mejdell Larsen, B., Brandsegg, H., Sivertsgård, R., and Karlsson, S. 2019. Downstream transport and seasonal variation in freshwater pearl mussel (*Margaritifera margaritifera*) eDNA concentration. Environmental DNA. **1**: 64–73.

This study surveyed the River Drakstelva (Trøndelag county) in Norway. It is a small wadeable stream (depth < 0.5 m; flow 0.02–0.04 m/s) in the headwater region of the watershed with clear, non-turbid water. Mussel surveys employed a timed-search approach. The objective of the study was to evaluate the relationship between the spatial distribution of mussels and eDNA concentrations in a lotic system. The authors surveyed eDNA concentrations above and downstream (1700 m) of small and large mussel aggregations and across seasons (spring and summer). The authors found complete eDNA detection at sites downstream (1700 m) of large mussel aggregations, but that detection was low downstream of small aggregations (13%). Summer yielded higher eDNA detections than spring.

106. Wegscheider, B., MacLean, H., Linnansaari, T., and Curry, R.A. 2019. Freshwater mussel abundance and species composition downstream of a large hydroelectric generating station. Hydrobiologia. **836**(1): 207–218.

A freshwater mussel survey was completed to assess the mussel community downstream of a large hydropower dam on the Saint John (Wolastoq) River. Twenty-eight sites were located across five reaches of the river beginning from the dam tailrace to 50 km downstream. The furthest river reach is a depositional area. Two people searched a 10 by 10 m area for one person-hour and the search area was surveyed with visual and tactile methods in a haphazard manner. Survey sites were 1.4 to 2.84 m in depth with moderate to high flow (1.57 to 46.36 cm/s). Sites deeper than 1.5 m were not sampled except at two locations where SCUBA divers searched for 30 minutes along an undefined course for 230 m and 330 m, respectively, at each site.

107. West Virginia Division of Natural Resources Wildlife Resources Section (WVDNRWRS). 2020. West Virginia Mussel Survey Protocols. Available from https://wvdnr.gov/wp-content/uploads/2021/07/2020-WV-Mussel-Survey-Protocols.pdf [accessed March 2022].

State-standardized protocols for mussels in support of development in West Virginia, USA. The protocols aim to assess presence/absence of mussels during impact review. WV Rivers are divided into four groups: Groups 1 and 2 cover small or shallow sites, and Group 3 (large rivers where endangered species are not expected), and Group 4 (large rivers where endangered species are expected) describe methods for larger, deeper sites. Site search area is defined by the area of the anticipated impact zone and is standardized by Group. Group 4 rivers/sites must be surveyed using a transect method; transects must be placed perpendicular to river flow. Search should occur along and between transects. Cells (quadrats) are recommended in large rivers only when evaluating a very small project area and cells should not be bigger than 100 m^2. “A transect survey must contain at least 500 m of transect search area and consist of a minimum of five transects, three of which must be placed within the area of impact”. Transects are placed up to 20 m apart. Visual and tactile methods are used to search along the transect and must include at least 1 minute/m^2 effort. Data from Group 4 transects will need to be supplemented with timed searches conducted within the mussel concentration area (10-minute increments) until no new species are found in six consecutive samples. Authors recommend randomizing sampling to increase statistical power. Search effort should increase in heterogenous habitat compared to homogenous habitat. Surveys should be conducted in high-visibility, low-flow conditions.

108. White, S., Koehane, I., Woodford, E., and Burstein, J. 2019. Congaree River mussel habitat sonar survey. Final report submitted to USFWS and to Congaree National Park.

Sonar surveys were used to evaluate mussel habitat in a large coastal plain river, the Congaree River, in South Carolina. The survey area covered 15 river miles and used a Ping DSP 450 3D side-scan sonar unit to create digital maps of mussel habitat and predict mussel habitat. Survey sites were reported as 1 to 4.8 m in depth with high flow. Sediment was collected for grain size analysis. No mussels were sampled during the program. Digital habitat models were overlaid on existing mussel distribution maps.

109. Wisniewski, J., M., Shea, C., P., Abbott, S., and Stringfellow, R., C. 2013. Imperfect Recapture: A Potential Source of Bias in Freshwater Mussel Studies. Am. Midl. Nat. **170**(2): 229–247.

This study evaluated how incomplete detection of individuals in a population can bias the results of freshwater mussel presence/absence surveys. The authors evaluated seven years of mark-recapture data to measure recapture probabilities and to estimate survival. The results suggest that only a limited number of mussels at a site are collected during a given sampling event, even though similar number of mussels may be collected between samples. Recapture rates were influenced by species, time, and shell length. Therefore, studies that do not account for incomplete detection may bias interpretation of survey results. Mussels used in the study were collected from a wadeable stream (<1 m) where mussel density was high. A fixed-area, timed-search survey design was followed. Only 16.5% of all mussels in the study were captured on more than two sampling occasions.

110. Zieritz, A., Gum, B., Kuehn, R., and Geist, J. 2012. Identifying freshwater mussels (Unionoida) and parasitic glochidia larvae from host fish gills: a molecular key to the North and Central European species. Ecol. Evol. **2**(4): 740–750.

This report provides a complete molecular identification key for European freshwater mussel species. The method can be applied to adult and larval specimens. While freshwater mussels used in this study came from a variety of habitats including lake and ponds, the method of collection is not provided.