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PERSPECTIVE

Approaches and research needs for advancing the protection and recovery of imperilled freshwater fishes and mussels in Canada[1]

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Abstract: Effective conservation requires that species recovery measures are informed by rigorous scientific research. For imperilled freshwater fishes and mussels in Canada, numerous research gaps exist, in part owing to the need for specialized research methods. The Canadian Freshwater Species at Risk Research Network (SARNET) was formed and identified or implemented approaches to address current research gaps, including (1) captive experimental research populations, (2) nonlethal methods for estimating abundance and distribution, (3) nonlethal field methods to measure life-history parameters, (4) species distribution models informed by co-occurring species, (5) conservation physiology to inform habitat and threat science, (6) evidence syntheses to evaluate threats and recovery measures, (7) disease-transmission models to understand mussel–host relationships, (8) experimental mesocosms and manipulative experiments to evaluate key habitat stressors, (9) threat and hazard models for predictive applications, and (10) rigorous evaluation of surrogate species. Over a dozen threat- and recovery-focused SARNET research applications are summarized, demonstrating the value of a coordinated research program between academics and government to advance scientific research on, and to support the recovery of, imperilled freshwater species.

Résumé : Des mesures de rétablissement reposant sur des travaux de recherche scientifique rigoureux sont nécessaires à une conservation efficace. Pour les espèces de poissons d'eau douce et mulettes en péril au Canada, il existe de nombreuses

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lacunes dans la recherche, dues partiellement à la nécessité de méthodes de recherche spécialisées. Le réseau canadien de recherche sur les espèces d'eau douce en péril (SARNET) a été créé et il a cerné ou appliqué différentes approches visant à combler ces lacunes, dont les suivantes : (1) des populations expérimentales captives de recherche, (2) des méthodes non létales d'estimation de l'abondance et de la répartition, (3) des méthodes de terrain non létales de mesure de paramètres du cycle biologique, (4) des modèles de répartition des espèces intégrant des données sur les espèces cooccurrentes, (5) la physiologie de la conservation pour soutenir les travaux scientifiques sur les habitats et les menaces, (6) des synthèses de données probantes pour évaluer les menaces et measures de rétablissement, (7) des modèles de transmission des maladies pour comprendre les relations mulettes–hôtes, (8) des mésocosmes expérimentaux et des expériences de manipulation pour évaluer des facteurs de stress clés des habitats, (9) des modèles de menaces et de dangers pour des applications prédictives et (10) l'évaluation rigoureuse d'espèces substitutives. Un résumé de plus d'une douzaine d'applications des travaux de recherche du SARNET axées sur les menaces et le rétablissement est présenté, qui démontre l'utilité d'un programme de recherche au sein duquel les travaux de chercheurs universitaires et gouvernementaux sont coordonnés afin de faire avancer la recherche scientifique sur les espèces d'eau douce en péril et soutenir leur rétablissement. [Traduit par la Rédaction]

Introduction

An important goal of conservation biology is to prevent species extinctions caused by human activity (Soulé 1985). When populations decline and become imperilled, various recovery measures are often enacted to rebuild populations and address the threats that led to imperilment. Imperilled species conservation is most successful when recovery measures are informed by rigorous scientific research (Dee Boersma et al. 2001), so it is imperative to identify knowledge gaps and associated research bottlenecks that limit progress on recovery efforts (Drake et al. 2021). Although research gaps can occur due to funding constraints, poor research engagement, or a disconnect between knowledge generators (e.g., researchers) and (or) knowledge users (e.g., conservation practitioners and decision makers), gaps also exist due to technical limitations, a lack of suitable methods to address key uncertainties, and (or) simple failures of regulatory oversight. These issues are particularly problematic for recovery measures that are complex, involve multiple steps and stakeholders, and require active intervention with significant socioeconomic consequences such as mitigation of threats (Cattarino et al. 2015) and species reintroduction (Cochran-Biederman et al. 2015; Lamothe et al. 2019*c*). As recovery efforts require long-term commitment of resources, it is critical to make use of the best available knowledge. For imperilled species in particular, the paucity of suitable specialized research methods is compounded by uncertainty about basic life-history traits, sparse populations (limited in number, distribution, and (or) abundance) and the need for nonlethal sampling, all of which suggest that new approaches are needed to advance research progress. Given the high levels of imperilment among freshwater organisms, there is an urgent need to identify effective strategies based on rigorous scientific research for reversing biodiversity loss and recovering populations (Tickner et al. 2020).

In Canada, imperilled species are federally protected when they are listed under the *Species at Risk Act* (SARA 2002); however, to date, the recovery of SARA-listed species has been variable (Favaro et al. 2014; WWF-Canada 2020). This reflects, at least in part, an increased volume of species listed under the Act and scientific gaps that limit recovery progress (Drake et al. 2021). There are several challenges that management biologists and conservation practitioners face related to restricted scientific knowledge and experience, including making permitting and protection decisions with high degrees of uncertainty, delaying action or decision-making due to these uncertainties, being unable to confidently recommend remedies, mitigation, or set quantitative recovery targets, and limited background information to support the funding of stewardship projects. Addressing research gaps at the scientific level could reduce many of these uncertainties and increase scientific knowledge to support management decisions.

To identify research gaps that may be impeding species recovery and to facilitate the development of an integrated freshwater species at risk research program in Canada, an analysis of research for SARA-listed freshwater fishes and mussels was performed, focusing on progress within four focal areas: population ecology, habitat, threats, and recovery science (Drake et al. 2021). Based on the analysis, a workshop was held to review priority research actions that have yet to be implemented for freshwater fishes and mussels listed under SARA due to a lack of scientific or technological means (McNichols-O'Rourke et al. 2021). The workshop identified priority knowledge gaps that could be resolved with available or emerging technologies, tools, and approaches, and a collaborative government–academic research network (Canadian Freshwater Species at Risk Research Network, SARNET) was formed to deliver the research program. This paper provides a synthesis of the expert-identified research approaches and describes the novel approaches used by SARNET scientists to address the priority gaps since 2017. These approaches demonstrate that research partnerships between academia and government can be an efficient model for developing science in support of species recovery. Lastly, we identify key outstanding knowledge gaps and potential research approaches to support recovery of SARA-listed freshwater fish and mussel species in Canada.

Methods

Following an analysis of research progress against existing recovery and management plans for SARA-listed freshwater fish and mussel species in the Great Lakes basin (Drake et al. 2021), a two-day workshop organized by Fisheries and Oceans Canada and the University of Toronto Scarborough was held on 15–16 February 2017, in Oakville, Ontario (McNichols-O'Rourke et al. 2021), to survey experts with knowledge of SARA-listed species and related research expertise (genetic methods, habitat modelling, population modelling, reintroduction science, conservation physiology). The purpose of the workshop was to discuss outstanding scientific uncertainties and establish research priorities for SARA-listed fishes and mussels. A total of 29 scientific experts and managers from a variety of government and academic institutions participated, creating the potential for an impactful identification of needs and approaches through diverse participation of researchers, research users, and potential funders (Dey et al. 2020).

Experts were assigned to breakout groups to discuss and consider specific research gaps for a subset of identified subdisciplines (Tables 1–4). Discussions were moderated and focused on how existing approaches, as well as new methods, tools, or technologies, could be applied to resolve identified knowledge gaps (McNichols-O'Rourke et al. 2021). The main discussion points and approaches to address the gaps are explored in this manuscript.

Following the workshop discussions, academic researchers were invited to submit funding proposals to conduct research within the threat and recovery science envelopes. Fourteen research studies were funded in part by Fisheries and Oceans Canada, and SARNET (https://fwsarnet.ca/) was formed. Studies investigated physiological responses to threat-induced stresses, modelled species co-occurrence using fish community data, and estimated the potential for genetic

Table 1. Key research gaps and approaches discussed during the expert workshop for the population ecology of SARA-listed freshwater fishes and mussels, based on analysis of completed research by Drake et al. 2021.

Subtheme	Research gaps	Approaches
Life history	1. Difficult to obtain data on fecundity, egg sizes, sex ratios, age at first maturity, and age structure with nonlethal sampling 2. Difficult to estimate age-specific mortality, especially for early life stages 3. Reproductive behaviour poorly understood, direct observation difficult 4. Dispersal and home range of small-bodied species difficult to assess. Movement of subadults also difficult to assess 5. How to increase consistency of age interpretations among readers? How to validate aging structures with nonlethal methods?	Prioritize simple natural history studies Nonlethal technology for measuring life-history parameters in the field: • Portable magnetic resonance imaging (MRI), ultrasound machines • Viewing windows (mussels) • eDNA (detect life stages, infer spawning activity) Surrogate species where appropriate Captive experimental research populations (CERPs): • Measure life-history traits • Validate nonlethal methods Artificial streams; whole-stream experimental manipulations
Abundance	1. How to estimate population trajectory and abundance for species with poor monitoring data or sporadic captures? 2. How to develop tag-recapture approaches for species at low abundance? 3. Utility of new methods to quantify abundance and refine sonar approaches for shallow, noisy, and vegetated environments? Other automated approaches, eDNA? 4. How to refine extinction thresholds across populations? 5. How to extrapolate density-based estimates when extent of suitable habitat is unknown? 6. How to confirm if reproducing populations exist (detection of early life stages difficult, adults often inaccessible during spawning season and compounded by low population abundance)?	No quick fix; life-history advancements will lead to improvements Nonlethal technology: • eDNA (quantitative assays; detect life stages and gametes) ◦ Temporal eDNA surveys to detect seasonal activity and gamete release • Underwater cameras • Real-time outputs of tags • Surrogates (presence of host fish) • Sonar (but constraints in shallow and noisy environments)
Distribution	1. Inferring distribution at historical and extant sites compounded by general sampling issues; how to quantify occupancy given low probability of detection? Utility of eDNA?	Occupancy models account for detection probability Prioritize standardized protocols and programs Improving digital data transfer for historical and current specimen records across institutions (regional, provincial, national, and international natural history museums holding Canadian aquatic species) Traditional ecological knowledge Combine eDNA with habitat occupancy modelling Sediment core eDNA analysis to identify historical distribution Infer distribution with life-history traits of populations at different climates
Genetics	1. Exploring genetic variation within and among populations. What are the implications of very small samples sizes? How can continued advances (e.g., SNIPS) lead to improved genetic understanding? 2. Undertaking genetic research to inform captive rearing and relocation — what are the next steps?	Identify intraspecific designatable units (DUs) and distributions Infer historical population sizes and connectivity from neutral genetic markers; contrast with non-neutral genetic variation (evidence of selection or local adaptation) Potential of eDNA to identify intraspecific variation, population abundance Resolve mating systems and relatedness in wild populations; use to inform establishing CERPs Mating trials to investigate benefits of genetic rescue vs. outbreeding depression risk from mating with allopatric source–rescue populations
Species interactions	1. How to determine abundance of host fishes required to support mussel populations? 2. How to evaluate mechanisms of glochidial attachment (carrying capacity on host gills)? 3. How to evaluate mussel dispersal ability based on interactions with host fishes? 4. Evaluating competitive and predatory interactions within fish community — how to conduct food web studies (including gut contents) with nonlethal sampling?	Evaluate the transferability of lab studies to natural populations Borrow from disease-transmission and host–parasite modelling to understand mussel–host dynamics Improve our ability to determine, nonlethally, the host fishes for SAR freshwater mussels through rapid identification of glochidia attached to gills or fins of fishes using latest DNA Evaluate timing and access of mussels to host species; fish–host traits for glochidia maturation Lethally sample predators and examine gut contents Stable isotope analyses with and without invasive species; bioenergetics Mesocosms, experimental populations, and field manipulations

Table 2. Key research gaps and approaches discussed during the expert workshop for habitat science of SARA-listed freshwater fishes and mussels, based on analysis of completed research by Drake et al. 2021.

Subtheme	Research gaps	Approaches
Habitat associations by life stage	1. Identify stage-based associations to inform critical habitat and develop predictive models of species–habitat associations. How to construct habitat suitability models with sparse captures? Utility of MaxEnt or similar approaches? 2. How to infer habitat use by early life stages (reliable identification of eggs + early life stages) 3. How to determine correlation vs. causation in habitat selection for animals with no or poor reference condition? 4. How to evaluate physiological thresholds without lab studies? Field-based approaches? Utility of surrogates?	Species distribution models involving species co-occurrences Habitat suitability model validation: • Gut content analysis (nonlethal) • eDNA to detect species presence DNA barcoding to identify eggs and larvaee DNA to detect habitat occupancy by cryptic life stages Laboratory studies: • Surrogates • Captive experimental research populations Field studies: • Comparative studies • Enclosures • Experimental rivers and waterbodies • Seasonal habitat occupancy (eDNA) to identify critical habitat Otolith microchemistry Prioritize basic questions such as random vs. nonrandom habitat associations Physiological tolerance nonlethal methods: • Cortisol (blood, fin, scales) • CT_{max} • Thermal shock proteins • Electronic tags (heart rate, temperature) • Mussel-specific sensors (respiration, movement, digging) • Tissue samples, gill biopsies
Habitat supply	1. Mapping habitat quality and quantity within historical and current sites 2. What is state of available technology to map habitat features (substrate, macrophytes, water quality, riparian features)? Utility of sonar-based approaches, drones, LIDAR? What are the operational constraints (shallow, noisy, high velocity, vegetation)?	Consider species-specific habitat quality Several technologies can now be applied in smaller systems: • High-resolution side and bottom imaging with portable sonar technology • Remote sensing • Drones • ROVS • LIDAR • Acoustic doppler • Incremental instream monitoring tools (in development) Bayesian mapping

maintenance in, and synthesized the evidence of the effectiveness of, captive breeding programs, among other topics (Leclair et al. 2020; Turko et al. 2020, 2021; Firth et al. 2021; VanTassel et al. 2021; Zinn et al. 2021; Luck 2020; Lum 2020; McDonnell et al. 2021; Rodríguez et al. 2021; Rosencranz et al. 2021; Rosenfeld et al. 2021; Rytwinski et al. 2021).

Results: expert-identified research approaches and SARNET priorities

Workshop experts identified research gaps and many potential approaches and methods to fill the knowledge gaps determined by Drake et al. (2021) by thoroughly addressing the questions presented (Tables 1–4). There was much overlap identified in approaches and in some novel applications of approaches across themes and subthemes. Threat and recovery sciences were determined to be the most data and research deficient, and SARNET projects were implemented to fill these knowledge gaps. Ten research needs that overlapped across disciplines were identified and deemed to be a priority for advancing threat and recovery research for imperilled freshwater species. The 10 research priorities, in no particular order, are (1) developing captive experimental research populations to identify life-history traits, reproductive ecology, and species responses to threats, (2) developing and implementing methods to improve nonlethal abundance and distribution estimates (e.g., cameras and eDNA), (3) developing and implementing non-invasive methods to measure life-history parameters in the field (e.g., ultrasound), (4) developing species distribution models involving co-occurring species, (5) integrating conservation physiology into habitat and threat science, (6) using rigorous evidence syntheses to evaluate threats and recovery measures, (7) developing disease-transmission models to understand mussel–host relationships, (8) using experimental mesocosms and other manipulative experiments to evaluate key habitat

Table 3. Key research gaps and approaches discussed during the expert workshop for threats to SARA-listed freshwater fishes and mussels, based on analysis of completed research by Drake et al. 2021.

Subtheme	Research gaps	Approaches
Mechanisms	1. Identifying threat mechanisms. Findings often based on speculation or weak correlations — causation usually unknown. Cause–effect experiments difficult or impossible in field (lack of replicates, low population abundance). Lab experiments promising (need access to range of life stages), but protocols lacking — how to develop husbandry techniques for fishes? 2. Mechanism of impact due to invasive species. How to isolate components of food web change that influence fishes and mussels (e.g., direct vs. indirect effects) — how to isolate mechanisms driving vital rates?	Experimental populations, streams, watersheds Mesocosm experiments when suitable Borrow predictive modelling techniques for catastrophic events (e.g., earthquakes and tsunamis); human behaviour modelling; mine existing spill databases Surrogate species, field or lab Physiology endpoints, field or lab Consider novel stressor interactions (e.g., microplastics, pharmaceuticals) Food web models; focus on rigorous baseline Interdisciplinary approaches and research teams
Probability and magnitude of impact	1. How to link environmental stressors to population responses (e.g., develop better relationship between stressor and vital rates). Environmental stressors include urbanization and water quality degradation, climate change, invasive species, vegetation removal and chemical application, agricultural drain maintenance, and variation in hydrologic regime	Large-scale analysis and assessment; mechanistic models coupled with empirical validation Continual observational sensors and studies Physiology endpoints, field or lab Stress physiology research on CERPs (chronic or acute) Risk mapping and pathway analysis; forecasting scenarios (climate change and aquatic invasive species)

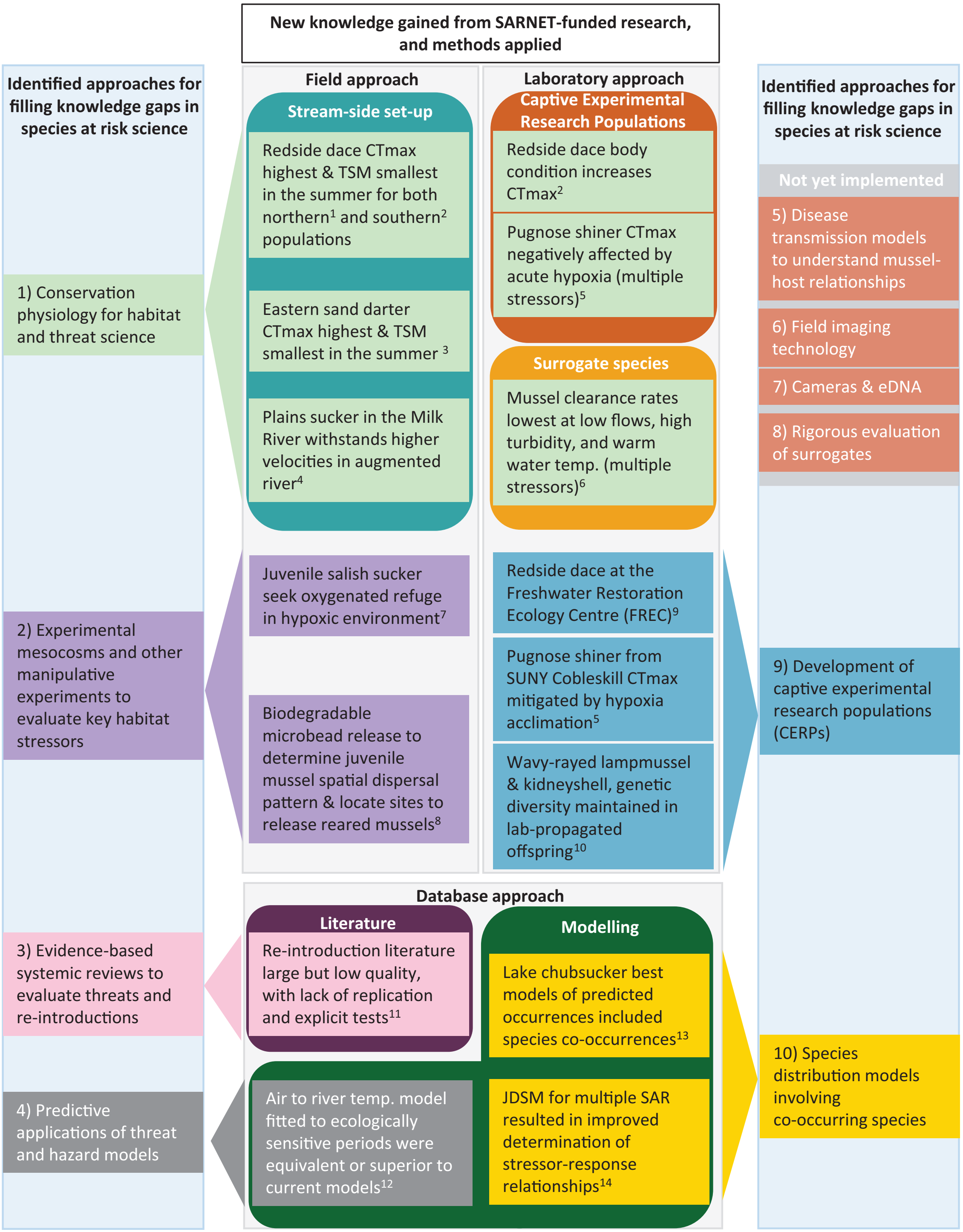
Table 4. Key research gaps and approaches discussed during the expert workshop for the recovery (threat mitigation; reintroductions) of SARA-listed freshwater fishes and mussels, based on analysis of completed research by Drake et al. 2021.

Subtheme	Research gaps	Approaches
Threat mitigation	1. Difficult to conduct multiyear field studies to develop "dose–response" relationships, imposes uncertainty about populations responses 2. Research needed that links stressor to mitigation measure to vital rates; would allow population responses to be predicted. Examples of stressors include climate change, invasive species, urbanization, flow regimes, pollutants; evaluate response of species to mitigation measures, especially flow mitigation and agricultural drain maintenance (suffer from low replicates) 3. Opportunities and limitations of stocking to mitigate transient vs. chronic threats? 4. Evaluate response of species to habitat restoration. How to estimate amount of restoration for population-level response? Dose–response relationships unknown, replicates difficult 5. Little research into invasive species control tactics; need to understand trade-offs in invasive species control or when incidental SAR mortality an issue	Monitor broad-scale stressors and mitigation projects Structured framework with uncertainty Post-mitigation monitoring to determine species response Consider threat avoidance from a landscape perspective rather than a site-level perspective Proactive rearing for anticipated threats Evidence-based conservation: • Systematic reviews • Meta-analyses Consider missing linked stressors Laboratory experiments for species interactions with AIS and AIS mitigation methods Cost–benefit analysis of threat mitigation, including AIS
Reintroductions	1. Feasibility of translocations and repatriations. Husbandry methods not developed for most SARA-listed freshwater fishes or mussels. How to complete life cycle in captivity? What are research priorities — understand hormonal treatments? Identify lab protocol to test host fishes or mussels 2. Site selection and stocking — Need jurisdictional body to oversee culture and reintroduction decisions. Stocking dynamics (when, how many, what release method to pursue). Impact of large stocked cohorts on remnant populations? Genetic and environmental implications of translocations and repatriations?	Need facilities in Canada to develop and prioritize husbandry of SARA-listed species Success of reintroduction is context-dependent Long-term monitoring Genetic diversity (founding diversity and adaptive potential) Efficacy of "headstarting" vs. captive breeding for producing propagules Mark–recapture Electronic tagging (biotelemetry and biologging) Structured approaches to identify reintroduction sites Propagule pressure from invasion biology to inform stocking density for reintroductions

stressors, (9) using threat and hazard models for predictive applications, and (10) rigorously evaluating the use of surrogate species. Of these 10 research priorities, SARNET research implemented six on several freshwater species at risk and surrogate species (Fig. 1). The approaches, ideas, and applications from the workshop, grouped by theme, are summarized in the Discussion section, along with focused research undertaken by SARNET.

Given the relatively limited progress on threat and recovery science to date, projects within SARNET focused on (*i*) field, lab, and analytical experiments to better understand threat mechanisms

Fig. 1. Ten identified approaches and applications for filling knowledge gaps in species at risk science. Coloured boxes represent each solution and their respective new knowledge gained through SARNET research. Projects are grouped by approach and methods used. Four approaches in red boxes have not yet been implemented by SARNET. [1]Leclair et al. (2020); [2]Turko et al. (2020); [3]Firth et al. (2021); [4]T. MacLeod (unpublished thesis); [5]McDonnell et al. (2021); [6]Luck (2020); [7]Zinn et al. (2021); [8]Lum (2020); [9]Turko et al. (2021); [10]VanTassel et al. (2021); [11]Rytwinski et al. (2021); [12]Rosencranz et al. (2021); [13]J. Bontje (unpublished thesis); [14]Rodríguez et al. (2021).



(causative factors) and impacts (consequences of causative factors), including relationships between multiple interacting stressors and species responses, and (*ii*) research to develop captive-rearing techniques for listed freshwater fishes and mussels, including the development of captive experimental research populations (CERPs), specifically to fill knowledge gaps in threats and recovery science. A variety of the research approaches were used, including manipulative field and lab experiments, modelling, and a systematic review (Fig. 1). The scientific knowledge gaps filled by SARNET research contribute to the general body of knowledge of how various threats and habitat stressors influence aquatic animals, which can be used to inform on-the-ground conservation actions.

Discussion: workshop discussion and SARNET's implementation of research priorities

The following sections provide an overview of the workshop discussion and SARNET-implemented research. Summaries of all the approaches suggested by the experts are outlined in Tables 1–4 for each research theme. Details on the approaches that have been identified as SARNET research priorities are reviewed by theme, and evidence on how these approaches can be used to inform species at risk science is provided through examples of completed SARNET projects.

Population ecology: approaches for estimating demographic parameters for imperilled freshwater species

Predicting population responses to stressors or habitat change is fundamental to advancing the management of imperilled species, yet many research gaps remain. Population modelling requires data to estimate model parameters (e.g., fecundity, mortality, or growth rates in different habitats), which may be difficult for rare and endangered species (Table 1). Conventional approaches for estimating life-history parameters for imperilled species are challenging due to their reliance on lethal sampling, sample-size issues of small populations, and the potential to deplete wild populations when collecting individuals for lab studies (Bennett et al. 2016; Costello et al. 2016). There is also concern regarding the extent to which parameters estimated in the laboratory reflect those in the wild. Surrogate or substitute species (or populations), whether from field or lab studies, can provide solutions in some cases (e.g., borrowing demographic parameters from other species; Caro and O'Doherty 1999; Caro et al. 2005; Cooke et al. 2017*a*), but rigorous evaluation of surrogates is required — a remaining SARNET priority (Fig. 1). Closely related species and different populations in geographically distinct areas can have very different life histories, physiological traits, and (or) environmental tolerances (Caro and O'Doherty 1999; Wiens et al. 2008; Gray et al. 2016), and thus, a set of criteria for a "good" surrogate species needs to be outlined for aquatic species at risk in Canada (US Fish and Wildlife Service 2015).

As an alternative, nonlethal methods to measure life-history traits (fecundity, egg size, sex ratio) are available in the form of portable, field-based imaging units — a remaining SARNET research priority (Fig. 1). Examples of nonlethal field methods include semi-portable magnetic resonance imaging (T. Pitcher, personal communication), portable ultrasound machines (Hildebrandt et al. 2003; Chiotti et al. 2016), viewing windows for marsupial pouches in mussels, and examination of marsupial gills in live unionid mussels by prying open the valves (Beaver et al. 2019) using custom-made valve-prying pliers (A. Martel, personal communication). Life-history stages such as eggs or larvae that can be difficult to detect or identify in the wild can be identified using DNA barcoding (Hebert et al. 2003; Hubert et al. 2008) or detected using environmental DNA (eDNA) assays, thereby informing the timing and location of reproduction events and allowing conservation practitioners to establish protection measures at the appropriate time and location (Bylemans et al. 2017; Gallage 2020).

Establishing and developing CERPs (Fig. 1) provides unique opportunities to obtain life-history, behavioural and physiological data without endangering wild populations, as well as providing other potential benefits for species restoration. A CERP is a population of the focal species that is obtained from the wild, ideally from the population in question, or a subpopulation that is less at risk (Turko et al. 2021); however, in cases where local adaptation or other strong intraspecific differentiation is suspected, CERPs must be specific to the population of concern. Establishing and maintaining CERPs, where needed, would provide applied knowledge relating to captive breeding and rearing, which is important for reintroductions or population rehabilitation efforts. The ability to conduct controlled experiments is also critical for testing hypotheses about threat mechanisms and the vulnerability of specific life stages. One example of a CERP developed through SARNET is a redside dace (*Clinostomus elongatus*) population housed at the University of Windsor Freshwater Restoration Ecology Centre (FREC). The FREC population consists of hundreds of individuals (both juveniles and adults) collected from parts of the range where the species is less imperilled. This CERP has been used to examine captive-breeding protocols (e.g., induction of gametes) and the response of redside dace to a variety of anthropogenic stressors (see threats and recovery sections; Turko et al. 2020). FREC has also begun developing the holding-tank design, overcoming practical biological issues (i.e., husbandry), and understanding reproductive skew of captive breeding of the endangered lake chubsucker (*Erimyzon sucetta*). The lake chubsucker CERP will be developed to facilitate threat-based experimental research and support future reintroduction programs. One limitation of CERPs is that stressors (e.g., habitat degradation, impacts of invasive species) associated with species declines (e.g., growth, survival) are difficult to replicate in the laboratory. Mesocosm studies (Pagnucco et al. 2016), including experimental ponds and streams using CERPs, can increase realism of the experimental setting, thereby increasing confidence that species responses reflect those in the wild (Fig. 1).

The challenge of estimating distribution and abundance of rare taxa can be partially resolved with emerging, nonlethal methods, whether based on eDNA (McKelvey et al. 2016; Lacoursière-Roussel et al. 2016; Castañeda et al. 2020*a*) or underwater cameras (Castañeda et al. 2020*b*, 2020*a*; Vargas Soto et al. 2021) — a remaining SARNET research priority (Fig. 1). Although eDNA requires ongoing development before it can be routinely applied (Beng and Corlett 2020), it has the potential to detect rare and cryptic species (Boothroyd et al. 2016; Currier et al. 2018), identify specific life stages and spawning activity (Bylemans et al. 2017), quantify abundance (Lacoursière-Roussel et al. 2016; Tillotson et al. 2018; Spear et al. 2020), and assess intraspecific genetic variation (Marshall and Stepien 2019). Genetic methods can be used to estimate historical effective population sizes (Roesti et al. 2015) and current effective number of breeders (Hunter et al. 2020), which may provide abundance baselines for recovery strategies, and to identify the correct ecotype for (re)stocking (Cochran-Biederman et al. 2015; He et al. 2016). Underwater cameras have been shown to be useful in describing the distribution and abundance of imperilled species but are limited by water transparency and lengthy video processing (Ellender et al. 2012; Castañeda et al. 2020*b*). Other technological advances to estimate abundance and dispersal include small telemetry tags for small-bodied fishes and subadults (Cooke 2008; Clark 2016), although interpretation of tagging data remains difficult when sample sizes are small. Telemetry tags can also provide information on natural mortality and other demographic parameters that are needed to inform population modelling (Pine et al. 2003; Whoriskey et al. 2019; Lees et al. 2021).

Understanding species interactions, whether in the form of predator–prey dependencies, vulnerability to invasive competitors, or host–symbiont relationships (e.g., mussel glochidia), may be pivotal for endangered species recovery. eDNA and metabarcoding are emerging as useful tools for analyzing samples obtained by swabbing fish gills to test for glochidial attachments or assessing gut contents of predators (Ruppert et al. 2019). Occupancy models can evaluate the strength of positive or negative associations with co-occurring species and provide insight into potential dependencies (e.g., Lamothe et al. 2019*a*, 2019*b*). Glochidia–host interactions are key to the recovery of freshwater mussels, and the application of disease-transmission (Grenfell and Dobson 1995) and host–parasite models (Anderson and May 1978) to address key knowledge gaps in the reproductive ecology of freshwater mussels is a remaining SARNET research priority (Fig. 1). Using occupancy and host–parasite models would allow researchers to determine parameter sensitivities, which would aid in developing focused hypotheses for subsequent field and lab studies to inform the biological necessities for reintroduction (Restif et al. 2012).

Habitat science: approaches for determining habitat dependencies of imperilled freshwater species

Laboratory and field experiments and surveys are common methods to estimate habitat selection and habitat productivity (Reid et al. 2005; Drake et al. 2008; Thompson et al. 2017). As with vital-rate estimation, there are inherent difficulties with these approaches such as the transferability of lab results to wild populations and the challenges of manipulative field experiments (Table 2). Habitat preferences for many imperilled freshwater species are not fully known; therefore, conventional field research on habitat variables where the species are abundant is still necessary. Further, when population sizes are depressed, there may be constraints in dispersing to suitable habitat, potentially causing an imperilled species to inhabit suboptimal habitat (Caughley 1994), so field results require careful interpretation (Hirzel and Le Lay 2008).

The paucity of information about spawning and early life-stage habitat has limited the identification and protection of these critical habitats, so more effective sampling methods and modelling approaches are necessary. eDNA or eDNA metabarcoding can identify spikes of eDNA that could signal the occurrence, timing, and general location of reproductive events (Bylemans et al. 2017; Takeuchi et al. 2019) and relate their occurrence (or relative abundance) to habitat features. Developing habitat suitability or productivity models with sparse captures is difficult (e.g., Potts et al. 2021*b*), but in many cases, testing for evidence of nonrandom habitat associations can be used to develop hypotheses for subsequent fieldwork (Restif et al. 2012); occupancy models and their extensions can be incorporated here using measured habitat covariates (MacKenzie et al. 2018). Another modelling technique that can be used to understand habitat associations for species with sparse capture is co-occurrence modelling (Fig. 1), where strength is borrowed from a common species that is often found with the rare species, acting as a statistical surrogate. This approach provides additional predictive capabilities beyond the typical abiotic variables used in species distribution models (SDM) (Vezza et al. 2015). The addition of co-occurrence information provides opportunities to incorporate information related to biotic interactions involving the focal species and may help to identify stressor–response relationships by determining the occurrence of focal species in relation to abiotic and biotic factors. A SDM based on abiotic variables and another also including co-occurring species were developed to assess threats impacting lake chubsucker and fish communities (multiple SARA-listed species), respectively (Fig. 1). The best models at both local and regional scales were those incorporating species co-occurrence information into predictions of lake chubsucker occurrence (J. Bontje, unpublished thesis). It is possible that the limited range of the environmental data available may have reduced the influence of these variables within the models. As well, the decision to balance the number of observations with occurrence and absence information will increase the error rate (Olden et al. 2002) but provides more realistic estimates of the reliability of the resulting models. In another SARNET project, joint species distribution models (JSDMs) that combined information from extensive standardized field sampling of species relative abundances and literature data on species traits and phylogeny were used to quantify the responses of vulnerable species to environmental stressors. This work provided insight into threats to focal SARA-listed fishes (Rodríguez et al. 2021). The JSDM approach provided more precise estimates of species responses than single-species analyses and, thus, better defined the shape (linearity, thresholds) of the responses (Norberg et al. 2019). The results were similar to expert-derived judgements of habitat associations and threats found in COSEWIC reports (Rodríguez et al. 2021), indicating that COSEWIC reports and recovery strategies may benefit from future applications of the JDSM approach. The JSDM developed for multiple SAR simultaneously resulted in improved determination of stressor–response relationships, which also provides opportunities for threat mitigation (Rodríguez et al. 2021).

Integrating conservation physiology into habitat science (Fig. 1) will provide a clearer understanding of the connection between species and the productivity and quality of habitat (Ames et al. 2020). Nonlethal methods and measurements to evaluate physiological performance and tolerance exist (Table 2), including measures of cortisol (blood, fin, or scale samples), critical thermal maximum (CT_{max}), and heat shock proteins (Lutterschmidt and Hutchison 1997). CT_{max} is the temperature at which a fish exhibits a loss of equilibrium and is the endpoint of a thermal performance curve, which defines a species' fundamental thermal niche (Becker and Genoway 1979; Molnár et al. 2017). CT_{max} is critical to understanding the range of habitats that a species can occupy, given that it defines the point at which overall physiological function ceases and can inform population growth (Molnár et al. 2017). Measures of CT_{max} and acclimation capacity of CT_{max} are increasingly being used to predict organism responses to anthropogenic threats such as climate change and changing thermal gradients (Comte and Olden 2017; Morley et al. 2019). The study of CT_{max} to various stressors is explored in the threats section below. Electronic tags (both biotelemetry and biologging platforms) for fishes are now available to record heart rate, locomotor activity, and body temperature and have been used to inform conservation decisions (Wilson et al. 2015). Similar devices exist, or are in development, for mussels that record respiration, movement, and digging behaviour (Robson et al. 2009). Physiological knowledge derived from the aforementioned methods can be linked to habitat features using mechanistic species distribution modelling (Evans et al. 2015).

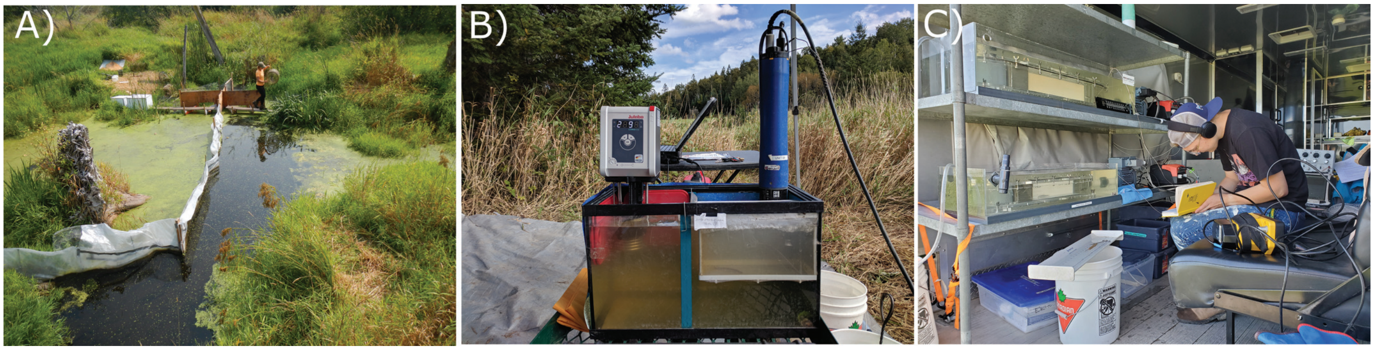
As with population ecology, the use of CERPs and experimental and artificial streams or watersheds can inform habitat-related questions (e.g., Gilliam and Fraser 1987; Giannico 2000; Gray et al. 2014; Turko et al. 2020). Such populations can be used to conduct manipulative lab or field experiments to disentangle causal associations between species and habitat. For example, Turko et al. (2020) used a CERP of redside dace to demonstrate a cause-and-effect relationship between condition factor and their thermal tolerance. Giannico (2000) manipulated food availability and woody debris in two small streams and found that juvenile coho salmon (*Oncorhynchus kisutch*) prefer open foraging areas interspersed with woody debris. Such information is important for habitat restoration and identification of critical habitat. Using experimental streams and CERPs enhances knowledge of habitat quality and quantity for species at risk.

Estimating habitat supply requires working knowledge of the habitat requirements of a species and a systematic habitat measurement approach at multiple spatial (reference sites) and temporal scales (at both historically and currently occupied sites). Several technological and statistical approaches for measuring habitat supply that were discussed by the workshop participants are listed in Table 2.

Threat science: approaches to identifying the mechanism and magnitude of threats to imperilled freshwater species

In most cases, the threats affecting freshwater species have been identified, but there is a lack of knowledge about the underlying threat mechanism, the scale and timing of the threat, and the species response (vital rates such as survival or fecundity implicated in decline and magnitude or periodicity of decline; Table 3). Current knowledge of chronic stressors usually centers

Fig. 2. Unique sampling methods and techniques used by SARNET research: (A) manipulative field experiments (photo by J. Rosenfeld, B.C. Ministry of the Environment); (B) streamside $\mathrm{CT_{max}}$ unit (photo by A. Leclair, University of Toronto); (C) streamside flow chamber unit (photo by M. Poesch, University of Alberta).



around extrapolating the effects of acute stress studies and often focus on one stressor. Less is known about effects of long-term exposure to stressors and the interactive effects of multiple stressors. Understanding threat mechanisms is paramount to determining how the implicated threat acts on the species in question, for example, whether aquatic invasive species impose competitive or habitat-related effects (Gallardo et al. 2016). Alternatively, the magnitude of impact can determine how the threat influences population viability.

Conventional correlative field surveys and laboratory experiments are the standard approach to identifying threat mechanisms and impacts (e.g., Mims and Olden 2013; Gray et al. 2014; Jackson et al. 2016; Raab et al. 2018), but these suffer from the same issues described for population ecology and habitat science (weak inference from observational field studies, nonlethal sampling constraints, poor realism of lab studies). To circumvent these challenges, laboratory results ideally should be validated with field results and vice versa. Surrogate species or CERPs can be used to study threat mechanisms and responses, with the key advantage being that captive populations are sufficiently large to overcome sample-size limitations.

Creative mesocosm design, coupled with experimental streams or watersheds, can provide additional realism to threat studies (Fig. 1). When large populations of focal species exist in the field, experimental enclosures or other manipulations can be used to examine individual and population responses to perturbations and allow for replication. An important threat to several species at risk is hypoxia. The propensity for hypoxia is greatly exacerbated by nutrient inputs in urban and agricultural landscapes that trigger eutrophication and drive up biological oxygen demand, leading to severe hypoxia (Mallin et al. 2006). Summer low flows, in conjunction with high temperatures and eutrophication from nutrient inputs, synergize to exacerbate hypoxia in agriculture-dominated landscapes (e.g., in lower Fraser Valley in British Columbia and in southern Ontario). Climate projections for warmer temperatures and longer summer low-flow periods will make these impacts worse, as will increased water demands for irrigation and domestic supplies (Pardo and García 2016; Rosenfeld et al. 2021). A SARNET project that tested the effects of hypoxia on Salish sucker (*Catostomus* sp. cf. *catostomus*) used flow manipulation to transform a natural stream to an experimental system where dissolved oxygen could be manipulated (Zinn et al. 2021). Using this unique experimental field setup (Fig. 2A), behaviour and growth responses of individual Salish sucker and coho salmon were recorded. Although Salish sucker likely has physiological and behavioural mechanisms that provide some degree of tolerance to low dissolved oxygen, its preference for deep pool habitats that are particularly vulnerable to hypoxia is a significant management concern. While the preference for deep pool habitat may be adaptive in a natural landscape, it can become an ecological trap under eutrophication in human-modified landscapes (Rosenfeld et al. 2021; Zinn et al. 2021).

Conservation physiology can also inform threat science (Fig. 1; Birnie-Gauvin et al. 2017). Nonlethal measures are available to understand species stress responses in the environment, which can provide an integrative understanding of individual performance in relation to the threat. Understanding organism-level responses to these stressors is a powerful approach for predicting population-level responses (Wikelski and Cooke 2006; Cooke et al. 2013; Bergman et al. 2019). Experiments exploring both independent and interactive effects of environmental stressors may be key to understanding threat mechanisms (causative factors) and their impacts. Further, the cumulative effects, and synergistic or antagonistic effects of multiple stressors, are important to understanding the magnitude of threat impacts (Piggott et al. 2015; Jackson et al. 2016). Such studies can identify changes to life-history traits in response to changes in environmental parameters to inform threat mitigation with insights into future threats (e.g., climate change) and have relevance to reintroduction efforts such as thermal hardening (Bergman et al. 2019).

The application of conservation physiology to inform threat mechanisms and impacts has been hampered by the lack of suitable field protocols. To overcome this limitation, several SARNET projects employed a unique streamside laboratory to measure physiological endpoints without having to transport and acclimate endangered fish species in the lab. To understand the implications of warming water temperatures, a key habitat stressor associated with climate change (Woodward et al. 2010; Winfield et al. 2016; Arthington et al. 2016; Reid et al. 2019), a streamside unit was used to measure $\mathrm{CT_{max}}$ (Fig. 2B) and the thermal safety margin (TSM) of redside dace at its northern range limit in Canada (Leclair et al. 2020) and a non-imperilled population farther south in Ohio, USA (Turko et al. 2020). The streamside unit was also used to test the $\mathrm{CT_{max}}$ and TSM of the eastern sand darter (*Ammocrypta pellucida*) in Ontario across seasons and in environments differing in turbidity (Firth et al. 2021). The streamside unit offered the opportunity to obtain information about the thermal tolerance of imperilled species in the wild, the effect of seasonal acclimation on parameter estimates ($\mathrm{CT_{max}}$ and TSM), and the implications of other co-occurring (multiple) stressors bounding physiological performance.

As with water temperature, changes in water flow are also hypothesized to affect the viability of several SARA-listed fishes and mussels. Flow augmentation has been hypothesized as a dominant threat for the plains sucker (*Pantosteus jordani*), as well as other species at risk such as Rocky Mountain sculpin (*Cottus* sp.) (Veillard et al. 2017; Rudolfsen et al. 2018). The impact of flow augmentation on plains sucker in the wild was investigated using a streamside flow chamber (Fig. 2C). The comparative field study involved a natural stream experiencing flow manipulation to meet irrigation needs and a natural stream without flow manipulation (T. MacLeod, unpublished thesis), with the goal of understanding the mechanisms underlying the decline of plains sucker in the Milk River. The study also provided baseline biological information about populations of plains sucker and potentially identified differences based on environmental stressors (T. MacLeod, unpublished thesis). Considerable evidence indicates that water flow also affects mussel clearance rates (vanden Byllaardt and Ackerman 2014) and responses to other stressors (Tuttle Raycraft and Ackerman 2019). These studies add to a growing body of literature on the impacts of flow modifications in freshwater systems, the potential limits to patterns of species dispersal and habitat connectivity (Veillard et al. 2017; Neufeld et al. 2018), and ultimately, changes in species distributions (Rudolfsen et al. 2019) and genetic structure (Ruppert et al. 2017).

For other SARNET projects, wild populations were not available for experimentation, but CERPs allowed threat mechanisms to be investigated (Fig. 1). Using the redside dace CERP housed at FREC, S. Gaffan (unpublished thesis) examined the effect of varying concentrations of suspended sediment on swimming performance of redside dace schools, and C.L. Madliger (unpublished) examined behavioural stress caused by different colours of artificial light pollution. A pugnose shiner (*Notropis anogenus*) CERP in a pond at SUNY Cobleskill (Lake Ontario source population; Carlson et al. 2019) provided an opportunity to evaluate the effects of elevated water temperature on thermal tolerance and the metabolic rate of pugnose shiner in the laboratory (Potts 2021*a*). Together, the CT_{max} experiments indicate that three fishes — redside dace, eastern sand darter, and pugnose shiner — are living close to their physiological limits during the summer months, and the thermal acclimation capacity for CT_{max} may not be sufficient to mitigate the negative effects of high temperature exposure (Potts 2021*a*; Leclair et al. 2020; Turko et al. 2020; Firth et al. 2021; McDonnell et al. 2021). Given these results, recovery actions that minimize fluctuations in summer water temperatures are likely to benefit the species; however, the CT_{max} studies demonstrated that redside dace are more thermally tolerant when acclimated to higher temperatures, implying that acute changes to temperature may cause more severe impacts than chronic warning (Turko et al. 2021). Overall, these studies illustrate the merits of CERPs to allow potentially invasive experiments to be conducted that would otherwise be impossible with wild Canadian populations (Turko et al. 2021). Additional CERPs would allow similar experiments to be conducted for other SARA-listed species.

To understand and predict effects of human activities on the viability of SARA-listed fishes, the interactive effects of multiple stressors must be determined. For fishes, two stressors that are likely to interact are high water temperatures and hypoxia because both affect oxidative metabolism — fish metabolic rate and thus their oxygen demand increase with water temperature, while hypoxia limits oxygen available for uptake (McBryan et al. 2013). Exposure to both stressors may have negative effects, but it is also possible that exposure to either high temperatures or hypoxia could improve tolerance to the alternative stressor (referred to as cross-tolerance). Using the pugnose shiner CERP, McDonnell et al. (2021) conducted an acclimation experiment to determine whether acclimation to hypoxia affects thermal tolerance. The findings showed that acute hypoxia exposure lowers thermal tolerance in pugnose shiner, a pattern also observed by Potts (2021*a*), but acclimation to hypoxia mitigates the negative effect of hypoxia on CT_{max}. The results offer opportunities for improving thermal resistance during captive rearing and reintroduction efforts and (or) identifying reintroduction source populations with greater thermal tolerance. Pugnose shiner from SUNY Cobleskill have been used to successfully re-establish a population in Chaumont Bay, Lake Ontario (Carlson et al. 2019; Haynes et al. 2019). While this is encouraging, future reintroduction efforts may benefit from using an artificial captive-breeding environment to promote environmental matching compatible with climate warming and associated stressors such as hypoxia; however, the fitness trade-offs of higher thermal or hypoxia tolerance need to be further explored prior to integration into breeding programs.

In some cases, wild caught surrogate species were used to investigate the effects of multiple stressors (Fig. 1). Because flow conditions are important for freshwater mussels that rely on flow to filter feed, the feeding physiology (clearance rates measured by change in chlorophyll concentration and oxygen consumption measured using the In/Ex (O_2 difference between inhalant and exhalant apertures) method) of fatmucket (*Lampsilis siliquoidea*) (surrogate species) was investigated using a laboratory flow chamber. Experiments also determined the interactive effect of sediment content (e.g., total suspended solids, TSS) and water temperature (Luck 2020), expanding on previous efforts involving TSS and flow (Tuttle-Raycraft et al. 2017). Understanding the response of species to multiple stressors under different flow conditions was necessary to determine the interactive effects of land-cover change (e.g., agriculture) on species responses and allows managers to determine when certain variable combinations will lead to heightened responses.

In other cases, borrowing predictive modelling methods for hazards and catastrophic events such as those used for earthquakes and tsunamis may help to forecast threat impacts for SARA-listed species. These types of models were identified as a SARNET research priority (Fig. 1). The predictive models can be coupled with existing data sources such as databases of contaminant spills available through regulatory agencies. Modelling human behaviour can also help to predict the occurrence of threats (e.g., the spread of invasive species or bycatch of listed species; Drake and Mandrak 2010, 2014) and can be coupled with risk mapping and pathway analysis. The timing and magnitude of the threats remain poorly studied, e.g., the ability to predict periods of thermal stress in watersheds occupied by SARA species. Water temperature is often modified by anthropogenic factors (e.g., timing of dam water release) that, in turn, have impacts on life-history events such as spawning. There are models to predict river temperature from air temperature (Caissie 2006), but these models have not been evaluated using metrics that are relevant to the life history of focal species. A SARNET project used black redhorse (*Moxostoma duquesnei*) to inform the standards of evaluation (e.g., temperature during the spawning period), and with this approach, Rosencranz et al. (2021) determined if model performance differed for variables and time periods critical to the success of the species (Fig. 1). The air-to-river temperature models, fitted with respect to ecologically sensitive periods (Rosencranz et al. 2021), had less predictive error and bias compared with other recent studies (Laanaya et al. 2017; Zhu et al. 2018), shedding light on threat timing and scale. In the future, the Rosencranz et al. (2021) approach can be used to estimate water temperatures when only air temperatures are available and to examine the thermal implications of changes in management (e.g., timing of dam water release to mitigate heat stress).

The approaches identified by the workshop experts (Table 3) are paramount for developing dose–response relationships for key threats, which will allow the effect on population viability to be estimated and the extent to which the threat must be mitigated to achieve recovery to be determined.

Recovery science: approaches for optimizing habitat restoration and reintroductions of imperilled freshwater species

In some cases, there is uncertainty about how to mitigate key threats (i.e., specific approaches, how to implement, likelihood of success; Table 4). Although threat mitigation is hampered by basic scientific knowledge gaps, most approaches previously identified (e.g., experimental populations and watersheds, conservation physiology, or rigorous application of surrogates; Tables 1–3) would improve progress towards threat mitigation by increasing the available knowledge base. Additionally, multiyear field studies to document responses to mitigation efforts can be done at local scales, but methods need to be developed to monitor broad-scale stressors and mitigation projects (Table 4). Adaptive management appears to be the most promising opportunity for improving threat mitigation but must be clearly enacted from the outset, otherwise opportunities for learning are reduced (Table 4).

Additional issues relate to carrying out species reintroductions, particularly for small-bodied species that lack established rearing and release methods (Lamothe and Drake 2019; Lamothe et al. 2019*c*; Table 4). Reintroductions are often recommended in situations where local extirpations have occurred and the cause of decline can be reversed. Although there is a well-developed body of literature concerning species introductions and stocking, reintroductions of SARA-listed freshwater species have occurred infrequently (Lamothe et al. 2019*c*). Lamothe and Drake (2019), a SARNET study, reviewed the reintroduction progress for imperilled freshwater fishes in Canada and highlighted 10 key questions to be answered to reduce scientific uncertainties. Many uncertainties for reintroductions center around the lack of captive populations of most SARA-listed species, including few facilities for rearing warmwater species in Canada. Fundamental knowledge gaps for imperilled species include suitable husbandry methods and culture techniques, including reproductive behaviour (microhabitat selection, mate choice) and captive breeding and rearing. Some information on culture techniques could be borrowed and adapted from the salmonid-rearing literature for coldwater species (Stark et al. 2014), from the centrarchid-rearing literature for warmwater species (Morris and Clayton 2009), and from existing operations in the United States (e.g., Conservation Fisheries in Tennessee).

There has been strong progress on the rearing of freshwater mussels in Canada, from identifying host fishes to captive breeding. Research directed at the identification of host fishes of SARA-listed unionid mussels in Canada was first developed at the University of Guelph in 2000 and expanded to facilitate experimental designs (J. Ackerman, personal communication). Subsequently, over 130 fish and mussel combinations (19 fish species; 12 mussel species) were examined experimentally with varying rates of host detection (0%–45%) in one nonlisted and nine SARA-listed mussels (e.g., McNichols 2007), identifying differences among host fish species and among female mussels in glochidia transformation rates (McNichols et al. 2011). Host–fish relationships often differed from those reported from other geographic regions indicating the need for local testing. The experiments also determined that the invasive round goby (*Neogobius melanostomus*) would reduce reproductive output of unionids because of high infestation rates but very poor metamorphosis rates (Tremblay et al. 2016). Experimentation on different juvenile rearing techniques was applied to the artificially reared juvenile SAR mussels in both the laboratory and the field; the former effort was hampered by flatworm predation. The techniques developed are used by the Ontario Ministry of Natural Resources and Forestry Fish Culture Section, who have scaled up the methods for hatchery production (K. Loftus, personal communication). Since 2013, the Fish Culture Section has been working to develop expertise in the culture of four species of mussels, including wavy-rayed lampmussel (*L. fasciola*), kidneyshell (*Ptychobranchus fasciolaris*), snuffbox (*Epioblasma triquetra*), and northern riffleshell (*E. rangiana*). Each effort involved the capture of mature females in the wild and transfer to the fish culture station, where glochidia were manually extracted, host fish were infested, and juveniles were subsequently collected and reared using protocols and specialized equipment. Over the years, the Fish Culture Section has provided and (or) sold reared mussels to support toxicity and genetic research; however, successfully reared mussels could not be released in the wild due to the lack of relevant policy.

Other considerations for captive breeding and maintaining CERPs include selecting appropriate source populations (Houde et al. 2015) and avoiding negative genetic consequences such as founder effects, inbreeding, and selection for captivity (Frankham et al. 2017; Lamothe et al. 2019*c*). To determine potential deleterious genetic effects, a SARNET project completed analyses of microsatellite genotypes for wavy-rayed lampmussel and kidneyshell. The work did not detect any significant differences between genetic diversity of wild and captive-reared offspring, and the captive-reared offspring were not significantly differentiated from the natal population (VanTassel et al. 2021). These findings support current genetic guidelines (Jones et al. 2006; Hoftyzer et al. 2008) and indicate that freshwater mussel propagation efforts should follow the existing guidelines in Canada.

A number of knowledge gaps also need to be addressed with respect to optimal release methods (life stage; number and frequency of propagules released; hard vs. soft releases), as well as transport-related stress (Harmon 2009). Experimental releases of propagated animals could resolve these uncertainties and help identify other information needs for facilitating re-establishment success (Jachowski et al. 2016). To locate appropriate sites for the release of SARA-listed mussels, habitat features that are required by juveniles after they have passively settled via hydrological conditions need to be identified. In the absence of CERPs and surrogate species, field experiments can be completed using synthetic materials that mimic biological systems. A SARNET project used nontoxic biodegradable microbeads that mimic the dispersal of juvenile mussels, where the density and diameter of the particle can be changed (Lum 2020). Preliminary particle-release experiments in rivers indicated that the rate of dispersion of the particles is similar to that of other macroinvertebrates (J. Ackerman and C. Farrow, personal communication). Additional experiments are planned to determine how near-bed hydrodynamics affect the spatial pattern of dispersal. Additionally, the relationship between local hydrodynamics and juvenile mussels in riverbeds was examined, and a statistical model to predict where they occur was developed (Lum 2020). In addition to identifying suitable habitat for juvenile mussels, this study provides insights about where captive-bred juvenile mussels should be released to support reintroduction initiatives. Another SARNET project (Lamothe et al. 2021) evaluated the trade-offs associated with harvesting source populations from the wild for subsequent translocation to release sites. This work identified important harvest and stocking thresholds for the eastern sand darter and, more broadly, indicated that mortality during fish transport to the release site had a large bearing on reintroduction success.

For a more comprehensive understanding of species responses to habitat restoration and effectiveness of reintroduction programs, systematic reviews and meta-analyses can point to generalizable patterns (Cooke et al. 2017*b*). A SARNET project involved developing a systematic map of the effectiveness of captive-breeding programs for imperilled freshwater fishes and mussels, which revealed that the body of literature is relatively large for fishes but comparatively smaller for mussels (Rytwinski et al. 2021). Where evidence did exist, it was generally of low quality. Many studies lacked adequate replication and were simple narratives of captive breeding rather than explicit tests with a reasonable comparator. There are several topics and endpoints that have been reasonably well studied (e.g., those related to fish growth rates), while many other topics have received little research attention. The outputs from the systematic map provide a first step towards improving our understanding of the ability of captive-breeding programs to achieve conservation targets in the wild and inform future research activity — both where additional effort is needed and how to improve the quality of science so that the evidence base is stronger (Rytwinski et al. 2021).

Although CERPs and related genetic research have provided insight into how captive breeding might support reintroductions, several questions remain about the suitability of any given reintroduction (Table 4). Research on in-hatchery enrichment to enhance poststocking survival is needed to ensure that the culture environment produces fish that are behaviourally more fit at the time of release, provided that suitable habitat is available (Brown and Day 2002; Johnsson et al. 2014). It will also be important to ensure that founding numbers encompass sufficient genetic resources to protect the adaptive potential of re-established populations (Frankham et al. 2017). The risk of disease transfer should be minimal if proper procedures are followed and if translocations from extant wild populations for reintroduction in other waterbodies do not originate from waterbodies with known pathogens.

Future directions: what knowledge gaps remain?

The expert workshop highlighted research approaches to address knowledge gaps for SARA-listed species, and SARNET subsequently filled many of those gaps (Fig. 1). Knowledge gaps were addressed using the approaches recommended by experts at the workshop, for example, using CERPs to integrate conservation physiology into habitat and threat science and streamside experimental setups (Figs. 1, 2). The approaches were implemented because of the dedicated infrastructure and collaborative academic–government research structure provided by SARNET. Although the SARNET research projects tended to focus on individual species, many inferences can be extrapolated, creating a body of scientific work relevant to other species at risk in Canada. SARNET produced research output that will inform recovery strategies and action plans for on-the-ground conservation of freshwater species across Canada.

Despite scientific progress made by the SARNET research, not all approaches identified at the workshop were implemented (Fig. 1); hence, opportunities remain to address outstanding knowledge gaps. Certain life-history traits are unknown for many listed species, creating additional uncertainty in modeling recovery potential and population-level responses to threats. The development of novel imaging technology such as portable ultrasound for field measurement of life-history traits would hasten the collection of this missing information, e.g., reproductive status and sex. A rigorous evaluation of surrogates for imperilled species should be undertaken to appropriately supplement missing life-history information. The application of nonlethal sampling methods such as underwater cameras and eDNA for estimating abundance and distribution was not explored through SARNET; however, there have been important advances in these methods (Castañeda et al. 2020*b*; Loeza-Quintana et al. 2020; Vargas Soto et al. 2021). A stronger understanding of the importance of species co-occurrence, specifically species interactions and host–glochidia relationships using disease-transmission and host–parasite modelling techniques, is required to increase knowledge of mussel population dynamics and, potentially, reintroduction success. Additional questions remain about the extent to which environmental change is driving differences between the populations (genetics, physiological adaptation, trophic implications).

Although there are opportunities for new technology and methods to fill remaining knowledge gaps, in many cases, existing methods are underutilized. Basic natural and life-history field studies are vital to address existing questions concerning the population ecology and habitat attributes of listed species, while manipulative field experiments can determine the response of individuals and populations to key threats. Unfortunately, research on SARA-listed species is often perceived as having a low return on investment due to challenges discussed herein (e.g., sampling difficulties). This, in part, explains the difficulty of obtaining funds and generating publication interest in basic ecological research such as the measurement of life-history traits, even with new methods. There are also research gaps in conservation social science, e.g., the human dimensions and social aspects of species recovery, that, if addressed, would lead to a better understanding of actions, policies, and decision-making (Rudd et al. 2016; Bennett et al. 2017).

Research conducted by SARNET employed numerous creative approaches to answer priority research questions for SARA-listed freshwater fishes and mussels in Canada. Such research was possible only through government commitment to develop a government–academic research network and willingness on behalf of academic participants to address government research priorities. The information gained through the scoping workshop and dedicated research projects has made a fundamental contribution to the research knowledge base to inform recovery strategies for SARA-listed freshwater fishes and mussels. Nonetheless, it is important to review outstanding research gaps, and research priorities should be revisited on a regular basis to support ongoing recovery actions. Although addressing research and information gaps must occur in conjunction with sound legislation, policy, and on-the-ground implementation of recovery actions, addressing the research gaps identified by Drake et al. (2021) is a critical step towards improving the recovery of imperilled species in Canada.

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