Canadian Science Publishing

INVITED REVIEW

# What evidence exists for evaluating the effectiveness of conservation-oriented captive breeding and release programs for imperilled freshwater fishes and mussels?[1]

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**Abstract:** Captive breeding programs are widely applied by conservation practitioners as a means of conserving, reintroducing, and supplementing populations of imperilled freshwater fishes and mussels. We conducted a systematic map to provide an overview of the existing literature on the effectiveness of captive breeding and release programs. A key finding is that there is limited evaluation of the effectiveness of such programs at all three stages (i.e., broodstock collection, rearing/release methods, and post-release monitoring). We identified clusters of evidence for evaluating supplementation associated with rearing/release methods for fish growth and survival metrics, and the monitoring stage for fish genetic diversity, growth, and survival metrics, primarily focused on salmonids. However, many studies had inadequate experimental designs (i.e., lacked a comparator). Overall, there was a paucity of studies on the effectiveness of captive breeding programs for imperilled freshwater mussels, highlighting the need to make such information broadly available when studies are undertaken. Outputs from this systematic map (i.e., the map database and heatmaps) suggest that the effectiveness of captive breeding and release programs requires further systematic evaluation.

**Résumé :** Des programmes de reproduction en captivité sont largement utilisés par les spécialistes de la conservation comme moyen de conservation, de réintroduction et de supplémentation de populations menacées de poissons d'eau douce et de mulettes. Nous avons réalisé une cartographie systématique pour dresser un portrait du corpus documentaire existant sur l'efficacité des programmes de reproduction en captivité et de lâcher. Une des constatations clés est le fait que l'évaluation de l'efficacité des trois étapes de tels programmes (c.-à-d., prélèvement de géniteurs, méthodes d'élevage/de lâcher et surveillance après le lâcher) est limitée. Nous avons cerné des groupements de données probantes pour les évaluations de la supplémentation associées aux méthodes d'élevage/de lâcher pour des paramètres reliés à la croissance et la survie des poissons, et l'étape de surveillance pour des paramètres reliés à la diversité génétique, la croissance et la survie des poissons, axés principalement sur les salmonidés. Le schéma expérimental de nombreuses études est toutefois inadéquat (c.-à-d., absence de comparateur). Globalement, les études sur l'efficacité des programmes de reproduction en captivité pour les mulettes menacées sont rares, soulignant la nécessité d'assurer une large disponibilité de ce type d'information quand de telles études sont réalisées. Les extrants de cette cartographie systématique (c.-à-d., la base de données en découlant et les cartes de densité) font ressortir la nécessité d'une évaluation systématique plus poussée de l'efficacité de programmes de reproduction en captivité et de lâcher. [Traduit par la Rédaction]

Received 31 August 2020. Accepted 4 April 2021.

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*Andrew R. Drake served as a Guest Editor at the time of manuscript review and acceptance; peer review and editorial decisions regarding this manuscript were handled by Donald Jackson.

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[1]This paper is part of a special issue entitled "Science to support Canada's SARA-listed freshwater species", which brings together articles on threats and reintroductions for at-risk freshwater fishes and mussels in Canada.

## Introduction

Global freshwater biodiversity has declined by over 80% since the 1970s, to the point where it is clearly in crisis (Harrison et al. 2018). Freshwater fishes have some of the highest extinction rates worldwide among vertebrates (Burkhead 2012), which is mostly reflected in range and population declines of specialized species (Mueller et al. 2018). On a global scale, freshwater mussel fauna is estimated to have among the highest extinction and imperilment rates of any group of organisms on Earth (Geist 2011; Haag and Williams 2014; Lopes-Lima et al. 2017). In the USA alone, 10% of freshwater mussels are classified as Extinct, and 28% are federally listed as imperilled (independent assessments estimate that upwards of 65% are imperilled) (Haag and Williams 2014). Similarly, in Canada, 35% of freshwater mussel species have been assessed by the Committee on the Status of Endangered Wildlife in Canada (COSEWIC) and listed under the *Species at Risk Act* (SARA) as "Special Concern" to "Extirpated" (i.e., 9% Special Concern, 4% Threatened, 20% Endangered, 2% Extirpated; Canadian Endangered Species Conservation Council 2016). Threats facing freshwater biodiversity are numerous (Dudgeon et al. 2006; Reid et al. 2019) and often interact in complex ways (Birk et al. 2020). Among the most common threats facing freshwater fishes and mussels are habitat destruction, loss of habitat connectivity, pollution, overharvest, the introduction of disease, climate change, flow modification, and invasive species (Dextrase and Mandrak 2006; Venter et al. 2006; Reid et al. 2019).

Given the dire state of freshwater biodiversity, Tickner et al. (2020) outlined an emergency action plan to restore freshwater biodiversity with a focus on addressing underlying drivers. It will take time for such a plan to be realized, and in the interim, certain species will continue to decline, with some likely to become extirpated or even globally extinct. As such, captive breeding programs (i.e., ex situ conservation; Rahbek 1993) have an important role in maintaining and restoring biodiversity. For freshwater fishes and mussels, captive breeding programs have increasingly been identified in recovery strategies to complement existing recovery measures and achieve recovery objectives, particularly in North America and Europe (Lamothe and Drake 2019; Lamothe et al. 2019; Strayer et al. 2019). Captive breeding programs are typically recommended, in conjunction with other recovery measures such as habitat restoration and strategies for reducing threats to the species in the wild (e.g., bycatch mitigation, changes to fishery regulations, control of invasive species, or improved habitat condition; Naish et al. 2007; McMurray and Roe 2017). Captive breeding programs are often considered a last resort, given their potentially high financial costs (Snyder et al. 1996) and issues associated with genetic diversity (Araki et al. 2007; Hoftyzer et al. 2008), and the recognition that captive breeding on its own fails to address underlying threats (Rahbek 1993; Snyder et al. 1996). In Canada, for example, the federal agency responsible for such activities (i.e., Fisheries and Oceans Canada) notes that captive breeding programs should only be considered in periods of very low survival, when management interventions can be planned to augment the low survival, or when environmental conditions associated with low survival are predicted to change (DFO 2008).

Captive breeding programs, by definition, breed animals in controlled environments to establish a stock of artificially propagated individuals, and are usually conducted to achieve one of three overarching goals, each with different quantifiable measures of success: (1) supplementing an existing population (i.e., bolstering small or declining populations in the wild to decrease the likelihood of local extirpation; e.g., Fraser 2008); (2) re-establishing a population where local extirpation has occurred (i.e., reintroduction; Wilson and Price 1994); or, (3) establishing an Ark population (e.g., zoo or hatchery stock; Bowkett 2009) to ensure a safe haven for genetic material and propagules. The overall objectives of conservation-oriented captive breeding programs are to reduce the risk of local extirpation (or sometimes global extinction), produce individuals that are genetically and phenotypically similar to wild populations, and increase the total number of individuals reproducing successfully in the wild (Araki et al. 2007).

Captive breeding programs often have several stages, including broodstock collection (i.e., the collection of individuals or gametes from the wild to be used for breeding purposes in captivity), spawning of reproductively prepared individuals (this can occur naturally in captivity or by human intervention), rearing of juveniles, releasing individuals to the wild, and monitoring the success of the released individuals. Evidence from North America and Europe indicates that captive breeding programs for the conservation of freshwater fishes and mussels have been successful on numerous occasions; however, there can be negative consequences of captive breeding programs at both the individual and population level, including impacts on donor populations (e.g., see Rakes et al. 1999; George et al. 2009; DFO 2016). Poorly conceived projects can waste resources and harm populations and ecosystems (Strayer et al. 2019). Despite these considerations, captive breeding programs offer tremendous promise for the conservation of imperilled species globally (Araki et al. 2007; Bowkett 2009).

Given the increased interest and use of captive breeding programs for freshwater fishes and mussels, particularly in temperate regions, there is a need to summarize the relevant evidence to inform and refine best practices and to identify key knowledge gaps. To that end, we created a systematic map to provide resource managers and scientists with a collated global summary of the existing body of literature addressing the effectiveness of conservation-oriented captive breeding and release programs for imperilled freshwater fishes and mussels in the wild in temperate regions. Here, we acknowledge that conservation targets for captive breeding and release programs are often related to big-picture conservation outcomes (e.g., evidence of reproducing populations in the wild, or the re-establishment of populations). However, programs and studies could involve more specific or stepping-stone conservation outcomes (e.g., successfully rearing juveniles in captivity) that lead to improvements in our understanding and ability to achieve broader outcomes. Therefore, we mapped the existing evidence base on the effectiveness of captive breeding and release programs in achieving program objectives (i.e., big- or small-picture conservation outcomes, as defined by the program/study) for imperilled freshwater fishes and mussels. We acknowledge that restoration of ecosystem functions and services (e.g., through increasing water filtration by enhancing mussel populations) is another important conservation outcome, though not explicitly examined in this mapping exercise unless directly associated with outcomes related to imperilled mussels. Through this mapping exercise, we describe the quantity and key characteristics of the available evidence, and we identify evidence clusters (subsets of evidence that may be suitable for secondary research, e.g., full systematic review) and knowledge gaps (topics that are under-represented in the evidence base that require future primary research); two mapping functions that are necessary first steps to drive more effective actions. Systematic maps are particularly appropriate for topics that are wide in scope and too diverse (e.g., variety of outcomes to evaluate effectiveness) for an individual systematic review. Although procedurally similar to a systematic review, systematic maps do not aim to provide a quantitative or qualitative answer to a particular question, but instead, an overview of research that has been undertaken, indicating where and how (Haddaway et al. 2016; James et al. 2016). Systematic maps have a variety of uses across research, policy, and practice by providing assessments about knowledge clusters and gaps, as well as identifying deficiencies in the evidence base regarding study methods, which can lead to recommendations that promote best practice (Haddaway et al. 2016; James et al. 2016).

**Table 1.** Article inclusion and exclusion criteria summarized from the protocol (Donaldson et al. 2019).

| Included | Excluded |
|---|---|
| **Subject (population)** | |
| Any imperilled freshwater fish or mussel population that has a captive breeding program occurring in northern (23.5°N to 66.5°N) or southern (23.5°S to 66.5°S) temperate regions around the globe. This included all fishes and mussels that have at least one stage of their lives in freshwater, including both migratory and non-migratory species. All life stages were considered relevant. Imperilled freshwater fish and mussel species, defined as those with conservation status in their relevant jurisdiction, in temperate regions around the world. In Canada, this included species assessed by the Committee on the Status of Endangered Wildlife in Canada (COSEWIC) as extirpated, endangered, threatened, and of special concern, as well as those listed under the *Species at Risk Act* (SARA), plus relevant provincial and territorial assessments. In other countries, species inclusion was based on the relevant conservation listing process (e.g. IUCN 2020; federal, state-level, and international including IUCN Red List). Studies providing no formal classification but instead describing the species as being "at risk" based on the author's own description (e.g., the species is experiencing rapid population decline) | Marine-only and oceanodromous species. Studies that stated the species was not at risk (e.g., species was common, stable population). *Studies that did not provide an indication of the conservation status for the species of interest using (i) a formal classification in their relevant jurisdiction, or (ii) the author's own description of the population (local or otherwise) AND that species/population was not deemed to be at risk on existing lists (a) COSEWIC listing for all fishes and molluscs in Canada (*Canadian Endangered Species Conservation Council 2016*), (b) IUCN Red List of threatened species (*IUCN 2020*), or (c) a list of species at risk from existing studies included in this systematic map database, generated at the end of the data extraction process [i.e., the species or species population from a similar geographical area was not already reported as at risk (using a formal classification or the author's own description) from a study captured in our database of existing studies]* |
| **Intervention/exposure** | |
| Studies reporting on a captive breeding program, or any component thereof, that have a conservation management objective(s), including (but not limited to): (*i*) propagation (i.e., producing a refugia population, rearing animals in captivity); (*ii*) supplementation (or augmentation) of an existing population (Waples et al. 2007) [i.e., to grow individuals that will support (and add to) existing populations of the same species in the wild]; (*iii*) re-introduction of a population where local extirpation has occurred and (or) establishing an Ark population (Frankham et al. 2004) [i.e., the purpose of rearing the animals was to re-introduce them back into the wild into areas where (*a*) they used to live but have been extirpated from, or (*b*) in to a new area/location/environment]. Relevant stages of the captive breeding program include articles related to the collection of broodstock, rearing and release methods, and monitoring of the released individuals in the wild. *Studies that do not explicitly and (or) clearly discuss the details of an existing captive breeding program but do report on (i) a species at risk in relation to a component of a captive breeding program (e.g., broodstock, rearing, releasing, or monitoring), (ii) small experimental/opportunistic studies focusing on a species at risk but not associated with a specific captive breeding objective (e.g., lab-based rather than hatchery-based experiments, optimization of cryopreservation techniques, glochidia infection trails, diet optimization) with the purpose of gathering information as opposed to evaluate effectiveness per se (referred to as exploratory studies), or (iii) the genetic status of a population in the wild and the potential for the establishment of a new program* | Studies reporting on a captive breeding program, or any component thereof where the objective is solely for the creation/enhancement of sport fishing opportunities, often termed "hatchery augmentation"; or for commercial and/or aquaculture purposes for the food industry (Naish et al. 2007) |
| **Outcome** | |
| Any qualitative or quantitative outcome measure related to evaluating the effectiveness of a captive breeding program, or any component thereof, for any relevant or intermediate stage of the program (e.g., ability to raise the organism in captivity, husbandry techniques), including (but not limited to) metrics related to: abundance, behaviour, genetic diversity, growth, recruitment, or survival | *Studies that do not provide a qualitative or quantitative outcome measure. Studies that report escapes from hatcheries without an assessment on the effect on the wild population* |
| **Comparator** | |
| Relevant comparators included: (*i*) wild individuals and (or) populations of the same species within the same geographical area or close proximity; (*ii*) individuals within the same captive breeding program that experience different conditions (e.g., water temperatures, release methods); or (*iii*) population status before the onset of a captive breeding program. Studies that do not include a comparator (e.g., studies reporting on rearing conditions of a particular imperilled species). | *No studies were excluded based on a comparator (or lack thereof)* |

**Table 1** (*concluded*).

| Included | Excluded |
|---|---|
| **Study design** | |
| Any study design used to investigate the effectiveness of a captive breeding program, or any component thereof, including (but not limited to): before/after (BA; e.g., population status assessment conducted before the captive breeding program and after individuals were released into the wild); control/impact (CI; e.g., population status assessment was conducted at a control population/site and at an impacted site); as well as studies combining these types of comparisons, before/after/control/impact (BACI) studies and randomised controlled trials [RCT; e.g., laboratory tanks of individuals allocated at random to receive one of several treatments (e.g., fungicide concentration), one of these treatments being a control]. After-only (PT) designs (i.e., a single post-treatment monitoring period, or a temporal correlation design using multiple post-treatment monitoring periods without true before data), and trend designs that look at the association/correlation between an outcome and an intervention that include a control (TRENDS-0; e.g., correlation between individual survival and the concentration of fungicide, including no fungicide) and those that do not include a control (TRENDS; e.g., correlation between individual growth and rearing temperature). *Opportunistic designs (OPPORT) in the case the genetic status of a population in the wild and the potential for the establishment of a new program* | Theoretical studies, review papers, and policy discussions. |
| **Language** | |
| English at full-text | Any study that is not in English at full-text |

**Note:** Further criteria for consideration that were developed post-publication of the protocol are shown in italic font.



## Materials and methods

### Search strategy

This systematic map followed detailed methods described in the systematic map protocol by Donaldson et al. (2019). In doing so, this map was performed according to the guidelines of the Collaboration for Environmental Evidence (2018), and conforms to ROSES reporting standards (i.e., detailed forms for ensuring evidence syntheses report their methods to the highest possible standards; see Haddaway et al. 2018) (Supplemental Material A, Table S1[2]). Any deviation from the protocol is highlighted below and (or) described in Supplemental Material B, Tables S1–S8[2]. The systematic map was based on literature searches conducted in 2018 (i.e., commercially published and grey literature) using five publication databases accessed from Carleton University's institutional subscriptions, two search engines, and 13 specialist websites (see Supplemental Material B[2], Tables S1–S8[2] for further details). English search terms were used to conduct all of our searches. Reference sections of 69 relevant reviews were hand-searched to evaluate relevant titles that were not found using the search strategy. We issued a call for evidence to target sources of grey literature through relevant mailing lists, social media, and distribution by the Advisory Team (i.e., a project-specific consultation group composed of ten topic experts from government, academy, and a non-governmental organization) to relevant networks and colleagues.

### Article screening and study eligibility criteria

Articles found by database searches, search engines, and specialist websites were screened in two distinct stages: (1) title and abstract, and (2) full-text. Articles or datasets found by other means (i.e., searching bibliographies of relevant reviews, social media, etc.) were entered at the second stage of this screening process (i.e., full-text). Prior to screening all articles, a consistency check was done at each stage on a subset of articles and discrepancies discussed (see Supplemental Material B[2] for further details on consistency checks).

All of the articles were screened according to the established eligibility criteria developed in consultation with the Advisory Team (Table 1). Articles were included only when all six criteria were met. No formal study validity assessment (i.e., study susceptibility to bias) was performed on the included articles. However, metadata on aspects of study design were extracted from included articles to provide a very basic overview of the robustness and relevance of the evidence (i.e., internal validity) and incorporated into the discussion of results to provide recommendations for future research needs and considerations.

### Data extraction strategy

Following full-text screening of articles, relevant studies were extracted from the included articles. When multiple studies were reported in a single article they were entered as independent lines in the database (i.e., if an article reported a different (*a*) study design, (*b*) captive breeding program/study objective, (*c*) captive breeding program/study stage, (*d*) captive breeding facility, and (or) (*e*) experimental condition (refer to Appendix A, Table A1, for term definitions). Attempts were made to identify supplementary articles and combine them with the most comprehensive article (i.e., primary study source) during data extraction.

In developing the map data extraction form and codebook (i.e., code sheet for all codes used in extraction form), the following key variables were identified through scoping activities and discussion with the Advisory Team: (*i*) bibliographic information; (*ii*) population details, i.e., taxa (fish or mussel), species name and family, life stage assessed, conservation status (e.g., endangered, threatened, special concern, data deficient), group or policy providing conservation status (e.g., IUCN, SARA, COSEWIC, author's description); (*iii*) captive breeding study details, i.e., objective (e.g., propagation, re-introduction, supplementation), facility name and country, stage of program/study (broodstock collection/selection, rearing and release methods, monitoring); (*iv*) study design and

[2]Supplementary data are available with the article at https://doi.org/10.1139/cjfas-2020-0331.

control type; and (*v*) outcome type. Coding options within these key variables were then compiled in a partly iterative process, expanding the range of options as they were encountered during scoping and extraction (see Supplemental Material B[2] for the details on consistency checks).

### Data mapping method

A searchable and accessible systematic map database was developed to describe the quantity and key characteristics of the existing literature on the effectiveness of captive breeding and release programs at achieving program objectives for imperilled freshwater fishes and mussels in the wild (Supplemental Material C, Table S1[2]). The distribution and frequency of the evidence base was also compiled into a structured heatmap showing linkages between examined stages of a captive breeding program/study (grouped by the objective of the program/study) (rows), and fish and mussel outcomes (columns). As studies within individual articles can examine links between more than one intervention and outcome type, individual studies were mapped to more than one cell when applicable (i.e., referred to as cases; see Appendix A, Table A1). Note, the systematic map does not quantify or validate the effectiveness of captive breeding programs/studies but rather aims to describe the distribution of research effort. Specifically, the systematic map database and heatmap were used to identify possible knowledge clusters (defined here as linkages with >25 cases) and gaps (linkages with ≤25 cases) (see Supplemental Material B[2] for further details on defining thresholds).

## Results

### Literature searches and screening

A total of 524 studies from 460 articles met our inclusion criteria and were subsequently included in the final map (see Supplemental Material D, Fig. S1[2], for flow diagram of inclusion/exclusion process results). Most of the 524 studies focused on temperate imperilled freshwater fishes (476 studies from 422 articles), with comparatively fewer studies involving mussels (48 studies from 38 articles). Article publication dates ranged from 1979 to 2018, with the majority published in the last decade (51% and 55% of articles for fishes and mussels, respectively; Appendix A, Fig. A1). Commercially published literature accounted for a higher frequency of included articles than grey literature in all decades for both fishes and mussels (Appendix A, Fig. A1). Coded data for all included studies are included in Supplemental Material C, Table S1[2]. All articles excluded at full-text along with reasons for their exclusion can be found in Supplemental Material E, Table S1[2].

### Summary of the evidence base

#### *Fishes*

Studies on the effectiveness of captive breeding programs for imperilled fishes were predominantly from North America, Europe, and Asia (Fig. 1*a*). Of the 33 included countries, those with the most studies were the USA (64% of studies), Canada (8%), and Australia (4%). The most frequent objective reported involved supplementing an existing population, with the majority of these focusing on monitoring in the wild (e.g., status of captive-bred individuals in the wild after supplementation), and rearing/release methods (Fig. 2*a*). Half of all studies (237/476) did not explicitly and (or) clearly discuss the details of an existing captive breeding program, but did report on an imperilled species in relation to (*i*) a component of a captive breeding program (e.g., broodstock, rearing, releasing, or monitoring), (*ii*) small experimental or exploratory studies primarily used to gather information rather than to evaluate the effectiveness per se (e.g., diet optimization), or (*iii*) the genetic status of a population in the wild and the potential for the establishment of a new breeding program. Fifty-eight percent of studies used a temporal and (or) spatial comparator in their study design (i.e., BACI, BA, RCT, CI, TRENDS-0; see Table 1 for definitions) (Fig. 2*c*). Control-impact (CI) designs were the most common (40% of studies), with comparisons made between wild-born individuals or populations of the same species (control group) and captive-born individuals (impact group), or individuals within the same captive breeding program that experience different conditions [e.g., current best practice rearing condition (control group) compared with new/different rearing conditions (impact group)]. Reported outcome measures were numerous and diverse. As a result, responses were grouped into seven broad outcome categories: abundance, behaviour, genetic diversity, growth, recruitment, survival, and other (Appendix A, Table A2). Within each outcome category, metrics were grouped into more closely related outcome subcategories (Appendix A, Table A2). Outcome metrics related to genetic diversity were the most frequently studied for fishes, with the majority focusing on metrics related to heterozygosity (e.g., expected/observed heterozygosity, $F_{st}/F_{is}$) and alleles (e.g., allelic frequency, richness) (Fig. 2*e*).

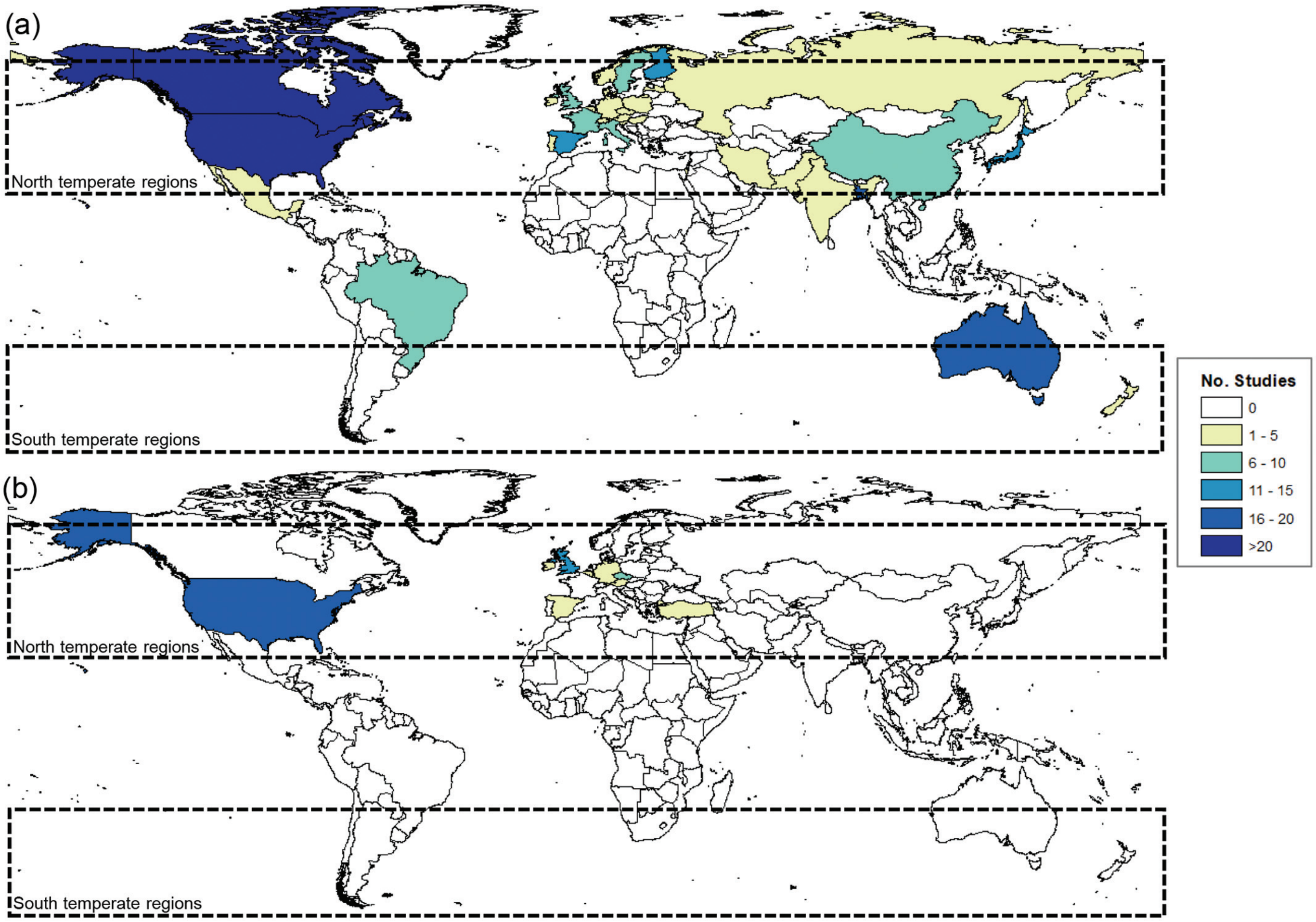
Captive breeding program studies investigated 126 species (8 subspecies therein) from 81 genera and 36 families. Salmonidae were the most common family studied (56% of cases), including most frequently the genera *Oncorhynchus*, *Salmo*, and *Salvelinus* (Fig. 3*a*). Most salmonid studies had the conservation objective of supplementation. Many studies also focused on the Acipenseridae family (13% of cases), most often from genera *Acipenser* and *Scaphirhynchus*, with most of these focusing on supplementation (Fig. 3*a*). The most frequently studied species were rainbow trout (*Oncorhynchus mykiss* and subspecies therein; 13% of cases) and Chinook salmon (*Oncorhynchus tshawytscha*; 10% of cases), primarily in the Pacific Northwest of USA. Commonly investigated non-salmonid imperilled fish species included lake sturgeon (*Acipenser fulvescens*; 3% of cases) and razorback sucker (*Xyrauchen texanus*; 2% of cases). Most studies were conducted on adult fish (36% of cases), followed by juveniles (e.g., age-0, age-1, 31%), mixed life stages (24%), and eggs (10%).

#### *Mussels*

Studies of captive breeding programs for imperilled mussels were conducted on three continents, with the majority of studies being conducted in Europe and North America (Fig. 1*b*). Of the 10 included countries, most studies were from the USA (36% of studies), UK (21%), and Czech Republic (17%). For mussels, propagation was the most commonly reported captive breeding objective, most often related to rearing methods (Fig. 2*b*). Many studies were also more exploratory in nature (e.g., optimization of cryopreservation techniques, glochidia infection trials, diet optimization), and commonly related to rearing methods (Fig. 2*b*). There were no studies included on re-introduction and genetic status in the evidence base. Similar to the existing literature on fish, half of all studies (23/48) did not explicitly and (or) clearly discuss details of an existing captive breeding program, but did however report on a species at risk in relation to more broadly defined captive breeding activities (e.g., small experimental/exploratory studies on cryopreservation techniques). Most studies used an after-only study design, where mussels were investigated or monitored after the treatment of interest was imposed, either for a single sampling period or repeated over more than one time period (69% of studies) (Fig. 2*d*). Only 25% of studies used a comparator. No mussel studies included a before and after captive breeding study comparison. Outcome metrics related to mussel survival and growth were the most frequently studied responses (Fig. 2*f*).

There were two families of mussels included in the map: (1) Unionidae (river mussels), with 15 genera and 21 species represented, and (2) Margaritiferidae (pearl mussels), with a single species investigated, *Margaritifera margaritifera* (called the "freshwater pearl mussel" in Europe, and the "Eastern pearlshell" in North America) (Fig. 3*b*). The most common genus of imperilled freshwater mussels investigated was *Margaritifera* (freshwater pearl mussels; 31% of cases), for a variety of captive breeding program

**Fig. 1.** Geographic distribution of evidence, displaying the number of studies per country for (*a*) fishes, and (*b*) mussels. Studies undertaken across more than one country are counted within each study country. Note, only temperate regions were considered for this map, and are identified with dashed boxes. The map was generating using Esri ArcGIS Desktop 10.7 (Esri 2018), and the World Countries (Generalized) shapefile from Esri (Esri 2019). [Colour online.]






objectives (Fig. 3*b*). A number of studies also focused on species from the genera *Epioblasma* and *Unio* (13% of cases each, respectively), primarily focusing on propagation and exploratory objectives (Fig. 3*b*). Most studies were conducted on juvenile mussels (49% of cases), followed by glochidia (33%), adults (14%), and mixed life stages (4%).

**Intersection of captive breeding study objectives and outcomes**

Figures 4 and 5 present heatmaps of the distribution and frequency of evaluations on the effectiveness of conservation-oriented captive breeding and release study objectives for imperilled freshwater fish and mussel outcomes. These heatmaps, along with the metadata previously described, were used to identify possible knowledge clusters and gaps outlined below. Conservation practitioners and managers may find the map useful to: (*i*) identify areas where there may be sufficient coverage on a specific topic to permit a full systematic review (see the section on *Knowledge clusters* below); and (*ii*) provide an indication of the extent of the current evidence base, and thus determine how to allocate future research funding to address knowledge gaps (see the section on *Knowledge gaps* below).

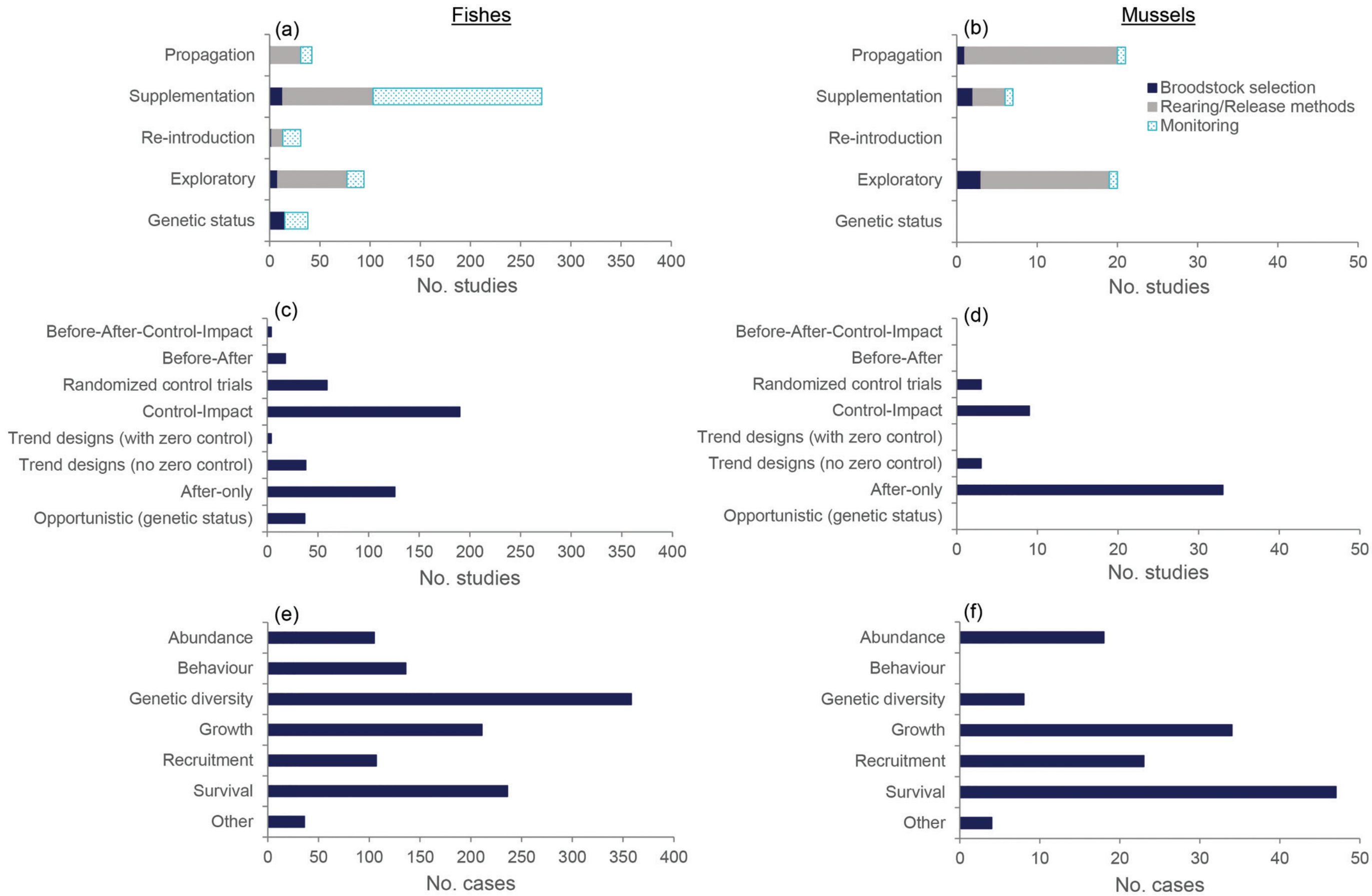
**Knowledge clusters**

This map suggests a number of subtopics that may warrant future evidence synthesis (Fig. 4). The following subtopics have a sufficient evidence base to permit full systematic reviewing, although the majority relate to evidence that, in general, may be susceptible to bias (i.e., study designs that lack true comparators):

***Fishes***

1. The effectiveness of supplementation-focused captive breeding studies during the rearing/release stage for imperilled freshwater fishes: (*a*) growth (i.e., mass) of reared or released propagules [47 cases, ~28% lacking appropriate comparators, including 18 species belonging to 10 genera, most cases (32/47) related to salmonids]; (*b*) survival of reared or released propagules [38 cases, ~45% lacking appropriate comparators, including 18 species belonging to 10 genera, most cases (25/38) related to salmonids]. Refer to Appendix A, Figs. A2 and A3, for separate heatmaps for salmonids and non-salmonid fishes.
2. The effectiveness of supplementation-focused captive breeding studies during the monitoring (i.e., post-release) stage for imperilled freshwater fish: (*a*) genetic diversity metrics related to (*i*) heterozygosity [36 cases, with ~17% lacking appropriate comparators, including 16 species belonging to 11 genera, most cases (26/36) related to salmonids], and (*ii*) allelic frequency/richness [46 cases, ~17% lacking appropriate comparators, including 18 species belonging to 11 genera, most cases (37/46) related to salmonids]; (*b*) growth (i.e., mass) of released propagules [40 cases, ~35% lacking appropriate comparators,

**Fig. 2.** Number of studies (or cases) in relation to: (*a* and *b*) captive breeding study objectives by captive breeding stage; (*c* and *d*) study design; and (*e* and *f*) outcome categories, for fishes (left panel) and mussels (right panel). Note the difference in scale for the panels for fishes and mussels. [Colour online.]




including 17 species belonging to 7 genera, most cases (26/40) related to salmonids]; (*c*) survival of released propagules [∼47 cases, ∼51% lacking appropriate comparators, including 17 species belonging to 9 genera, most cases (35/47) related to salmonids]. Refer to Appendix A, Figs A2 and A3, for separate heatmaps for salmonids and non-salmonid fishes.

3. The effectiveness of general exploratory research (e.g., diet optimization, various rearing conditions) in relation to captive breeding activities during the rearing/release stage for imperilled freshwater fishes: (*a*) reproductive recruitment (i.e., reproduction/fecundity) [37 cases, ∼24% lacking appropriate comparators, including 23 species belonging to 18 genera, most cases (29/37) related to non-salmonids]; (*b*) reproductive survival (i.e., survival for ≤age-0 fish) [37 cases, ∼24% lacking appropriate comparators, including 22 species belonging to 17 genera, most cases (35/37) related to non-salmonids]. Refer to Appendix A, Figs. A2 and A3, for separate heatmaps for salmonids and non-salmonid fishes.

### *Mussels*

No knowledge clusters were identified for mussels, owing to insufficient numbers for a full systematic review (Fig. 5).

## Knowledge gaps

This map identified a number of understudied subtopics that may correspond to knowledge gaps, which could benefit from primary research.

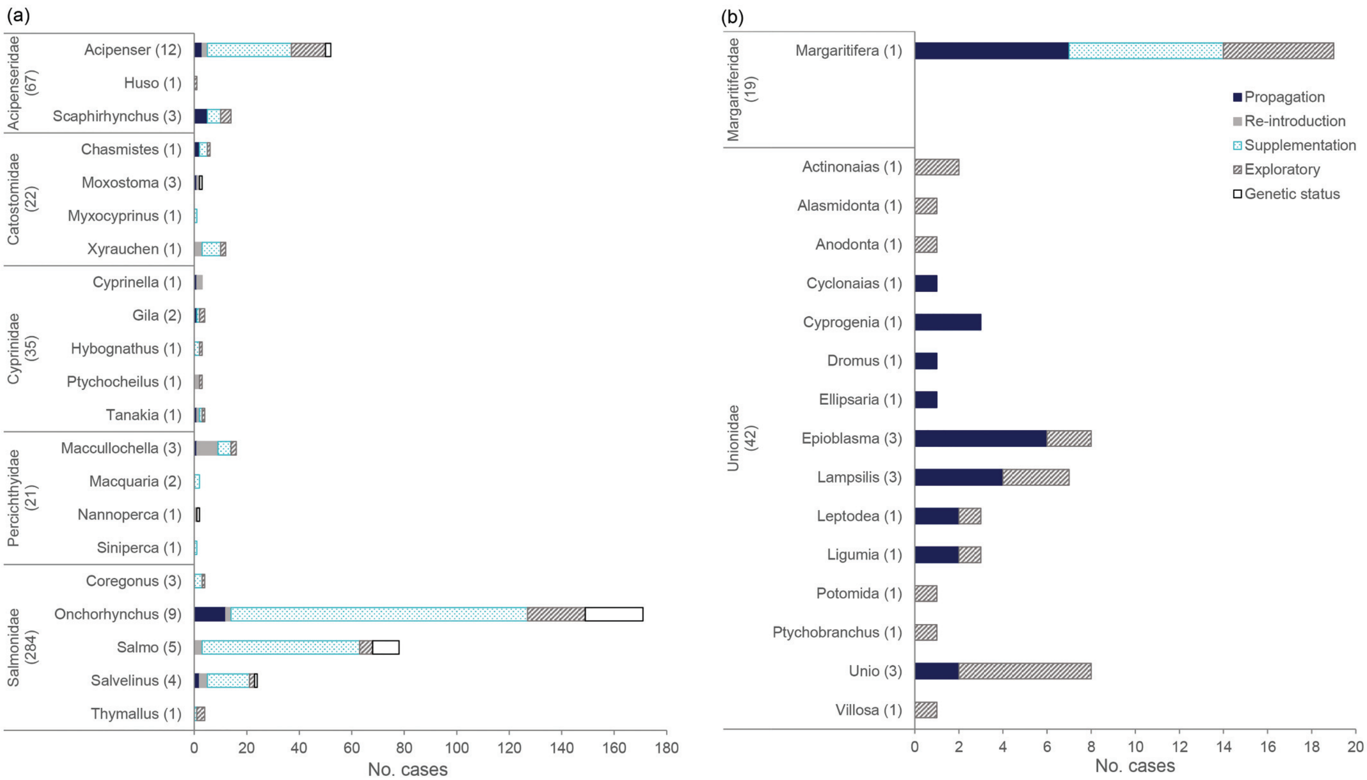
### *Fishes*

1. Geographic coverage beyond USA (Fig. 1*a*).
2. The ability to evaluate the effectiveness of any single captive breeding program objective (i.e., propagation, re-introduction, or supplementation) across all three stages of a program (i.e., broodstock collection, rearing/release methods, and post-release monitoring) for any outcome.
3. The effectiveness of broodstock collection (all objectives) on imperilled fishes (all relevant outcomes).
4. The effectiveness of propagation and reintroduction-focused captive breeding studies (all stages) for imperilled fishes (all relevant outcomes).
5. The effectiveness of captive breeding studies (all objectives, all stages) on metrics related to imperilled fish abundance, behaviour, and recruitment.
6. The effectiveness of captive breeding studies (all objectives, all stages, all relevant outcomes) for imperilled non-salmonid fish species (except for general exploratory studies at the rearing/release stage for reproductive recruitment and reproductive survival outcomes).

### *Mussels*

Overall, the available evidence base on the effectiveness of conservation driven captive breeding and release programs for imperilled freshwater mussels is extremely limited. This systematic mapping exercise suggests that all aspects of imperilled freshwater mussel captive breeding and release programs (Fig. 5),

**Fig. 3.** Number of cases in relation to captive breeding objective for (*a*) the five most commonly studied fish families and up to five genera therein, and (*b*) the only two studied mussel families and all captured genera therein. The number of cases per family is shown in parentheses below the family name. The number of species per genus is shown in parentheses adjacent to the genus name. Note, the difference in scales between (*a*) and (*b*). [Colour online.]




from all relevant geographical locations (Fig. 1*b*), require further primary research.

## Discussion

### Implications for management and research

The outputs from this systematic map (i.e., the map database and heatmaps) provide an up-to date global picture of the existing evidence of the effectiveness of conservation-oriented captive breeding and release programs for imperilled freshwater fishes and mussels. This mapping exercise highlights some general points of consideration for management agencies and researchers.

First, for fishes, there were clear clusters of evidence for supplementation evaluations related to rearing/release methods for fish growth and survival metrics, and the monitoring stage for fish genetic diversity, growth, and survival metrics, with evidence primarily focused on salmonids. Although the map suggests there may be suitable numbers for full systematic reviewing of these knowledge clusters, because many studies had inadequate experimental designs (i.e., lacked a comparator), it is unclear whether there will be sufficient high-quality data to allow for a quantitative synthesis. As such, alternate forms of evidence synthesis should be compared and considered for future secondary reviews (e.g., a narrative synthesis approach).

Second, this systematic map clearly highlights the overall paucity of studies on the effectiveness of captive breeding programs for imperilled freshwater mussels, suggesting further primary study is required. This could be because very few freshwater mussels have been evaluated for imperilled species status in many ecoregions (e.g., Australasian and Neotropical; reviewed in Lopes-Lima et al. 2018); providing no impetus to conduct captive breeding programs to begin with. Also, there are fewer researchers working on freshwater mussels than there are working on fishes. Furthermore, despite our best efforts to retrieve as much published and grey iterature as possible, we suspect that many evaluations have gone undocumented. Given that captive breeding programs are among the most expensive conservation interventions (see data in Bennett et al. 2015; Buxton et al. 2020), this issue has important implications for resource allocation in imperilled species programs more generally. Assessments of captive breeding program activities should therefore be disseminated in a manner that ensures they will be permanently and broadly available. One approach that might be of benefit is the use of journals that welcome practical field reports that document the outcomes of management practice (or field interventions) such as case study reports (e.g., Journal of Fish and Wildlife Management, Restoration Ecology, Environmental Management, Conservation Science and Practice, Case Studies in the Environment, Ecological Solutions and Evidence). Another approach could include forming collaborations between practitioners/facilities and scientists from universities, government agencies, or other organizations that may have more time and resources to help disseminate the information (Ramstead et al. 2012). Despite the small evidence base for mussels, we note that progress on the genetic implications of captive breeding for freshwater mussels has occurred through the Canadian Freshwater Species at Risk Research Network (VanTassel et al. 2021).

Third, this mapping exercise also highlighted that there is a need to improve our evaluations of captive breeding program effectiveness. Many studies were poorly documented (e.g., had unclear objectives) with inadequate experimental designs (i.e., lacked a comparator). Although improving evaluations may lead to a loss of efficiency for hatchery operations, it is vital to increase confidence about relevant interventions. Furthermore,

**Fig. 4.** Distribution and frequency of cases examining the effectiveness of the different stages of a captive breeding program (grouped by the objective of the study) for fish outcome categories and sub-categories (see Appendix A, Table A2 for example outcome metrics). In this matrix of counts (cases), darker coloured cells indicate a higher frequency of occurrence of the evidence, while lighter colours indicate a lower occurrence. [Colour online.]

No. cases: 0 10 20 30 40 50

FISH OUTCOMES

| Objective of captive breeding study | Stage | Abundance | | | | | | Behaviour | | | | | | | Genetic diversity | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Abundance (N) | Population counts | Density | Catch per unit effort | Habitat and occupancy | Other | Movement | Foraging | Spawning | Migration | Agonistic interactions | Anti-predator | Other | Population genetics | Heterozygosity | Allele metrics | Nucleotide metrics | Effective population | Haplotype metrics | Hybridization | Other |
| Propagation | Broodstock selection | | | | | | | | | | | | | | | | | | | | | |
| Propagation | Rearing/Release methods | 6 | | | | | | | | 1 | | | | | 3 | 6 | 5 | | 1 | | | 1 |
| Propagation | Monitoring | 2 | | | 1 | | 1 | | 1 | 2 | | 3 | | | | 1 | 2 | 1 | | 1 | | |
| Re-introduction | Broodstock selection | | | | | | | | | | | | | | 1 | 1 | 1 | | | | | |
| Re-introduction | Rearing/Release methods | 4 | | | | | | | | | | 1 | | 1 | | 1 | 1 | 1 | | | | |
| Re-introduction | Monitoring | 7 | | | 3 | 4 | | 4 | | 1 | | 1 | | | | 2 | 3 | | 1 | | | 1 |
| Supplementation | Broodstock selection | | | | | | | | | | | | | | 2 | 5 | 4 | | 1 | | 1 | 5 |
| Supplementation | Rearing/Release methods | 4 | | 1 | | 1 | 2 | 5 | 7 | 5 | 5 | 6 | 6 | 2 | 2 | 10 | 9 | 1 | 3 | | | 4 |
| Supplementation | Monitoring | 24 | 12 | 8 | 8 | 10 | 1 | 14 | 3 | 7 | 20 | 3 | 4 | | 11 | 36 | 46 | 2 | 10 | 7 | 3 | 18 |
| Exploratory | Broodstock selection | | | | | | | 1 | | | | | | | | | | | | 2 | | 2 |
| Exploratory | Rearing/Release methods | 5 | | | | 1 | | 11 | 3 | 3 | 1 | 5 | 5 | 2 | 3 | 9 | 5 | 2 | 2 | 1 | | 3 |
| Exploratory | Monitoring | | | | | | | | | | 2 | 1 | | | 1 | 6 | 6 | | 3 | 1 | 1 | 2 |
| Genetic status | Broodstock selection | | | | | | | | | | | | | | 4 | 8 | 7 | 1 | 1 | 5 | 1 | 4 |
| Genetic status | Rearing/Release methods | | | | | | | | | | | | | | | | | | | | | |
| Genetic status | Monitoring | | | | | | | | | | | | | | 10 | 18 | 17 | 3 | | 3 | 5 | 8 |

| Objective of captive breeding study | Stage | Growth | | | | | | Recruitment | | | | Survival | | | Other | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Mass | Condition | Mass of eggs | General growth | Shape | Other | Recruitment numbers | Recruitment rate | Reproductive recruitmen | Age structure | Survival | Reproductive survival | Recapture numbers | Disease/health | Physiology metrics | Other |
| Propagation | Broodstock selection | | | | | | | | | 1 | | | | | | | |
| Propagation | Rearing/Release methods | 16 | 1 | 3 | 5 | | | | | 8 | | 19 | 15 | | | | |
| Propagation | Monitoring | 1 | 1 | | | 1 | | | 1 | 1 | | 1 | 1 | 3 | | | |
| Re-introduction | Broodstock selection | | | | | | | | | | | 1 | | | | | |
| Re-introduction | Rearing/Release methods | 2 | 1 | | | | | | | | | 7 | 3 | | 1 | | |
| Re-introduction | Monitoring | 4 | | | 1 | | | | 1 | 2 | 1 | 5 | | | | | |
| Supplementation | Broodstock selection | 2 | | | | | | | | | 3 | | 2 | | | | 1 |
| Supplementation | Rearing/Release methods | 47 | 4 | 4 | 4 | 2 | 4 | | | 15 | 3 | 38 | 9 | 5 | 3 | 8 | 1 |
| Supplementation | Monitoring | 40 | 9 | 2 | 10 | 1 | 3 | 1 | 2 | 14 | 9 | 47 | 6 | 14 | | 4 | 5 |
| Exploratory | Broodstock selection | | | 1 | | | | 1 | | 4 | | 2 | 2 | | | | 1 |
| Exploratory | Rearing/Release methods | 25 | 3 | 1 | 5 | | 5 | | | 37 | | 12 | 37 | | 2 | 3 | 5 |
| Exploratory | Monitoring | 2 | | | | | 1 | | | 2 | 1 | 1 | | 6 | | | 2 |
| Genetic status | Broodstock selection | | | | | | | | | | | | | | | | |
| Genetic status | Rearing/Release methods | | | | | | | | | | | | | | | | |
| Genetic status | Monitoring | | | | | | | | | | | | | | | | |

to facilitate the knowledge base required for developing more effective captive breeding programs, we need to improve data reporting by providing comprehensive information on (*i*) the focal species i.e., the conservation status and life stage assessed, (*ii*) captive breeding study details i.e., objective (e.g., propagation, re-introduction, supplementation), facility name and country, stage of program/study (broodstock collection/selection, rearing and release methods, monitoring), and (*iii*) the outcomes evaluated. We recommend that data reporting according to the criteria outlined above should become mandatory for any future funding of a captive breeding conservation program, unless there is already sufficient evidence from previous related studies in which case authors should direct readers to that information.

Lastly, our ability to — rigorously or otherwise — evaluate the overall effectiveness of a captive breeding program is extremely limited due to a lack of study evaluations at all three stages of a program (i.e., broodstock collection, rearing/release methods, and post-release monitoring), for any outcome. For example, there were only a handful of species for which all three stages of a captive breeding program objective were evaluated (e.g., reintroductions of the trout cod, *Maccullochella macquariensis*, in Australia in relation to survival; supplementations of the Atlantic salmon, *Salmo salar*, across Spain, Canada, Finland, and Norway in relation to genetic diversity metrics; supplementing the Chinook salmon, *O. tshawytscha*, in the Pacific Northwest of the USA in relation to survival), and in all cases there were too few studies to allow for a quantitative estimate of captive breeding effectiveness. Therefore, to improve our evaluations of captive breeding program effectiveness, we advocate that future primary studies need to study all three stages of a captive breeding program for a given imperilled species so that the costs, benefits, and risks can be fully described, and for a variety of species, not just salmonids. Furthermore, given the limited evidence base on evaluations of captive breeding programs at all three stages of a program, an important goal of future systematic reviews is to determine the transferability of study results among species, particularly among the full complement of species for which captive breeding and reintroduction programs may be considered in the future (e.g., see Lamothe et al. 2019 for SARA-listed fishes in Canada). As many species under consideration for reintroduction in Canada are small-bodied and have not been the subject of active captive breeding efforts (i.e., most fish species evaluated were large-bodied and often recreationally or commercially targeted taxa such as salmonids and to a lesser extent, sturgeons), evaluating the transferability would provide critical insight about the extent to which species-specific investigations are required.

## Map limitations

There were a few potential limitations of this mapping exercise. First, the search strategy used to generate this map was designed to capture the breadth of relevant topics; however, the diversity of terminology used for captive breeding programs and their objectives, as well as for fish and mussel outcomes was such that this review may not be considered *completely* exhaustive (i.e., some terms may have been missed that could result in bias in our evidence map). Furthermore, due to the diversity of terminology, authors found coding of captive breeding objectives difficult at times. For example, there were relatively few studies evaluating the effectiveness of propagation and reintroductions for fishes and mussels on any outcome category. Although we included

**Fig. 5.** Distribution and frequency of cases examining the effectiveness of the different stages of a captive breeding program (grouped by the objective of the study) for mussel outcome categories and sub-categories (see Appendix A, Table A2 for example outcome metrics). In this matrix of counts (cases), darker coloured cells indicate a higher frequency of occurrence of the evidence, while lighter colours indicate a lower occurrence. [Colour online.]

No. cases: 0 10 20 30 40 50

MUSSEL OUTCOMES — Objective of captive breeding study

| Objective | Stage | Abundance: Abundance (N) | Population counts | Density | Catch per unit effort | Habitat and occupancy | Other | Behaviour: Movement | Foraging | Spawning | Migration | Agonistic interactions | Anti-predator | Other |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Propagation | Broodstock selection | | | | | | | | | | | | | |
| Propagation | Rearing/Release methods | 12 | | | | | | | | | | | | |
| Propagation | Monitoring | | | | | | | | | | | | | |
| Re-introduction | Broodstock selection | | | | | | | | | | | | | |
| Re-introduction | Rearing/Release methods | | | | | | | | | | | | | |
| Re-introduction | Monitoring | | | | | | | | | | | | | |
| Supplementation | Broodstock selection | | | | | | | | | | | | | |
| Supplementation | Rearing/Release methods | 2 | | | | | | | | | | | | |
| Supplementation | Monitoring | 1 | | | | | | | | | | | | |
| Exploratory | Broodstock selection | | | | | 1 | | | | | | | | |
| Exploratory | Rearing/Release methods | 2 | | | | | | | | | | | | |
| Exploratory | Monitoring | | | | | | | | | | | | | |
| Genetic status | Broodstock selection | | | | | | | | | | | | | |
| Genetic status | Rearing/Release methods | | | | | | | | | | | | | |
| Genetic status | Monitoring | | | | | | | | | | | | | |

| Objective | Stage | Genetic diversity: Population genetics | Heterozygosity | Allele metrics | Nucleotide metrics | Effective population | Haplotype metrics | Hybridization | Other |
|---|---|---|---|---|---|---|---|---|---|
| Propagation | Broodstock selection | | | | | | | | |
| Propagation | Rearing/Release methods | | | | | | | | |
| Propagation | Monitoring | | | | | | | | |
| Re-introduction | Broodstock selection | | | | | | | | |
| Re-introduction | Rearing/Release methods | | | | | | | | |
| Re-introduction | Monitoring | | | | | | | | |
| Supplementation | Broodstock selection | | 1 | 1 | | | | | |
| Supplementation | Rearing/Release methods | | 1 | 1 | | | | | |
| Supplementation | Monitoring | | | | | | | | 1 |
| Exploratory | Broodstock selection | | | | 1 | | | | |
| Exploratory | Rearing/Release methods | | 1 | 1 | | | | | |
| Exploratory | Monitoring | | | | | | | | |
| Genetic status | Broodstock selection | | | | | | | | |
| Genetic status | Rearing/Release methods | | | | | | | | |
| Genetic status | Monitoring | | | | | | | | |

| Objective | Stage | Growth: Mass | Condition | Mass of eggs | General growth | Shape | Other | Recruitment: Recruitment numbers | Recruitment rate | Reproductive recruitmen | Age structure |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Propagation | Broodstock selection | | | | | | | | | 2 | |
| Propagation | Rearing/Release methods | 11 | 1 | | 2 | | | | | 10 | |
| Propagation | Monitoring | 1 | | | | | | | | | |
| Re-introduction | Broodstock selection | | | | | | | | | | |
| Re-introduction | Rearing/Release methods | | | | | | | | | | |
| Re-introduction | Monitoring | | | | | | | | | | |
| Supplementation | Broodstock selection | 1 | | | | 1 | | | | | |
| Supplementation | Rearing/Release methods | 1 | | | 1 | | | | | | |
| Supplementation | Monitoring | | | | | | | | | | |
| Exploratory | Broodstock selection | | 1 | | | | | | | 1 | |
| Exploratory | Rearing/Release methods | 13 | | | | | 1 | | | 10 | |
| Exploratory | Monitoring | | | | | | | | | | |
| Genetic status | Broodstock selection | | | | | | | | | | |
| Genetic status | Rearing/Release methods | | | | | | | | | | |
| Genetic status | Monitoring | | | | | | | | | | |

| Objective | Stage | Survival: Survival | Reproductive survival | Recapture numbers | Other: Disease/health | Physiology metrics | Other |
|---|---|---|---|---|---|---|---|
| Propagation | Broodstock selection | | | | | | |
| Propagation | Rearing/Release methods | 20 | 4 | | | | |
| Propagation | Monitoring | | | 1 | | | |
| Re-introduction | Broodstock selection | | | | | | |
| Re-introduction | Rearing/Release methods | | | | | | |
| Re-introduction | Monitoring | | | | | | |
| Supplementation | Broodstock selection | | | | | | |
| Supplementation | Rearing/Release methods | 2 | 1 | | | | |
| Supplementation | Monitoring | | | | | | |
| Exploratory | Broodstock selection | | | | | | |
| Exploratory | Rearing/Release methods | 12 | 7 | | | 3 | |
| Exploratory | Monitoring | | | | | | 1 |
| Genetic status | Broodstock selection | | | | | | |
| Genetic status | Rearing/Release methods | | | | | | |
| Genetic status | Monitoring | | | | | | |

search terms specifically for these two objectives, and variations thereof (see Supplemental Material B, Tables S1–S8[2] for database search strings), we found that some of the literature used the terms reintroduction, supplementation, and variations of these words interchangeably. However, we carefully followed objective definitions, regardless of the terminology used in the articles, for differentiating between reintroduction and supplementation studies (see Table 1 for intervention definitions). Therefore, it is likely that this represents a "real" gap in knowledge. We acknowledge that this gap could be due in part to the fact that in some countries (i.e., USA, under the U.S. Endangered Species Act; e.g., Shirey and Lamberti 2010; McMurray and Roe 2017), the ecological criteria needed to undertake a reintroduction are extremely rigorous, indicating that most work will instead center around supplementation.

Another potential limitation of this map is that the search may have been biased towards Canadian at-risk species because the search string included known imperilled species names generated from the Canadian government's Species at Risk Public Registry (Government of Canada 2017) (see Donaldson et al. 2019 for further details and considerations). The rationale for this was that the words "fish" and "mussel" are not always included in the title, abstract, and (or) keywords of articles, and generating a global list of all imperilled fish and mussel species was not feasible. However, only 10% of the species eventually captured in this map database were species included from that Canadian species list (15/148 species), suggesting our search string was not biased towards Canadian species. Still, we recommend that future updates to this map consider additional, subtopic-specific (e.g., individual outcome category and sub-category metrics) and (or) taxonomic-specific terms in search for novel evidence.

An obvious consideration of this map is that the search was limited to English language literature. We recognize that more evidence likely exists in other languages; however, we did not have the resources to conduct these searches. A total of 18 non-English articles were identified by our search strategy (i.e., had English abstracts) but were excluded. It is unclear how many of these articles would have met all the inclusion criteria; however, the ability to include these untranslated articles would add strength to the accuracy of the map and any resultant syntheses.

Owing to the scope of the topic and the heterogeneous nature of the studies, this systematic map did not conduct a formal in-depth critical appraisal of each study's internal validity. Instead, metadata on aspects of the study design were extracted from the included studies to provide a very basic overview of the robustness of the evidence. The mapping exercise highlighted that many studies lacked true comparators (i.e., before data or control group information; Figs. 2c and 2d). Without an appropriate comparator, it is unclear whether any observed change in an outcome can be attributed to the intervention. In the absence of an in-depth assessment of study validity on the included studies, we cannot provide a clear picture of the overall reliability of the evidence base or determine whether there are sufficient high-quality quantitative data to allow for meta-analyses in any future syntheses.

Another limitation of the evidence base is that articles did not always report the conservation status of species that met our criteria for imperilment. This made screening articles for inclusion/exclusion in relation to defined species challenging. Our approach to address this issue was to be as inclusive as possible, including those that provided a formal conservation status of the study organism, as well as those for which no formal conservation status was reported, but rather a description of the species that suggested the species/population was or could be at risk (e.g., the species is experiencing rapid population decline). If the species was not captured in our database of existing studies, we also then cross-checked it with (*a*) the COSEWIC listing for all fishes and molluscs in Canada (Canadian Endangered Species Conservation Council 2016); and (*b*) the IUCN Red List of threatened species (IUCN 2020). From this process, we found that 46% of studies for fishes (239/520 cases) and 36% of studies for mussels (22/61 cases) reported a formal conservation status of the focal species, and 45% (fishes) and 51% (mussels) were provided a description by the author(s). The remaining studies did not provide an indication of the conservation status for the species of interest, but were included after cross-checking with existing lists (9% and 13% of cases for fishes and mussels, respectively). Therefore, to ensure articles related to evaluating the effectiveness of captive breeding programs for imperilled species are included in future evidence synthesis, we recommend authors provide an indication of the conservation status of focal species in all publications even when it is believed this information is common knowledge.

During the protocol development of this systematic map and through discussions with the project Advisory Team, a paucity of studies related to evaluating the effectiveness of captive breeding programs for imperilled species was anticipated. Furthermore, it was suspected that what evidence did exist was likely available as grey literature, especially for freshwater mussels. While this map suggests there was a limited evidence base for imperilled mussels, grey literature accounted for very little of the evidence base for both fishes and mussels (11% and 21% of articles). This suggests that (*i*) there is indeed a paucity of studies on this topic, in particular for freshwater mussels, and (or) (*ii*) many previously conducted projects have likely been undocumented. In the case of the latter, failure to document or share knowledge on past efforts is not unique to our review topic (e.g., Davies et al. 2008; Ramstead et al. 2012; Lintermans 2013; Rytwinski et al. 2019). Indeed, many management practitioners or facility researchers (e.g., in hatcheries or for non-profits) involved in captive breeding are not rewarded for publishing findings nor provided the necessary support to do so, and (or) do not necessarily value publishing their research, highlighting the important need for finding ways to make such information broadly available so that it can be used by others and included in evidence syntheses (ideally involving assessments of study validity) (see also Geist 2015; Strayer et al. 2019).

Lastly, there were some geographical biases in the data. Most studies were from the USA (64% and 36% of studies for fishes and mussels, respectively), potentially limiting the geographic scope of subtopics (and thus species) beyond this region.

## Conclusions

Our map highlights several main points of consideration for both the management of imperilled species and conducting research to support species reintroduction activities. First, there were many studies in the current evidence base that were poorly documented with inadequate experimental designs. For proper evaluation and interpretation of captive breeding program effectiveness, future primary studies should have explicitly stated objectives and study designs that (when possible) incorporate controlled investigations. Second, we speculate that many captive breeding program evaluations have likely gone undocumented. Evaluations of captive breeding program effectiveness should be published to ensure findings will be permanently and broadly available. Lastly, to improve our evaluations of captive breeding program effectiveness, we recommend that future primary studies evaluate captive breeding programs for a given imperilled species at all three stages (i.e., broodstock collection, rearing/release methods, and post-release monitoring), and for a variety of species including non-salmonid, non-sportfish or non-commercial fish species. Furthermore, future review is needed to determine the transferability of captive breeding and reintroduction study results among species. If transferability among species is poor, a strong focus on adaptive management for newly initiated captive breeding and release programs, in parallel with species-specific studies, would maximize learning and ensure strong progress towards meeting conservation objectives in Canada.

## Funding statement

Funding for this study was provided by Fisheries and Oceans Canada's Canadian Freshwater Species at Risk Research Network (CU-01). Additional support was provided by the Natural Science and Engineering Research Council of Canada (S.J. Cook), The Canada Research Chairs Program, and Carleton University.

## Acknowledgements

The authors thank Shannon Clarke, Teah Lizée, and Elizabeth Robson Gordon for help with website searches, article retrieval, and screening of articles and websites. We also thank Meagan Harper for help with article retrieval and creation of some figures, and the Carleton University library staff for help with article retrieval through the Interlibrary Loans program. The authors thank Patrick O'Reilly (Research Scientist, Salmon Population Geneticist, Fisheries and Oceans Canada, Dartmouth, Nova Scotia) who provided valuable insights that strengthened this project in its early stages. We also thank the reviewers for their constructive comments.

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## Appendix A: Definitions and descriptive statistics

**Table A1.** Definitions of terms used throughout the systematic map.

| Term | Definition |
|---|---|
| Article | An independent publication (i.e., the primary source of relevant information); used throughout the map |
| Supplementary article | An article that reported data that was also found elsewhere or contained portions of information that were used in combination with another more complete source; used throughout the map |
| Study | A single study from a single article, or when multiple studies were reported within a single article that differed with respect to: (*i*) study design; (*ii*) captive breeding study objective; (*iii*) captive breeding program/study stage; (*iv*) captive breeding facility; and (or) (*v*) experimental condition; used throughout the map |
| Case | Situationally defined in text/visual aids; e.g., multiple counts within a given study for different outcome categories for a given species, or the same outcome for >1 species evaluated. In such instances, the number of cases will exceed the total number of studies included (i.e., >524 studies). When speaking generally about descriptions, these are still referred to as studies (e.g., "Most *studies* were conducted on juvenile mussels. . ."; however, to distinguish these counts from individual study counts, we use the term *cases* when providing in-text descriptive statistics (e.g., "Most studies were conducted on juvenile mussels (49% *cases*). . .", and in figure/ table captions/ legends where applicable |

**Fig. A1.** Period of publication for the 460 articles in relation to source for (*a*) fishes, and (*b*) mussels. Note, the difference in scales between (*a*) and (*b*). [Colour online.]

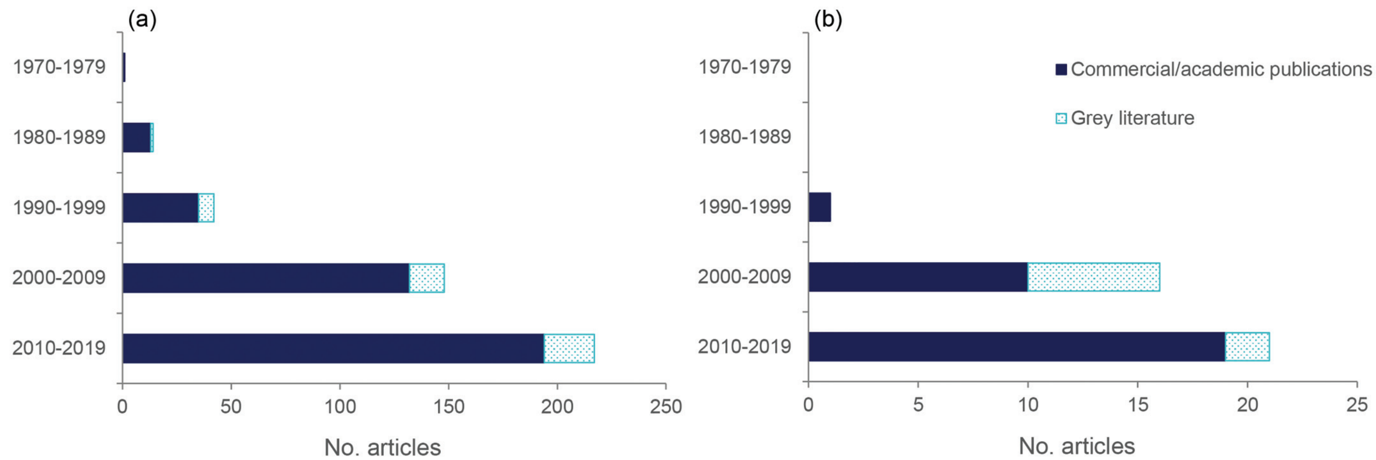



**Table A2.** Outcome categories, sub-categories, and example metrics used to evaluate captive breeding programs along with the number of cases for imperilled freshwater fishes and mussels.

| Outcome categories | Outcome sub-categories | Example metrics* | No. cases Fishes | No. cases Mussels |
|---|---|---|---|---|
| Abundance | Abundance (N) | Number of individuals | 52 | 17 |
| | Population counts | % Population, population growth | 12 | 0 |
| | Density | Density | 9 | 0 |
| | Catch per unit effort | CPUE | 12 | 0 |
| | Habitat and occupancy | Spatial distribution, habitat use | 16 | 1 |
| | Other | Sex ratio, wild:hatchery ratio | 4 | 0 |
| Behaviour | Movement | Swimming speed, mobility | 35 | 0 |
| | Foraging | Feed efficiency, prey selection | 14 | 0 |
| | Spawning | Spawning time, courtship behaviour | 19 | 0 |
| | Migration | Migration rate, timing, range | 28 | 0 |
| | Agonistic interactions | Aggressive behaviour, dominance | 20 | 0 |
| | Anti-predator | Fright response, predation risk | 15 | 0 |
| | Other | Reaction to light, duration of freezing | 5 | 0 |
| Genetic diversity | Population genetics | Genetic diversity, variation | 37 | 0 |
| | Heterozygosity | $F_{st}$, $F_{is}$, inbreeding coefficient | 103 | 3 |
| | Allele metrics | Allelic frequency, richness, number of alleles | 106 | 3 |
| | Nucleotide metrics | Nucleotide diversity, distance | 11 | 1 |
| | Effective population | $N_e$, effective breeders | 22 | 0 |
| | Haplotype metrics | Haplotype diversity, frequency | 20 | 0 |
| | Hybridization | Hybridization, introgression, admixture | 11 | 0 |
| | Other | Demographic or parentage analyses | 48 | 1 |
| Growth | Mass | Fork or shell length, weight, shell height | 139 | 27 |
| | Condition | Condition factor | 19 | 2 |
| | Mass of eggs | Egg size, weight, diameter | 11 | 0 |
| | General growth | Growth rate, mean growth | 25 | 3 |
| | Shape | Shape | 4 | 1 |
| | Other | Dorsal fin index, fin length | 13 | 1 |
| Recruitment | Recruitment numbers | CPUE | 2 | 0 |
| | Recruitment rate | Recruitment rate | 4 | 0 |
| | Reproductive recruitment | Fecundity, glochidia viability | 87 | 23 |
| | Age structure | Age structure, age at maturity | 14 | 0 |
| Survival | Survival | Survival beyond age 0/glochidia stage | 133 | 34 |
| | Reproductive survival | Survival $\leq$age 0/glochidia stage | 75 | 12 |
| | Recapture numbers | Recapture rate, return rate | 28 | 1 |
| Other | Disease/health | Disease, healing rate, infestation rate | 6 | 0 |
| | Physiology metrics | % Lipid, gill ATPase, $Na^+$ | 15 | 3 |
| | Other | Mark longevity, gastric lavage efficiency | 15 | 1 |

*For a full list of example outcome metrics, refer to Supplemental Material C[2].

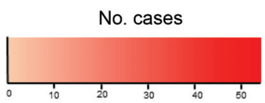
**Fig. A2.** Distribution and frequency of cases examining the effectiveness of the different stages of a captive breeding program (grouped by the objective of the study) for salmonid fish outcome categories and sub-categories only (see Appendix A, Table A2 for example outcome metrics). In this matrix of counts (cases), darker coloured cells indicate a higher frequency of occurrence of the evidence, while lighter colours indicate a lower occurrence. [Colour online.]

No. cases (colour scale: 0, 10, 20, 30, 40, 50)

SALMONID OUTCOMES

| Objective of captive breeding study | Stage | Abundance | | | | | | Behaviour | | | | | | | Genetic diversity | | | | | | | | Growth | | | | | | Recruitment | | | | Survival | | | Other | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Abundance (N) | Population counts | Density | Catch per unit effort | Habitat and occupancy | Other | Movement | Foraging | Spawning | Migration | Agonistic interactions | Anti-predator | Other | Population genetics | Heterozygosity | Allele metrics | Nucleotide metrics | Effective population | Haplotype metrics | Hybridization | Other | Mass | Condition | Mass of eggs | General growth | Shape | Other | Recruitment numbers | Recruitment rate | Reproductive recruitment | Age structure | Survival | Reproductive survival | Recapture numbers | Disease/health | Physiology metrics | Other |
| Propagation | Broodstock selection | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Propagation | Rearing/Release methods | | | | | | | | | | | | | | | 1 | 1 | | | | | 1 | 5 | 1 | 1 | 3 | | | | | 5 | | 5 | 5 | | | | |
| Propagation | Monitoring | | | | | | | | | 1 | | 1 | | | | | | | | | | | | | | | | | | | 1 | | 1 | | 2 | | | |
| Re-introduction | Broodstock selection | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Re-introduction | Rearing/Release methods | | | | | | | | | | | 1 | | 1 | | | | | | | | | 1 | 1 | | | | | | | | | 1 | | | | | |
| Re-introduction | Monitoring | 1 | | | | | | 1 | | 1 | | 1 | | | | | | | | | | 1 | | | | | | | | 1 | 1 | | 1 | | | | | |
| Supplementation | Broodstock selection | | | | | | | | | | | | | | | 3 | 3 | | | | 1 | 1 | 1 | | | | | | | | | | | 2 | | | | |
| Supplementation | Rearing/Release methods | 2 | | 1 | | 1 | 2 | 4 | 4 | 5 | 5 | 6 | 5 | 2 | 1 | 3 | 5 | | 2 | | | 3 | 32 | 2 | 4 | 4 | 1 | 4 | | | 12 | 3 | 25 | 6 | 5 | 3 | 6 | 1 |
| Supplementation | Monitoring | 20 | 9 | 7 | 5 | 9 | 1 | 6 | 1 | 6 | 20 | 2 | 4 | | 7 | 26 | 37 | 1 | 7 | 4 | 3 | 15 | 26 | 6 | 1 | 6 | 1 | 3 | | | 14 | 5 | 35 | 5 | 7 | | 4 | 3 |
| Exploratory | Broodstock selection | | | | | | | 1 | | | | | | | | | | | | 1 | | 1 | | | 1 | | | | 1 | | 4 | | 2 | 2 | | | | 1 |
| Exploratory | Rearing/Release methods | 1 | | | | 1 | | 2 | 1 | 2 | 1 | 4 | 1 | 1 | 2 | 5 | 2 | 1 | 1 | 0 | | 3 | 6 | 3 | 1 | 2 | | 1 | | | 8 | | 3 | 2 | | | 1 | 2 |
| Exploratory | Monitoring | | | | | | | | | | 2 | 1 | | | | 3 | 3 | | 1 | 1 | | 1 | 2 | | | | | 1 | | | 2 | 1 | | | 4 | | | 2 |
| Genetic status | Broodstock selection | | | | | | | | | | | | | | 1 | 2 | 3 | | | | | 2 | | | | | | | | | | | | | | | | |
| Genetic status | Rearing/Release methods | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Genetic status | Monitoring | | | | | | | | | | | | | | 9 | 18 | 17 | 3 | | 3 | 5 | 8 | | | | | | | | | | | | | | | | |

**Fig. A3.** Distribution and frequency of cases examining the effectiveness of the different stages of a captive breeding program (grouped by the objective of the study) for non-salmonid fish outcome categories and sub-categories only (see Appendix A, Table A2 for example outcome metrics). In this matrix of counts (cases), darker coloured cells indicate a higher frequency of occurrence of the evidence, while lighter colours indicate a lower occurrence. [Colour online.]




| | | NON-SALMONID OUTCOMES | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Abundance | | | | | | Behaviour | | | | | | | Genetic diversity | | | | | | | | Growth | | | | | | Recruitment | | | | Survival | | | Other | | |
| Objective of captive breeding study | | Abundance (N) | Population counts | Density | Catch per unit effort | Habitat and occupancy | Other | Movement | Foraging | Spawning | Migration | Agonistic interactions | Anti-predator | Other | Population genetics | Heterozygosity | Allele metrics | Nucleotide metrics | Effective population | Haplotype metrics | Hybridization | Other | Mass | Condition | Mass of eggs | General growth | Shape | Other | Recruitment numbers | Recruitment rate | Reproductive recruitment | Age structure | Survival | Reproductive survival | Recapture numbers | Disease/health | Physiology metrics | Other |
| Propagation | Broodstock selection | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | 1 | | | | | | | |
| Propagation | Rearing/Release methods | 6 | | | | | | | | 1 | | | | | 3 | 5 | 4 | | 1 | | | | 11 | | 2 | 2 | | | | | 3 | | 14 | 10 | | | | |
| Propagation | Monitoring | 2 | | | 1 | | 1 | | 1 | 1 | | 2 | | | | 1 | 2 | 1 | | 1 | | | 1 | 1 | | | 1 | | | 1 | | | 1 | | 1 | | | |
| Re-introduction | Broodstock selection | | | | | | | | | | | | | | 1 | 1 | 1 | | | | | | | | | | | | | | | | 1 | | | | | |
| Re-introduction | Rearing/Release methods | 4 | | | | | | | | | | | | | | 1 | 1 | 1 | | | | | 1 | | | | | | | | | | 6 | 3 | | 1 | | |
| Re-introduction | Monitoring | 6 | | | 3 | 4 | | 3 | | | | | | | | 2 | 3 | | 1 | | | | 4 | | | 1 | | | | | 1 | 1 | 4 | | | | | |
| Supplementation | Broodstock selection | | | | | | | | | | | | | | 2 | 2 | 1 | | 1 | | | 4 | 1 | | | | | | | | | 3 | | | | | | 1 |
| Supplementation | Rearing/Release methods | 2 | | | | | | 1 | 3 | | | | 1 | | 1 | 6 | 4 | 1 | 1 | | | 1 | 15 | 2 | | | 1 | | | | 3 | | 13 | 3 | | | 2 | |
| Supplementation | Monitoring | 4 | 3 | 1 | 3 | 1 | | 8 | 2 | 1 | | 1 | | | 4 | 10 | 9 | 1 | 3 | 3 | | 3 | 14 | 3 | 1 | 4 | | | 1 | 2 | | 4 | 12 | 1 | 7 | | | 2 |
| Exploratory | Broodstock selection | | | | | | | | | | | | | | | | | | | 1 | | 1 | | | | | | | | | | | | | | | | |
| Exploratory | Rearing/Release methods | 4 | | | | | | 9 | 2 | 1 | | 1 | 4 | 1 | 1 | 5 | 3 | 1 | 1 | 1 | | | 19 | | | 3 | | 4 | | | 29 | | 9 | 35 | | 2 | 2 | 3 |
| Exploratory | Monitoring | | | | | | | | | | | | | | 1 | 3 | 3 | | 2 | | 1 | 1 | | | | | | | | | | | 1 | | 2 | | | |
| Genetic status | Broodstock selection | | | | | | | | | | | | | | 3 | 6 | 4 | 1 | 1 | 5 | 1 | 2 | | | | | | | | | | | | | | | | |
| Genetic status | Rearing/Release methods | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Genetic status | Monitoring | | | | | | | | | | | | | | 1 | | | | | | | | | | | | | | | | | | | | | | | |