Fisheries and Oceans Canada / Pêches et Océans Canada
Science / Sciences

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Canadian Science Advisory Secretariat / **Secrétariat canadien de consultation scientifique**

Research Document 2008/026 / **Document de recherche 2008/026**

Protocol for the detection of fish Species At Risk in Ontario Great Lakes Area (OGLA)

Protocole pour la détection d'espèces de poissons en péril dans la région des Grands Lacs de l'Ontario (RGLO)

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ISSN 1499-3848 (Printed / Imprimé)

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ABSTRACT

This document provides protocols and methods for addressing the presence, or possible presence, of fish species at risk within the zone of impact of a project, and determining whether the project must be reviewed under the *Species at Risk Act* (SARA). Guidance on when to obtain a SARA permit for the collection and handling of species at risk is also provided. A brief introduction to the SARA is given. Guidance is provided on sampling gear and effort required to determine the presence, or probable absence, of fish species at risk and on methods of documenting species at risk identification.

This document is designed for use by persons undertaking a project that may impact a fish species at risk, or where the potential detection of a species at risk may affect a project. It is directed primarily toward species at risk that occur in Ontario.

RÉSUMÉ

Le présent document fournit les protocoles et les méthodes pour aborder la présence, ou la présence potentielle, d'espèces de poissons en péril dans la zone de répercussion d'un projet et pour déterminer si le projet doit être examiné en vertu de la *Loi sur les espèces en péril* (LEP). Le document offre également de l'aide pour savoir quand obtenir un permis de la LEP pour la collecte et le traitement des espèces en péril. On y trouve une brève introduction à la LEP. Le document fournit aussi des conseils sur l'effort et l'engin utilisé pour l'échantillonnage afin de déterminer la présence, ou la présence potentielle, d'espèces de poissons en péril, ainsi que sur les méthodes pour documenter l'identification des espèces en péril.

Le présent document doit être utilisé uniquement par des personnes entreprenant un projet qui peut influencer une espèce de poissons en péril, ou dans l'éventualité où la détection possible d'espèces en péril peut avoir des répercussions sur un projet. Cela vise directement les espèces en péril de l'Ontario.

1.0 INTRODUCTION

1.1 PURPOSE OF THIS DOCUMENT

This document provides protocols and methods for addressing the presence, or possible presence, of fish species at risk within a study area. By following these protocols and methods, the project proponent and Fisheries and Oceans Canada (DFO) can determine with reasonable certainty if a fish species at risk is within the zone of impact, and if the project must be reviewed under the Canadian *Species at Risk Act* (SARA).

Many of these protocols and methods will be necessary components of monitoring plans or fish salvage undertakings.

Guidance on when to obtain a SARA permit for the collection and handling of species at risk is also provided.

1.2 WHO SHOULD USE THIS DOCUMENT?

This document will be useful to persons undertaking a project that may impact a fish species at risk, or where the potential detection of a species at risk (SAR) may affect a project. It is, however, directed primarily toward species at risk that occur in Ontario.

1.3 HOW TO USE THIS DOCUMENT

Section 2.0 outlines the legislative environment relating to fish species at risk. Section 3.0 provides guidance for determining if a fish sampling program is required. Section 4.0 outlines how to obtain a SARA collection permit. Section 5.0 contains guidance on the design of field investigations with additional supporting information provided in Appendix A. Section 6.0 discusses the necessity of proper documentation of the presence of species at risk and gives guidance on the type of evidence required (voucher specimen, photographs) for fish species at risk that occur in Ontario. An example of a completed application form for a SARA permit is provided in Appendix B.

2.0 LEGISLATIVE ENVIRONMENT

The purposes of the SARA are to prevent wildlife species from being extirpated or becoming extinct, to provide for the recovery of wildlife species that are extirpated, endangered or threatened as a result of human activity, and to manage species of special concern to prevent them from becoming endangered or threatened. Currently, several fishes are among the identified species at risk in Schedule 1 of the SARA. Species listed in Schedule 1 will be periodically reviewed as ongoing studies and investigations provide evidence to justify their continued inclusion or removal. To obtain a current list of fish species listed in Schedule 1 of the SARA, refer to the SARA registry website (http://www.sararegistry.gc.ca).

Listing under SARA is based on species assessments conducted by the Committee on the Status of Endangered Wildlife in Canada (COSEWIC). This is the first step towards protecting species at risk. Subsequent steps include COSEWIC reporting its results to the Canadian government and the public, and the Minister of the Environment's official response to the assessment results. Species that have been designated by COSEWIC may then qualify for legal protection and recovery under SARA. At present, a total of 35 freshwater fish species in Ontario have been assigned a conservation status by the

Committee on the Status of Endangered Wildlife in Canada (COSEWIC), and it is expected that most will eventually be listed under the SARA (http://www.cosewic.gc.ca/).

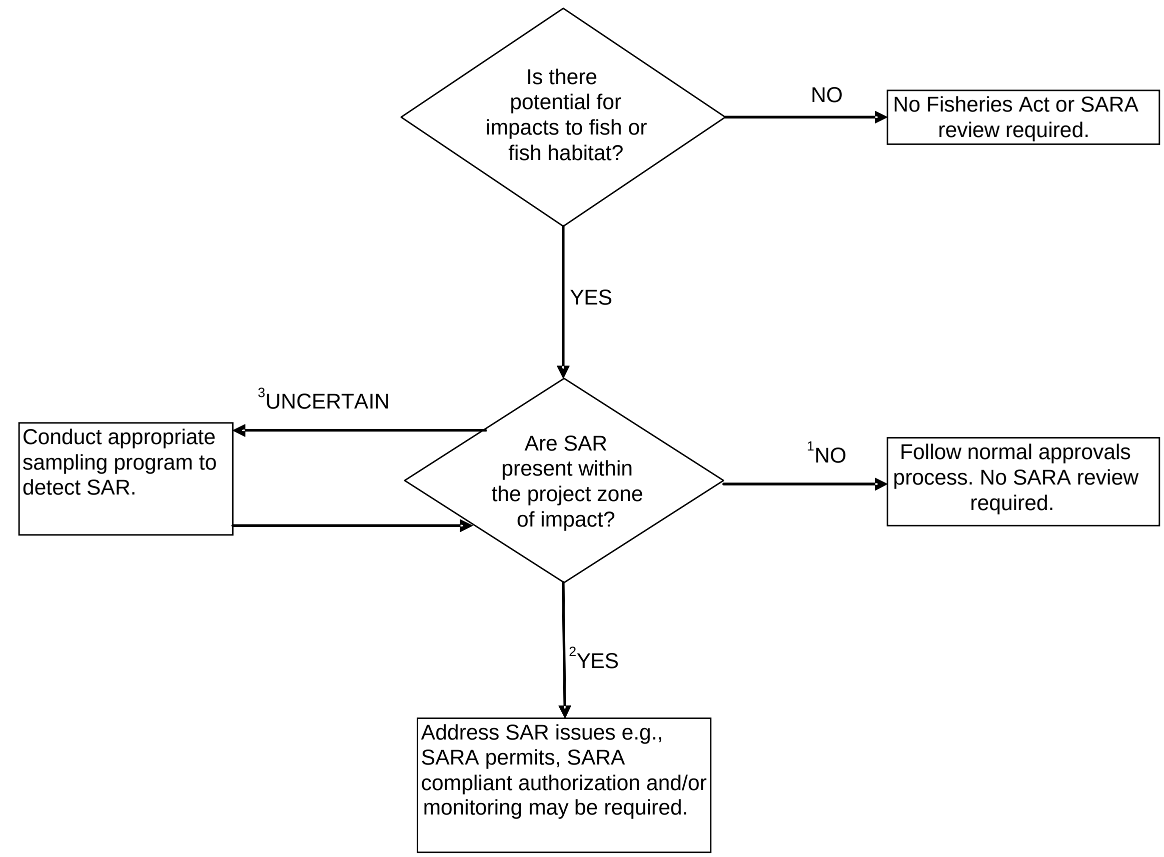
The Minister of Fisheries and Oceans is responsible for aquatic species listed under the SARA, including freshwater fishes and mussels. Once a species is listed under the SARA, it becomes illegal to kill, harass, capture or harm it in any way. Critical habitats are also protected from destruction. The Act also requires that recovery strategies, action plans and management plans be developed for all listed species. DFO is responsible for the coordination of recovery strategies and action plans for endangered or threatened aquatic species at risk. It is important to remember that there may be provincial and/or municipal and/or Conservation Authority policies that also pertain to species at risk.

The SARA Schedule 1 is the official list of wildlife species at risk in Canada. It includes species that are extirpated, endangered, threatened, and of special concern. Once a species is listed on Schedule 1, protection and recovery measures are developed and implemented. The SARA also amends the definition of "environmental effect" in the Canadian Environmental Assessment Act (CEAA) to include any change that a project may cause to a listed species, its critical habitat or the residences of individuals of that species, as defined in the SARA. Therefore, projects that require an environmental assessment under CEAA will have to take into account the project's effects on listed wildlife species and their critical habitats. The assessment must include recommendations for measures to avoid or reduce adverse effects and plans to monitor the impact of the project, if it goes ahead. The project plan must respect recovery strategies and action plans.

3.0 IS A SAMPLING PROGRAM NECESSARY?

To determine whether or not the habitat provisions of the SARA apply to a particular project, it is necessary to know if a species at risk or its habitat will be affected by the project. For some projects, it will already be known if a species at risk or its habitat is present. For others, it will be necessary to determine if this is the case. Figure 1 is provided to aid in determining the appropriate course of action.

A mapping tool, developed by DFO Science and Habitat Management, compiles all available information on the distribution of aquatic species at risk in southern Ontario. Maps, based on this tool, have been distributed to the Conservation Authorities, the Ontario Ministry of Natural Resources, the Ontario Ministry of Transportation and DFO offices. This mapping tool provides information on species at risk distributions at the stream segment level. Given the amount of sampling that has occurred in southern Ontario, if a species at risk has not been previously collected within a tertiary watershed, the potential for a fish species at risk to be present is considered low and a SARA review is not necessary. Therefore, the first step in assessing the need for SARA considerations is to consult this mapping tool.



[1] No - the project will not cause impacts to SAR or the species at risk mapping tool indicates that the project is not located in a stream segment where species at risk are considered potentially present

[2] Yes - species at risk mapping tool, or an appropriate sampling program, indicates that the project is located in a stream segment where species at risk are present and the project has the potential to cause impacts to SAR.

[3] Uncertain - there is uncertainty as to whether SAR are present or will be impacted.

Figure 1. Decision chart to determine the appropriate course of action to address project-specific species at risk issues.

If existing data indicate that a species at risk occurs, or may occur, within the study area, the next task is to determine, in conjunction with DFO, if a sampling program is required. Generally, sampling will be required in all cases, unless:

- a species at risk is known to occur within the study area; or,
- the proponent assumes that a species at risk is present in the study area when designing the project and agrees that review agencies will make the same assumption during review; or,
- it can be determined, without conducting sampling, that there is a very low probability of a species at risk occurring within the study area; or,
- it can be determined, without conducting sampling, that there is a very low probability of a species at risk being negatively impacted by the proposed activity.

If a species at risk is known to occur within the study area, then sampling to demonstrate its presence is redundant, unless it is thought that the species may have been extirpated since it was last captured. Similarly, if there is the potential for a species at risk to occur in a study area, and the proponent is prepared to assume that the species is present and to allow agencies to assume its presence during project evaluation, then sampling to demonstrate presence is not required. There may be other reasons to conduct sampling, for example, to determine the relative significance of specific areas, or as part of a monitoring program, but there is no need to sample in order to simply demonstrate presence again. Unnecessary sampling should be avoided, as it could cause harm to the species at risk.

There are situations in which the probability of a species at risk being present can be determined to be sufficiently low in a study area without undertaking any field sampling, even though the species in question is present within the stream segment. This may be the case in some areas of southern Ontario where there is a considerable amount of historic sampling data, and the species at risk has not been previously collected within the study area of the project. Many species at risk have very specific habitat requirements. If a species at risk is present in the tertiary watershed, but it can be demonstrated through adequate knowledge of the species' habitat requirements and of the habitats within the study area that no suitable habitat is present in the study area, then sampling is not required. Obviously, the likelihood of this criterion being met is lower for habitat generalists, and decreases as the size of the study area increases. Finally, if DFO determines that the risk posed by the proposed activity is sufficiently low, it may not be necessary to determine whether or not a species at risk is present within the study area.

4.0 OBTAINING A SCIENTIFIC RESEARCH/EDUCATION PERMIT UNDER SARA

Since the SARA was enacted on June 1 2004, it has been an offence under Canadian law to "kill, harm, harass, capture or take an individual of a listed species that is classed as extirpated, endangered or threatened". However, some of these activities prohibited under the SARA may be necessary to protect species at risk. Therefore, there is a provision in the SARA that allows such activities, at the discretion of DFO, if:

a) the activity is scientific research relating to the conservation of the species and conducted by qualified persons; or,
b) the activity benefits the species or is required to enhance its chance of survival in the wild; or,
c) affecting the species is incidental to the carrying out of the activity.

A SARA permit must be obtained if a proposed activity may contravene any one of the three SARA prohibitions. These are:

Section 32. (1) No person shall kill, harm, harass, capture or take an individual of a wildlife species that is listed as an extirpated species, an endangered species or a threatened species.

(2) No person shall possess, collect, buy, sell or trade an individual of a wildlife species that is listed as an extirpated species, an endangered species or a threatened species, or any part or derivative of such an individual.

Section 33. No person shall damage or destroy the residence of one or more individuals of a wildlife species that is listed as an endangered species or a threatened species, or that is listed as an extirpated species if a recovery strategy has recommended the reintroduction of the species into the wild in Canada.

Section 58. (1) Subject to this section, no person shall destroy any part of the critical habitat of any listed endangered species or of any listed threatened species — or of any listed extirpated species if a recovery strategy has recommended the reintroduction of the species into the wild in Canada — if

(a) the critical habitat is on federal land, in the exclusive economic zone of Canada or on the continental shelf of Canada;
(b) the listed species is an aquatic species; or
(c) the listed species is a species of migratory birds protected by the Migratory Birds Convention Act, 1994.

This includes any field sampling of species at risk, or prior to any fish salvage operations during a project, and **is in addition to a provincial scientific collection licence**. The local office of DFO must be contacted if it is suspected that a permit will be required.

A permit is obtained by submission of a standard application form. The most recent version of the application form can be obtained from the nearest DFO office or from the SARA Registry website (http://www.sararegistry.gc.ca). For a complete list of DFO office locations refer to http://www.dfo-mpo.gc.ca/oceans-habitat/habitat/aboutus-apropos/regions/index_e.asp. The applicant must demonstrate that they have sufficient expertise to be able to conduct the field survey, as well as identify the species at risk. An example of a completed application form is provided in Appendix B. If it is known or expected that the proposed activity does not pose a threat to the species at risk, or a species at risk does not occur within the area, a permit is not required.

5.0 SAMPLING STRATEGIES TO DETECT THE PRESENCE OF SPECIES AT RISK

While the presence of a species can be proven, absence never can. The most that can be achieved is to demonstrate that the presence of a species in a particular area is improbable, given that it has not been captured by a sampling program with which the probability of its capture, had it been present, was high.

The four key aspects of designing a sampling program are:

- knowledge of the biology of the species at risk
- delineation of the study area
- designing the sampling program (locations, gear and effort)
- species identification

5.1 KNOWLEDGE OF THE BIOLOGY OF THE SPECIES AT RISK

The known distribution of the target species, combined with its habitat requirements, must be used to determine if it is reasonable to assume that the target species occurs within the project study area. Knowledge of the biology of the target species is critical for sampling program design and for determining if the proposed activity may impact the species at risk.

Detailed information regarding the habitat requirements and life history of the target species at risk, including seasonal requirements, is essential in determining the timing, phasing, target habitats and methods of collection. COSEWIC status reports for individual species (http://www.cosewic.gc.ca), as well as recovery strategies if available (http://www.speciesatrisk.gc.ca), are good sources of species-specific information, and often provide a bibliography of additional references. DFO has a series of fact sheets for individual species at risk that can be obtained at local DFO offices. Regional texts, such as Scott and Crossman (1973), Trautman (1981), Becker (1983), Smith (1985), Coad *et al.* (1995), and Holm *et al.* (2008) can also provide very useful information. A search of the recent (post-status report or recovery plan) scientific literature should also be conducted. Relevant habitat and life history information must be included in the documentation of the sampling design.

5.2 DELINEATION OF THE STUDY AREA

The extent of impacts from the proposed project will delineate the area for which the presence or probable absence of a species at risk must be determined. In projects that only result in direct physical impacts, the study area may be limited to the project footprint. In projects that also result in indirect impacts, such as may result from changes in flow regime, water velocity, water quality, water temperature, etc., multiple habitat characteristics over large areas can be affected, and the study area must be sufficiently large to include the area where these potential impacts occur. Furthermore, the study area must include the areas affected by both permanent and temporary (i.e., during construction) impacts.

5.3 DESIGNING THE SAMPLING PROGRAM

5.3.1 Timing of field investigations

Seasonal use of potentially impacted habitat by species at risk must be considered. Sampling of specific habitats must coincide with the requirement of the target species at risk for that habitat. In some cases, there may be no seasonal variation in habitat use. To determine the appropriate timing for a field investigation, it is best to monitor the use of similar nearby habitats known to be utilized by the species at risk, taking into account possible temperature differences between stream reaches or watercourses. If no local or nearby populations of the target species at risk are available to help determine the timing of field work, then a thorough review of information from other locations, combined with knowledge of local water temperature, weather and flow conditions, must be used to estimate the appropriate timing of field work. Regardless of the quality of information used, a single site visit is generally not adequate to determine the absence of a species at risk, as slight shifts in environmental conditions can influence some activities such as spawning, or affect species distributions. Sensitivity to change in environmental and/or habitat conditions differs from species to species, and so the effort required to overcome the associated uncertainties must also be species-specific.

Many fishes engage in diurnal movements (Hynes 1970). Examples of fishes that move diurnally between shallow and deeper habitats are spottail shiner (*Notropis hudsonius*), mimic shiner (*Notropis volucellus*), and trout-perch (*Percopsis omiscomaycus*) (Scott and Crossman 1973). Where shallow habitats are present in the study area and the species in question can seek refuge in adjacent deep habitats that are difficult or impossible to sample, both day and night sampling may be necessary.

5.3.2 Sampling gear

A variety of fish sampling gears are available to collect fishes in a wide range of habitats. Table 1 presents a matrix of the list of species at risk found in Ontario, the types of aquatic habitats in which they are commonly encountered in Ontario, and sampling gears commonly used by investigators. *Net traps* refers to the family of similar gears that includes trap nets, fyke nets, hoop nets or others, but not minnow traps. *Portable electrofisher* refers to any electrofishing equipment that is used in wadeable waters, such as shore-based electrofishers, towed barge or punt electrofishers, and backpack electrofishers of various configurations. In all cases, the mesh size of all nets must be appropriate for the target fish species.

Any gear preferred by the investigator may be utilized if approved by the permitting agencies and stipulated in the collection licence. However, at the discretion of the responsible DFO personnel, a negative result (no catch of the targeted species at risk) will only be accepted as sufficient to demonstrate its probable absence if the appropriate gear(s) listed in Table 1 are employed, with sufficient effort (see below), under the direction of experienced personnel. A discussion of commonly used fish collection gears is available in Portt *et al.* (2006). Refer to Appendix A for a discussion of catchability.

5.3.3 Sampling Effort

An extensive sampling program is designed to determine whether or not a species is present within a study area and, if present, how widely it is distributed. For example, if a new dam was proposed that would alter habitat conditions over several kilometres of stream, then an extensive survey might be required to determine the probability that a species at risk was present and, if present, the extent of its distribution throughout the study area. If extensive sampling reveals that a species is present, intensive sampling may be required to provide additional information.

Intensive sampling programs are typically conducted for site-specific investigations or monitoring purposes, to determine the effectiveness of impact mitigation, or as part of CEAA reviews. For example, intensive sampling may be used to provide an index of abundance and/or habitat significance at a specific location, and may be repeated over a multi-season and/or multi-year time scale. Although not the focus of this document, some of the methods detailed here may be useful or necessary components of an intensive sampling program.

As stated previously, it is not possible to prove that a species is not present in a given area. The best that can be achieved is to demonstrate that there is a high probability that this is the case. As fishes are mobile creatures, a species may be present in a given area at some times and not at others. Table 2 provides recommendations for the amount of sampling effort required, as a minimum, to demonstrate that if no individuals of the species at risk are captured, there is a high probability that a species at risk is not present in the study area. Regardless of whether an intensive or extensive program is undertaken, if the species under investigation has specific and known habitat preferences, sampling should target those areas where the species is most likely to be present. For example, if a species is found only in riffles, then only the riffles in the prescribed area need be

Table 1. Sampling gear required to capture species at risk in different habitats.
Terminology defined on following page. Note: The list of fishes in Schedule 1 of SARA is revised on an on-going basis. Refer to the SARA Registry website (http://www.sararegistry.gc.ca) for the most recent Schedule 1 list. **Maximum gillnet mesh size (mesh perimeter) must not exceed the average adult girth of the target species, and more than two mesh sizes should be used. Maximum mesh size of ½ inch is suggested for active net gears, unless ¼ inch is stipulated.**

Scientific name	Common name	COSEWIC status	Lacustrine (<3m)	Lacustrine/ riverine (>3m)	Wetland	Riverine (1.5-3m)	Riverine (<1.5m low gradient)	Riverine (<1.5m pool/riffle)
Anguilla rostrata	American eel	SC	portable EF, boat EF, trapnet	trapnet	portable EF, boat EF, trapnet	boat EF, trapnet	portable EF, boat EF, trapnet	portable EF
Salvelinus fontinalis timagamiensis	Aurora trout	END	In a few known locations.	gillnet	na	na	na	na
Ictiobus cyprinellus	bigmouth buffalo	SC	net traps, boat EF	na	seine, boat EF, net traps	net traps, boat EF	seine, portable EF	na
Ictiobus niger	black buffalo	SC	net traps, boat EF	na	seine, boat EF, net traps	net traps, boat EF	seine, portable EF	na
Moxostoma duquesnei	black redhorse	THR	net traps, boat EF	na	na	net traps, boat EF	seine, portable EF	portable EF, seine(¼)
Coregonus nigripinnis	blackfin cisco	THR	na	gillnet	na	na	na	na
Fundulus notatus	blackstripe topminnow	SC	na	na	seine(¼), portable EF, net traps(¼)	na	seine(¼), portable EF	na
Notropis bifrenatus	bridle shiner	SC	seine(¼), portable EF, boat EF	na	seine(¼),boat EF, portable EF, net traps(¼)	seine(¼),boat EF	seine(¼), portable EF	na
Percina copelandi	channel darter	THR	seine(¼), portable EF	trawl(¼)	na	boat EF	seine(¼), portable EF	portable EF, seine
Ichthyomyzon castaneus	chestnut lamprey	SC	na	na	na	boat EF	seine(¼), portable EF	portable EF
Myoxocephalus thompsoni	deepwater sculpin	THR	na	gillnet, trawl, lighted/ baited traps	na	na	na	na
Ammocrypta pellucida	eastern sand darter	THR	seine(¼)	trawl(¼)	na	seine(¼)	seine(¼), portable EF	portable EF
Esox americanus	grass pickerel	SC	seine, portable EF, boat EF	na	seine, portable EF, boat EF, net traps	boat EF	seine, portable EF, boat EF	na

Scientific name	Common name	COSEWIC status	Lacustrine (<3m)	Lacustrine/ riverine (>3m)	Wetland	Riverine (1.5-3m)	Riverine (<1.5m low gradient)	Riverine (<1.5m pool/riffle)
vermiculatus								
Erimystax x-punctata	gravel chub	EXP	na	trawl(¼)	na	boat EF, seine(¼)	seine(¼), portable EF	seine(¼), portable EF
Coregonus kiyi	kiyi	SC	na	gillnet	na	na	na	na
Erimyzon sucetta	lake chubsucker	THR	seine, portable EF, boat EF	na	seine, portable EF, boat EF, net traps	na	seine, portable EF, boat EF	na
Noturus insignis	margined madtom	DD	seine, portable EF, boat EF	na	na	na	seine, portable EF, boat EF	portable EF
Ichthyomyzon fossor	northern brook lamprey	SC	na	na	na	na	seine, portable EF	portable EF
Noturus stigmosus	northern madtom	END	seine, portable EF, boat EF	trawl	na	net traps, boat EF	seine, portable EF, boat EF	portable EF
Polyodon spathula	paddlefish	EXP	na	gillnet	na	gillnet	na	na
Opsopoeodus emiliae	pugnose minnow	SC	seine(¼), portable EF	na	seine(¼), portable EF, net traps(¼)	na	seine(¼), portable EF	na
Notropis anogenus	pugnose shiner	END	seine(¼), portable EF	na	seine(¼), portable EF, net traps(¼)	na	seine(¼), portable EF	na
Clinostomus elongatus	redside dace	SC	na	na	na	na	seine(¼), portable EF	portable EF, seine(¼)
Moxostoma carinatum	river redhorse	SC	net traps, boat EF	gillnet	na	net traps, boat EF	seine, portable EF	portable EF
Coregonus zenithicus	shortjaw cisco	THR	na	gillnet	na	na	na	na
Coregonus reighardi	shortnose cisco	THR	na	gillnet	na	na	na	na
Macrhybopsis storeriana	silver chub	SC	net traps, boat EF	gillnet, trawl	na	net traps, boat EF	seine, portable EF	na
Notropis photogenis	silver shiner	SC	na	na	na	net traps(¼), boat EF	seine(¼), portable EF, boat EF	portable EF, seine(¼)
Lepisosteus oculatus	spotted gar	THR	boat EF	na	seine, portable EF, boat EF, net traps	net traps, boat EF	seine, portable EF, boat EF	na
Minytrema	spotted sucker	SC	seine, net traps,	na	seine, portable EF,	net traps, boat	seine, portable EF,	portable

Scientific name	Common name	COSEWIC status	Lacustrine (<3m)	Lacustrine/ riverine (>3m)	Wetland	Riverine (1.5-3m)	Riverine (<1.5m low gradient)	Riverine (<1.5m pool/riffle)
melanops			portable EF, boat EF		boat EF, net traps	EF	boat EF	EF, seine
Lepomis gulosus	warmouth	SC	na	na	seine, portable EF, boat EF, net traps	na	na	na

Table Notes and Definitions

- One or more of the fishing methods listed is required. Others may be used, but in the absence of a positive result, one of the listed methods must be used to consider the result valid.
- Mesh size is expressed in inches, as this is how nets are sold commercially.
- Net traps - includes all larger passive entrapment gears such as fyke nets, hoop nets, pound nets, but not minnow traps.
- Portable EF - includes all shallow water electrofishing methods such as backpack electrofishers, barge electrofishers, and shore units.
- Boat EF - refers to an electrofisher mounted on a powerboat.
- Where destructive sampling method is required (e.g., gillnet), a less destructive method must be attempted first (e.g., underwater video).
- Lacustrine (inshore) - from shore to a depth that can be fished by net traps, boat electrofisher, etc. (< 3 m.)
- Lacustrine/riverine (deepwater) - deeper than what can be fished with a net traps, boat electrofisher, etc. (> 3 m).
- Wetland - from shore to a depth that can be fished by net traps, boat electrofisher, etc.
- Riverine (moderately deep) - deeper than wadeable (1.5 - 3 m).
- Riverine (shallow low gradient, and pool/riffle) - wadeable (<1.5 m).
- EXP (Extirpated) - A wildlife species no longer existing in the wild in Canada, but occurring elsewhere.
- END (Endangered) - A wildlife species facing imminent extirpation or extinction.
- THR (Threatened) - A wildlife species likely to become endangered if limiting factors are not reversed.
- SC (Special Concern) - A wildlife species that may become a threatened or an endangered species because of a combination of biological characteristics and identified threats.
- DD (Data Deficient) - A wildlife species for which there is inadequate information to make a direct, or indirect, assessment of its risk of extinction.
- na - not applicable because the species is unlikely to occur in this habitat.

sampled. A summary of the scientific literature and background information utilized to develop these strategies is provided in Appendix A.

5.3.4 Documentation of Sampling

Proper documentation is an essential part of any fish sampling program, and it is particularly critical when the objective is to determine the likelihood that a species is present or absent from a location. An example of a field collection record is provided in Appendix C. At a minimum, the documentation should include the following:

- the names and contact information of the people who conducted the sampling, and their affiliation
- the date and time of the sampling
- a clear description and map of the sampling location(s), including the waterbody name (if named), latitude and longitude, and preferably photographs, as well as information on access where this may be difficult,
- a clear and comprehensive description of the gear(s) utilized and the sampling effort expended (see Portt *et al* (2006) for guidance).
- a characterization of the habitat sampled, particularly as it relates to the known habitat preferences of the target species and the sampling efficiency, preferably with photographs.
- documentation of all fish species captured and, preferably the number of individuals of each species (for abundant species an approximate number may be adequate). If reproduction is an issue, then documentation of the size or size range of individuals may be important.

Section 6 (below) provides guidance on the means of verifying the capture of species at risk.

6.0 IDENTIFICATION OF FISHES

In all fisheries work, whether it be a simple fish community survey, a monitoring program, a population estimate, or a fish behavioural study, the accurate identification of fishes is one of the most critical aspects of the endeavour. This is equally true in the search for species at risk, as the initial critical step in the protection of a species at risk population and its habitat is the verifiable identification of the species at risk from the fishes captured at a site. The considerable effort often required to determine the status of a species at risk within an area is wasted if the species at risk is misidentified or if, even though a species at risk was identified by a competent biologist, the identification cannot be verified with a voucher specimen or photographs if called into question.

Table 2. Preferred gears and minimum sampling effort required in order for failure to capture a species at risk to demonstrate that the species is probably not present in the study area.

Habitat	Gear[1]	Sampling distance/area
wadeable streams	backpack electrofisher	Greater of 250 m or 50 stream widths
	seine (where conditions suitable)	Greater of 250 m or 75 stream widths
non-wadeable rivers and streams	boat electrofisher	50 stream widths
nearshore or littoral habitats	backpack electrofisher	shoreline length of 50 times wadeable distance from shore
	seine (where conditions suitable)	shoreline length of 75 times wadeable distance from shore
	boat electrofisher	shoreline length of 50 times width of target depth-defined habitat, as measured perpendicular to shore
offshore shallow habitats (< 3 m)	multiple gears - trap nets, boat electrofisher, seine (from boat)	30 stations
wetlands	multiple gears - hoop nets, trap nets, seines.	50 sampling events/stations with appropriate gear(s)
deep habitats (lake or river)	video or other observation methods, gill nets, trawls, traps	Reasonable effort decided on a site-specific basis

[1] Conductivity must be considered when determining if electrofishing is a suitable method

The accurate identification of fishes is not easy, and overconfident field workers often miss rare species, or misidentify species because of the over-reliance upon one or two key identification features. The dichotomous keys that are part of any regional or national text of fishes, all require specialized tools that are unlikely to be available in the field such as microscopes, dissecting tools, and a variety of probes to aid in counting meristic features, or detecting morphometric features such as the presence of barbels or vomerine teeth. There are also critical points in any identification key that are often misinterpreted during the examination of certain species, leading to a misidentification. Furthermore, almost all keys have been developed using preserved museum specimens, and may rely on pigmentation or other markers that are not readily visible in live fishes.

There are two ways to identify a fish specimen to species. The traditional way is to preserve specimens of the fish, and include a waterproof label in the container that has, at a minimum, locality data (location description and geographical coordinates), date of collection, and the collector's name. The suspected species at risk is then submitted to a recognized expert for confirmation of identification. The second way is to adequately photograph and release the live fish, and send the photographs to a recognized expert for confirmation of identification. The specimen preservation method is by far the best, as it provides a voucher specimen that can be examined in any number of ways by experts. It also has a long history of establishing a species presence at a site, that is the practical basis of all museum collections upon which species distribution maps, natural history texts and studies are based. A properly labelled and preserved specimen is generally considered absolute proof that a species exists, or existed, at a particular location. Furthermore, preserving a specimen does not require much equipment and is essentially foolproof, since the act of positively identifying the specimen is deferred to when the specimen is delivered to an expert, or any number of experts. The one drawback is that some fishes are sacrificed. To minimize the number of specimens taken, it is important that someone who is capable of some level of fish identification be involved in the collection effort. This person can sort through the fishes captured and identify any suspected target species at risk, or minimally, be capable of recognizing how many species have been captured and preserve one adult specimen of each species. The taking of one specimen is inconsequential to a viable population.

If a specimen is kept for verification of species identity, it is important to preserve it in a way that is appropriate for the suspected species and life stage. All small juvenile fishes and all lampreys should be preserved in 70% ethanol. All other fishes can be preserved in 10% formaldehyde (10% concentration of the 37% formaldehyde solution that is sold commercially). A syringe should be used to inject preservative into the body cavity and bulky tissue areas of large fishes. To reduce the amount of pain, fishes should be sacrificed in an anaesthetic solution (e.g., sodium bicarbonate, tricaine methanesulfonate, clove oil) prior to preservation. For buffaloes (*Ictiobus* spp.), redhorses (*Moxostoma* spp.), ciscoes (*Coregonus* spp.) and deepwater sculpin (*Myoxocephalus thompsoni*), fin clips measuring 1 cm^2 must be preserved in 70% ethanol or dried and placed in scale envelopes for potential genetic analysis to confirm identification, if necessary, and for ongoing taxonomic studies.

Photography requires more time, equipment, and a certain amount of fish handling expertise and photographic ability. A camera capable of macro-photography must be available in the field and, in some cases, the fish must be anaesthetized to keep it still. The key identification characters differ from species to species and, therefore, the photographic views required also differ. The photographer must know what the key identification characters are for the species at risk so that they can be photographed, and the photographs must be of sufficient quality to allow someone else to positively identify the fish. Generally, it is easier to photograph large-bodied fish species. Photography to identify a species at risk has several drawbacks. It takes a higher level of identification expertise during the field collection of fishes, and a greater amount of time and expertise is required to anaesthetize and photograph the fish. If the photographs are inadequate, the species at risk will remain unidentified as there is no specimen to examine in greater detail. Finally, the fish may die during the increased handling and time required to obtain photographs, or after it is released. Sensitivity to handling varies from species to species. The only advantage with photography is the knowledge that an individual of a species at risk was not sacrificed, providing that it did not die during or after the process.

Table 3 provides guidance in regard to preserving and photographing specimens for confirmation of identification.

Table 3. Standards for the confirmation of species identification.

Common name	**Preserved specimen essential[1]**	**Photography recommended[2]**
American eel		Full side view.
Aurora trout		Full side view.
bigmouth buffalo	yes (juveniles)	Full side view showing fins and lateral line scales; close-up of dorsal fin and side view of head; close-up of mouth showing absence of barbels; side and ventral view of closed mouth showing lips.
black buffalo	yes (juveniles)	Full side view showing fins and lateral line scales; close-up of dorsal fin and side view of head; close-up of mouth showing absence of barbels; side and ventral view of closed mouth showing lips.
black redhorse	yes (juveniles)	Both sides of the caudal peduncle; dorsal and caudal fins spread out to see shape and colour; side view that shows each scale for a lateral line scale count; ventral view of closed mouth showing lips to see the traverse lines on the plicae.
blackfin cisco	yes	
blackstripe topminnow		Full side view showing fins; side view of head; close-up of mouth.
bridle shiner	yes	
channel darter		Full side view showing fins; close-up side view of head; close-up of mouth; downward frontal view of mouth showing protractile premaxillaries.
chestnut lamprey	yes	
deepwater sculpin	yes	
eastern sand darter		Full side view showing fins and side markings; close-up side view of head.
grass pickerel	yes	Full side view plus close-up side view of head showing suborbital bar and cheek scalation plus view of underside of bottom jaw showing sub-mandibular pores
gravel chub	yes	
kiyi	yes	
lake chubsucker	yes (juveniles)	Side view that shows each scale for a lateral scale count as well as fins and side pigmentation; close-up side view of head; ventral view of closed mouth showing lips.
margined madtom	yes	
northern brook lamprey	yes	
northern madtom	yes	
paddlefish		Full side view showing profile and fins; dorsal view showing snout and body shape.

pugnose minnow	yes	
pugnose shiner	yes	
redside dace	yes (juveniles)	Full side view showing fins and side colouration and markings; close-up side view of head.
river redhorse	yes (juveniles)	Both sides of the caudal peduncle; dorsal and caudal fins spread out to see shape and colour; side view that shows each scale for a lateral line scale count; ventral view of closed mouth showing lips to see the traverse lines on the plicae.
shortjaw cisco	yes	
shortnose cisco	yes	
silver chub	yes	
silver shiner	yes	
spotted gar	yes (juveniles)	Full side view showing fins, lateral scales, and side markings; full dorsal and ventral views showing body profiles and markings; close-up dorsal view of head; dorsal view between head and origin of dorsal fin to count mid-dorsal scales. Dry photographed area with cloth to show scales.
spotted sucker	yes (juveniles)	Full side view that shows each scale for a lateral line scale count as well as fins and side pigmentation; close-up side view of head; side and ventral view of closed mouth showing lips.
warmouth	yes (juveniles)	Full side view that shows body profile as well as fins and side pigmentation; close-up side view of head; close-up views of dorsal and anal fins.

[1]Preserved specimens are always recommended; however, this table provides guidance to allow confirmation of identification for some species using photography.

[2]All fin views assumed to be with spread or flared fins.

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APPENDIX A. SAMPLING CONSIDERATIONS FOR DETERMINING THE PRESENCE OR PROBABLE ABSENCE OF FISH SPECIES AT RISK

A.1 INTRODUCTION

While the presence of a species can be proven, absence never can. The most that can be achieved is to demonstrate that the presence of a species in a particular area is unlikely, given the failure of capture by a sampling program in which the probability of capture, had it been present, was high. The questions of what constitutes "adequate improbability" to assume absence, and how to achieve it, are important in the design of species at risk sampling programs.

The probability of capturing a species in a given sampling area (P_{capture}) is a function of the number of individuals present in the area sampled (n), the amount of sampling effort applied (e), and the efficiency of the sampling method, which is the same as the catchability of the species (q). Catchability varies among species and habitats, regardless of the sampling method used. Other things being equal, increasing one or more of n, e or q, should increase the probability of capture and, thus, reduce the likelihood that a null result is incorrect. In an investigation where an individual species at risk is the subject, methods can be employed that maximize the probability of catching that species only.

The following discussions of sampling gear and sampling effort draw primarily from studies on streams, but are broadly applicable to fish sampling in all habitat types.

A.2 SAMPLING GEAR

Catchability of a particular species can vary widely among gear types. Active gears are generally not as selective as passive gears because catchability in active gear is less affected by fish behaviour. Electrofishing and seines are the two active gears most often utilized in streams and nearshore lake habitats.

While electrofishing is less selective and more widely applicable than the other techniques for monitoring fish assemblages (Hughes *et al.* 2002), seines can also be used to collect fishes in shallow water (<1.5 m). Seines are much more variable in their capture efficiencies among species and habitat types and, overall, are often not as effective as electrofishers (Wiley and Tsai 1983; Parsley *et al.* 1989; Pierce *et al.* 1990; Poos *et al.* 2007). Benthic fishes are more likely to escape a seine than midwater and surface fishes, and coarse substrates provide more escape routes than smooth, fine-grained substrates (Parsley *et al.* 1989; Pierce *et al.* 1990). Poos *et al.* (2007) examined the effectiveness of bag seines and backpack electrofishers for sampling four species at risk (eastern sand darter, greenside darter, blackstripe topminnow and spotted sucker) in the Sydenham River, Ontario. They concluded that a backpack electrofisher was more effective at detecting the species at risk, detected higher relative abundances, and required fewer sampling stations to detect a species at risk in a watershed. Patton *et al.* (2000) compared seines to electrofishers in fine-substrate watercourses. Their results indicated that seining 200 m of stream was required to catch 90% of the species present, compared to 150 m for electrofishing. It was estimated that seining 300 m or electrofishing 200 m would result in 100% of the species present being captured. The watercourses in the study conducted by Patton *et al.* (2000) were particularly suited for seining, and the difference in efficiency between electrofishers and seines for sampling species richness would probably be much greater in streams with coarse substrates or greater structural habitat diversity.

Hughes *et al.* (2002) indicated that larger rivers are less effectively sampled by boat or raft electrofishers and, consequently, a greater relative length is required to determine species richness. The reduced sampling efficiency is due to factors such as greater depth, the presence of larger more mobile fishes, dangerous and inaccessible habitats, and sampling generally limited to nearshore areas. They estimated that sampling a stream length equivalent to 100 channel widths was required to yield an average of 85% of true species richness. Using the same dataset, Cao *et al.* (2001) calculated that an average of 214 channel widths (range=50-1027) would be required to yield 95% of true species richness, and that an average of 286 channel widths (range=70-1383) would be required to yield 100% of true species richness. Hughes *et al.* (2002) discussed the calculation undertaken by Cao *et al.* (2001) and suggested that 300 channel lengths should be used to determine species richness in large rivers.

Lapointe *et al.* (2006) examined the efficacy of different gear types in a portion of the offshore waters of the Detroit River, which was characterized as shallow (< 3 m), with substrates not particularly coarse, low flow velocity, and few snags. Of the six gears used, trap nets and minnow traps were deemed ineffective, while seine nets were the most effective, followed by hoop nets, boat electrofishing, and Windermere traps. They concluded that if assemblage data from similar ecosystems are required, and rare species will likely be removed from analysis, then a combination of seining and boat electrofishing was recommended. However, if synoptic surveys are the goal, adding passive gears would increase the number of species captured by targeting different (active and benthic) portions of the fish assemblage. Since their sampling sites had low or no flow velocity, these conclusions could also be applied to the littoral zone of lakes.

Surette (2006) collected fishes using five passive sampling gears (inshore and offshore hoop nets, minnow traps, Windermere traps, and trap nets), and two active sampling methods (bag and straight seines) from a large wetland in Point Pelee National Park on Lake Erie, Ontario. Of the gears employed, inshore and offshore hoop nets, trap nets, and bag seines were the most effective. Minnow traps were inefficient. All species, other than spottail shiner, which were very uncommon in Point Pelee, were caught by the inshore hoop nets. Inshore hoop nets also caught the greatest length range of fishes. Hoop nets were the only sampling gear that could be used effectively in both wadeable and non-wadeable situations.

In our experience, seines have an advantage over electrofishers where substrates are fine, the bottom profile is not too rough, there are few obstructions, and the water is turbid. Under turbid conditions, fishes are more easily captured by seine since they do not observe the seine and, thus, do not become agitated and attempt escape. In contrast, electrofishing in highly turbid conditions is not very efficient, because it is difficult to observe stunned fish unless they break the water surface and, consequently, netting efficiency is low.

A.3 SAMPLING EFFORT

The number of individuals present in the sampling area is, of course, unknown and outside of the control of the investigators. If the species is present in the area of investigation, and evenly or randomly distributed, then increasing the total area sampled will increase the total number of individuals susceptible to capture and, other things being equal, the probability of capture ($P_{capture}$). If the distribution of the species is expected to be patchy, and the patches where there is a higher probability of the species being present can be identified, then those are the areas that sampling should target. Sampling habitats where the species in question is unlikely to be present, even if it does occur in the larger area of interest, has little effect on $P_{capture}$ and, assuming the amount of effort that can be expended is finite, may actually decrease $P_{capture}$. For example, if a species is known to occur exclusively in riffles, sampling pool habitat is an inefficient use of limited time and resources.

The question that arises is 'How much area must be sampled without catching an individual in order to be reasonably confident that a particular species does not occur there?'. Some insight can be gained from studies that have attempted to determine how much sampling effort is required to determine species richness. In studies examining species richness, however, the sampling objective is to capture all, or a high percentage of, the species present. Thus the sampling methods must address all species and the determination of necessary effort is driven by the species with the lowest $P_{capture}$. Low Pcapture may be due to low abundance, low catchability, or both. Species at risk do not necessarily fit this profile. They can be locally abundant and, where they are present, easily captured, hence, the importance of targeting appropriate habitats when these can be determined. Nonetheless, the results of species richness studies provide some insight into sampling requirements when species at risk are present at low densities and difficult to capture, and some of these are reviewed, by habitat, in the sections that follow.

A.3.1 Rivers and Streams

The relationship between area or distance sampled and number of species, or proportion of the total number of species captured has been the focus of numerous studies in streams (Lyons 1992; Angermeier and Smogor 1995; Paller 1995; Patton *et al.* 2000; Cao *et al.* 2001; Hughes *et al.* 2002; Dauwalter and Pert 2003; Reynolds *et al.* 2003) and in small lakes (Jackson and Harvey 1997; Fago 1998). The applicability of some of these studies is questionable since the number of species is often much higher than the number that are present in Canadian watercourses, which influences the effort required to capture all species. Of the studies reviewed, a study in Wisconsin (Lyons 1992) and two studies of eastern streams located farther south (Angermeier and Smogor 1995; Dauwalter and Pert 2003) were considered most applicable because the habitat and surrounding land use were similar to those of streams in southern Ontario, although species diversity was generally higher. Only one published study was found that examined the effort required to sample larger rivers (Hughes *et al.* 2002). This study was conducted on Oregon rivers.

Lyons (1992) sampled ten reaches in nine different wadeable watercourses, ranging in mean width from 4.9 to 17.2 m. Electrofishing occurred between August and October under base flow conditions. All of the streams had a wide range of gradients in watersheds with a variety of land uses. All of the streams had relatively diverse warmwater fish communities, dominated by cyprinids, catostomids, ictalurids, centrarchids and percids, with numbers of species similar to what is commonly found in southern Ontario warmwater streams. The results of this study found that a single electrofishing pass was required over a stream length equal to 5.4 to 48.8 stream widths, with a mean of 32.3 stream widths, to capture 95% of the species present. Lyons (1992) recommended that a minimum length of watercourse equal to 35 stream widths be used in studies of species richness in wadeable streams. Angermeier and Smogor (1995) found that a distance equal to 45 to 90 stream widths was required in a wadeable stream to capture 95% of species in two passes with an electric seine. Dauwalter and Pert (2003) found that species richness had not reached an asymptote after sampling 75 stream widths in 11 of 15 Ozark highland streams sampled. On average, a distance of 54 stream widths was required to sample 95% of empirical species richness (total number of species actually captured by sampling a reach 75 stream widths long), while it was predicted that 102 stream widths were needed to obtain theoretical species richness (predicted total number of species present).

As reported above, Poos *et al.* (2007) found backpack electrofishing to be more effective than seining with a bag seine in capturing species at risk in the Sydenham River, Ontario. They electrofished in an upstream direction at a rate of 1 m^2 per 5 s. Sampling sites were stream reaches comprised of a pool:riffle sequence or, where there were no clearly defined pool:riffle sequences, a 60 m length of stream. Sites ranged from 208 m^2 to 1954 m^2 (mean=722 m^2) in area. Each site was subdivided into 10 subsections of equal length which were sampled sequentially. The number of sites and total area required to initially detect four at-risk species (the greenside darter is no longer considered at-risk) are presented in Table A1.

Table A1. Total area and number of sites required to initially detect each of four at-risk fish species in the Sydenham River, Ontario, with 95% probability using electrofishing and seining (Poos *et al.* 2007).

	ELECTROFISHING		**SEINING**	
	Sites (n)	**Area** (m^2)	**Sites** (n)	**Area** (m^2)
eastern sand darter	24	26,457	38	57,485
greenside darter	3	2,474	5	4,164
blackstripe topminnow	8	4,619	8	5,257
spotted sucker	19	13,273	nc*	nc*

*no individuals collected

Usually, investigators attempting to determine species richness will advocate sampling more area because the probability of encountering a new species is greater from increasing the area sampled than from re-sampling the same area with multiple electrofishing passes or seine hauls. This is true, in part, because increasing the area sampled increases the probability of encountering a new habitat. Also, given the same amount of resources, the probability of capturing species that are present at low densities usually increases as a result of increasing sampling area than by re-sampling the same area. For species at risk studies, however, the pertinent question is not how many species are present, but whether or not a particular species is present. Therefore, if the area of investigation is small, or if the amount of habitat that is suitable for the species in question is small, re-sampling may be appropriate.

In wadeable streams, a single electrofishing pass is nearly always adequate to determine the presence of abundant species, but the question arises as to how much effort is required to determine the presence of species that are not abundant. Meador *et al.* (2003) found that basin species richness estimates based on the first of two electrofishing passes were 80.7% to 100% of the estimated species richness based on two electrofishing passes when at least seven sites per basin were sampled. For individual sampling sites, however, additional species unique to the second pass were captured in 50.3% of the 183 samples.

The authors of this report determined the number of passes required to capture at least one specimen of species that were present in low densities, using data from Portt (1979), Mahon (1980), and Simonson and Lyons (1995). Mahon (1980) made six to eight electrofishing passes through six reaches of southern Ontario streams that had been isolated with blocking nets, and then treated the reaches with rotenone to collect any remaining fishes. Portt (1979) made seven to nine passes through four sections of southern Ontario streams that were isolated with blocking nets. Both used DC current produced by an electrofisher that was powered by a stream-side generator. Simonson and Lyons (1995) made three or four passes through nine sites using a DC tow-barge electrofisher, where a pass was a shocking run from the downstream blocking net to the upstream blocking net and back to the downstream net. The data for species of which ten or fewer individuals were captured at a site were examined. Mahon (1980) reported four cases of a species being collected following the application of rotenone that had not been captured by electrofishing. For the other 26 cases in which less than ten specimens of a species were captured at a site, at least one specimen had been captured by the third electrofishing pass (Figure A1). Portt (1979) reported that, in one case, the first capture of a species occurred during the sixth pass. In the remaining cases, the first specimen was captured during the first (nine cases) or second pass (three cases)(Figure A1). Simonson and Lyons (1995) reported 38 species for which a total of ten or fewer individuals were captured by three or four passes at a site. In 36 of these cases, at least one individual of the species was captured in the first pass. In one case, the first individual was captured in the second pass and, in the other, it was captured in the third pass.

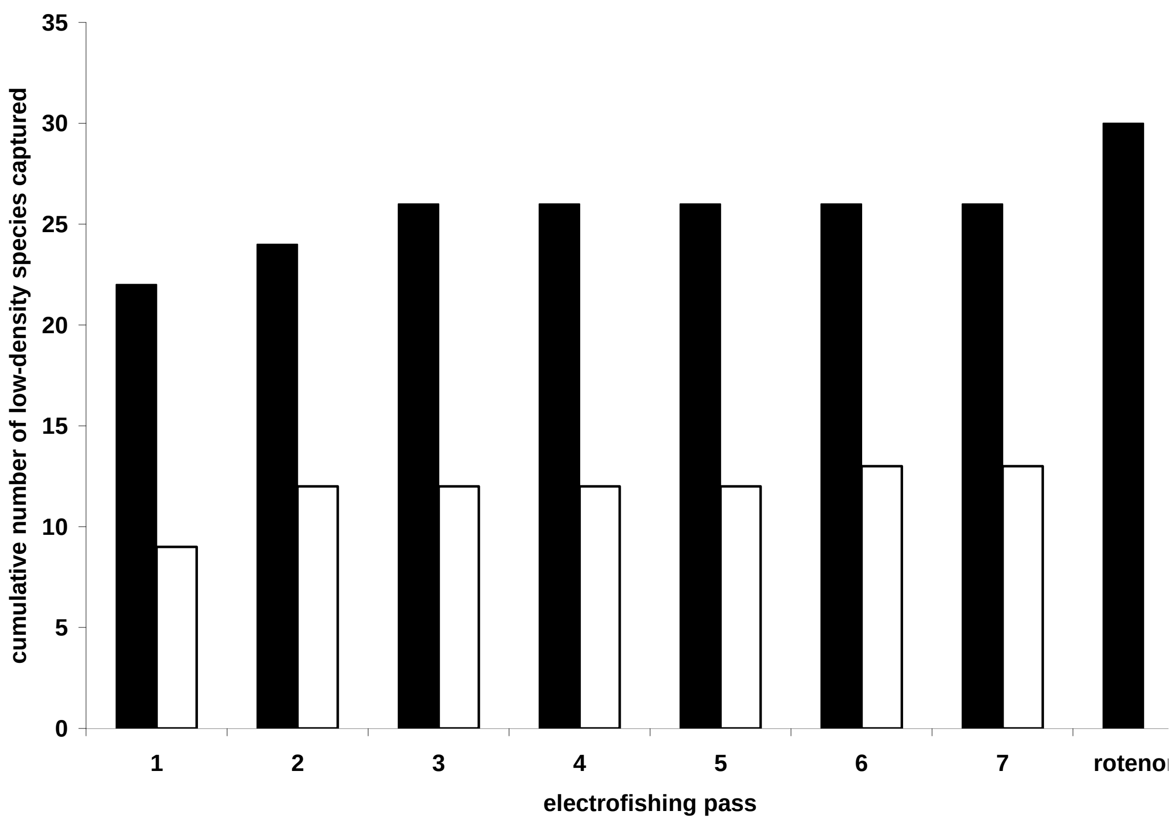

Figure A1. Cumulative number of low-density species captured after successive electrofishing passes (Portt 1979; 4 sites combined, open bars), and successive electrofishing passes followed by rotenone application (Mahon 1980; six sites combined, solid bars). Only data for species of which ten or fewer individuals were captured by 7 – 9 electrofishing passes are included. More abundant species were all captured during the first pass.

Based on the data from Portt (1979), Mahon (1980), and Simonson and Lyons (1995), it appears that in relatively small, wadeable southern Ontario or Wisconsin streams, most species present are captured in the first electrofishing pass and two or three electrofishing passes will result in the capture of nearly all of the species present. Where catchability is lower, as may be the case in habitats that are more difficult to sample, additional passes may be warranted; however, catchability often declines in successive electrofishing passes, so that sampling again on a different occasion is likely to be more effective than making many sampling passes in quick succession.

Paller (1995) made seven electrofishing passes through 25 reaches of streams in South Carolina. He continued to capture new species at some sites after each pass. The number of new species averaged less than 1 species per site after the third pass, and less than 0.5 species per site after the fourth pass (Figure A2). Nonetheless, on average, two more species were captured per site by seven electrofishing passes than by four passes. The differences between the results of Portt (1979) and Mahon (1980) and those of Paller (1995) may be due to the larger number of species present at the South Carolina sites, and the larger size of the sites themselves.

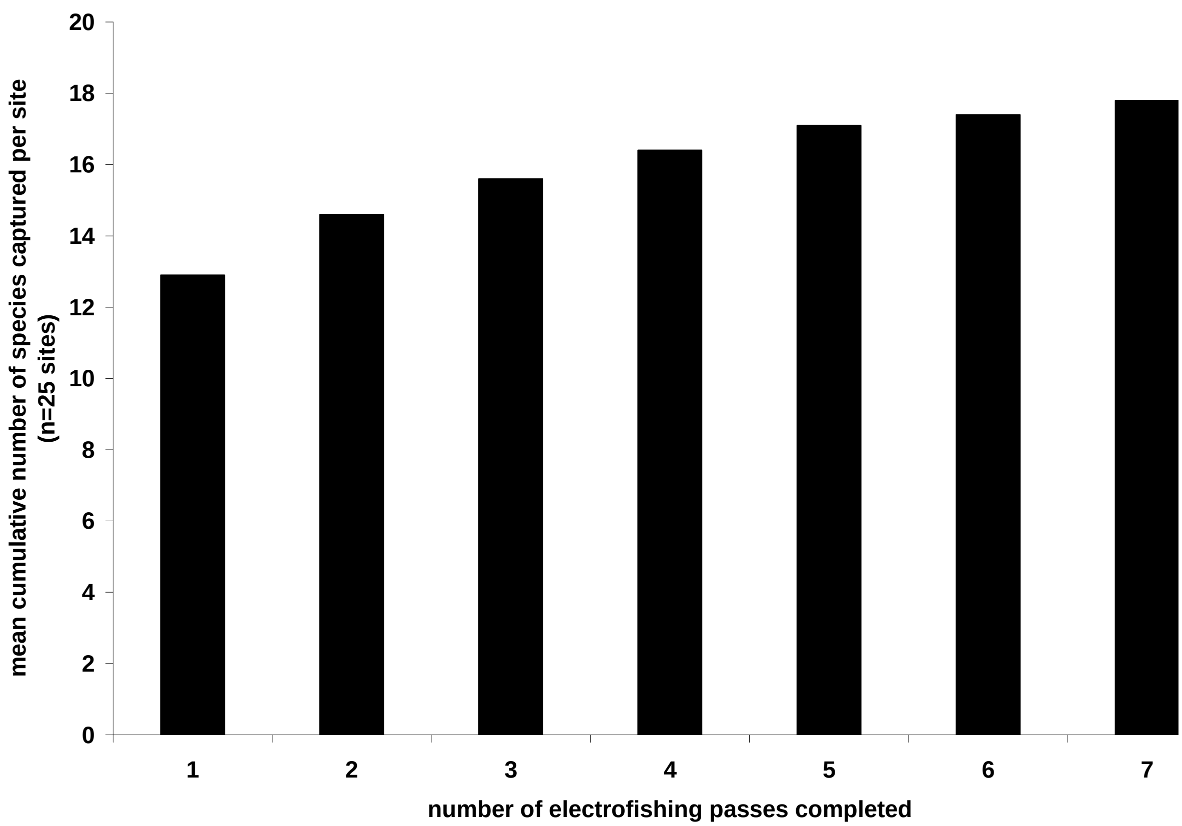

Figure A2. Mean cumulative number of species captured after each of seven successive electrofishing passes at 25 sites in South Carolina coastal streams. Data from Paller (1995).

A.3.2 Watershed scale investigations

Smith (2003) undertook a study to: (1) evaluate species lists derived from existing survey data; (2) determine the sampling effort needed to determine a target percentage of estimated species richness, and to determine if environmental factors, such as watershed size, influence sampling effort requirements; and (3) develop a protocol for the efficient assessment of species composition in Great Lakes watersheds by examining the allocation of effort within a watershed or stream. Her study was based on nine Great Lakes tributary stream systems that differed in location within the basin, physical characteristics and fish assemblage types. Watershed area ranged from 23.6 km^2 to 432.8 km^2, and streams were third to fifth order. Statistical analyses indicated that watershed size (km^2), estimated species richness, and the number of uncommon species did not apparently affect sampling effort requirements.

To determine sampling locations, Smith (2003) grouped stretches of stream by order and partitioned long stretches so that they were not under-represented in the selection process. Stretches of watercourse were randomly selected from each stream-order group, with equal sampling effort allocated to each group. To minimize unnecessary effort, the sampling location within each chosen stretch of watercourse was located at, or near, a direct access point, but the access point was randomly selected from all possible access points within that stretch. One electrofishing pass was made without block nets at each sampling location, over a stream length of 30 mean watercourse widths. A three-person field crew conducted sampling in a upstream direction, moving back and forth across the river while moving the anode in a continuous 'm' pattern with effort made to cover the entire stream reach area. Sampling effort was focused in areas where fishes were most likely to be found (e.g., around large woody debris) and moved quickly through unproductive areas (e.g., sandy open areas).

Smith (2003) estimated that between 9 and 25 sampling sites, with an average of 15.1 sites, were needed to detect 80% of the species estimated to exist within a watershed. To detect 90% of the species in a watershed, the estimate ranged from 17 to 49 sites, with an average of 30 sites. Between 30 and 98 sites, with an average 49 sites, were required to find 95% of the species in a watershed. It was estimated that, to detect 100% of species present, between 76 and 151 sites, with an average of 119, should be sampled.

A.3.3 Lake shorelines

Fish community sampling of lakes can be undertaken effectively only if multiple gear types are employed (Weaver *et al.* 1993; Jackson and Harvey 1997; Fago 1998; Vaux *et al.* 2000) with electrofishing being an essential component of any lake survey (Fago 1998; Vaux *et al.* 2000). Vaux *et al.* (2000) found that electrofishing alone, even utilizing a backpack electrofisher mounted on a small boat, captured a greater number of species than other gear types.

Fago (1998) compared mini-fyke nets with a combination of small-mesh seines and electrofishing in their ability to describe littoral fish assemblages in 19 Wisconsin lakes (110-2454 ha), evaluating his findings against known fish assemblages compiled from multiple lake surveys since 1970. Multiple sites, randomly selected, were sampled in each lake. At each location, a 15 m block net was used to isolate the sample area, following the 1 m depth contour along the offshore side, and this was sampled by two passes with a backpack electrofisher and finally a seine was pulled once through the blocked area. A fyke net, set perpendicular to shore with the lead staked at the shoreline, was fished for one approximately 24 hour period during the following week. Analysis of the results found that 18 or more locations, fished with all three gear types, were necessary to characterize the littoral fish assemblage of a lake.

King and Portt (1990) examined the increase in the total number of ‘pseudospecies’ captured (young-of-the-year fish and older fish of the same species were considered to be separate pseudospecies) in multiple seine hauls at the same locations in Penetanguishene Harbour, Georgian Bay. Three consecutive hauls were made with a 2 m high by 16 m long bag seine with 3 mm mesh at ten locations on each of four occasions, approximately two weeks apart. The mean number of pseudospecies captured in a single seine haul was five (range 0 – 12), the mean number of pseudospecies captured in two seine hauls combined was 7 (range 1 – 13), and the mean number of pseudospecies captured in three seine hauls combined was eight (range 2 – 15). From one to four additional pseudospecies were captured by a second seine haul and from zero to three additional pseudospecies were captured by a third seine haul. The increases would have been slightly less if young-of-the-year and adults were not treated separately, but it is clear that multiple seine hauls are necessary to capture all of the species present at a site.

A.3.4 Wetlands

Surette (2006) used inshore and offshore hoop nets, trap nets, bag and straight seines, and minnow traps to capture fish in a large (1113 ha) coastal wetland on Lake Erie. Five species at risk were captured, and, of these, four were quite rare: spotted gar, grass pickerel, lake chubsucker and bigmouth buffalo. The warmouth was relatively common. The catch data indicated differences in gear susceptibility among species (Table A2). Bigmouth buffalo were only captured in inshore hoop nets and trap nets, whereas all of the other at-risk species were also captured by seining. Species accumulation curves indicated that 157 sampling events would be required to detect 95% of the fish assemblage using inshore and offshore hoop nets, trap nets, and bag seines. Surette (2006) did not estimate how many samples would be required to capture individual species, but 13 bag seine samples were sufficient to collect three of the at-risk species in 2002 (spotted gar, grass pickerel and warmouth) and fifty bag seine samples detected all of the at-risk species except bigmouth buffalo in 2003. In 2002, 26 inshore hoop net sets were sufficient to detect all of the at-risk species except lake chubsucker (Table A2).

Table A2. Number of individuals of at-risk fish species captured and total effort with a variety of gears during two years in the Point Pelee, Ontario, wetland on Lake Erie (from Surette 2006).

	spotted gar	grass pickerel	bigmouth buffalo	warmouth	lake chubsucker	# sampling events
2002						
inshore hoop net	2	1	2	123	0	26
offshore hoop net	1	0	0	64	0	22
trap net	3	0	1	2	0	18
bag seine	1	1	0	7	0	13
straight seine	0	5	0	0	0	17
minnow trap	0	0	0	55	0	10
Windermere trap	1	2	0	103	0	20
2003						
inshore hoop net	1	3	3	257	20	169
offshore hoop net	0	0	0	34	1	52
trap net	1	0	7	1	0	15
bag seine	1	8	0	11	4	50

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APPENDIX B. EXAMPLE OF A COMPLETED APPLICATION FORM FOR A SARA PERMIT

Fisheries and Oceans Canada   Pêches et Océans Canada

Application for a Species at Risk Permit
Scientific Research/Education
Fisheries & Oceans Canada

1. Applicant Information:

Name:	John Doe
Organization:	Seaport and Company
Address: Street:	100 Main Street
City:	Ottawa
Province/State:	*Ontario*
Country:	*Canada* Postal Code/Zip: *Y0Y 0Y0*
Phone:	*999-999-9999* Cellular: Fax:
Email:	*jdoe@SandC.com*

Applicant Experience/Credentials *National Museum Div. of Fish (1985-1991); DFO research 1991-1992;*

Generic Inc. consulting fisheries biologist (1992-1997); Seaport and Company, consulting fisheries biologist

(1997 to present). BSc Guelph University. I have many years of experience collecting and identifying fishes, and know what is required to reduce the impact of collection and handling methods upon many species of fish, and to ensure their survival. I am presently working on a "SAR Recovery Plan" for two species of fish in the Ottawa River. I have taken the ROM fish id and fish SAR id courses.

2. Logistics of Proposed Research:

A Lead Investigator	*John Doe*
B Other researchers	*R. Bedsprings, Jim Kirk*
C Vessel / Platform Name:	*work is based from shore. No vessel required.*
CFV/Registration #:	
Country of Registration:	
D Locations and dates where research will be done	*Exactly 2.5 km of the Anyname River downstream of the Fish Dam. Sampling will be conducted on three occasions between mid May and Late September.*

E SARA Species to be included in	**Anticipated # of mortalities for each species**

Permit *eastern sand darter* <u>*Ammocrypta pellucida*</u>	none

3.	**Description of Proposed Research and Potential Impacts on SARA listed species:**
A	Objective/Purpose of Research
	Monitoring presence and number of eastern sand darters in the nearshore areas of a section of the Anyname River, to determine the outcome of habitat compensation and mitigation measures that were constructed/initiated to protect the habitat requirements of a local population of eastern sand darters. This field program monitors eastern sand darter presence in a constructed habitat adjacent to the Fish Dam GS tailrace, at a pre-existing spawning area immediately downstream of the tailrace, and at nearshore areas for 2.5 km downstream. This monitoring is a condition of approval for the DFO authorization of the construction of the Fish GS.
B	Briefly explain field collections/study techniques
	Pre-determined areas are electrofished with a backpack unit (Smith-Root Model 12). All fish are identified and counted, and released alive at the point of capture. Eastern sand darter capture locations are measured for substrate type, water depth, and flow velocity. An annual report is submitted to the Burlington, Ontario, DFO office. All SAR will be photographed and released.
C	Describe anticipated or potential disturbances to each of the SARA-listed species in **2E,** include impacts on habitat(s) used by the species: List the species, the nature of harm, and the likelihood of harm or encounters (High, Medium, Low)
	No impacts to habitat are anticipated, other than the harm done by wading in the nearshore habitats. We believe the amount of harm done by wading is "Low" (negligible). *Electrofishing can potentially damage individual eastern sand darters. In 2005 we held the eastern sand darters in a bucket for approximately 1 hour after collection. All of them appeared normally active when they were released. We therefore believe that electrofishing using the appropriate unit settings poses a "Low" risk in causing individual harm to eastern sand darters.*
NOTE: Please attach the project workplan/proposal to this application	

4.	**Following from the criteria in Section 73 of SARA ; if impacts on a listed species are likely, the proponent should specify:**
A	What alternatives to the proposed method of conducting the activity have you considered? How is the chosen method the best solution to reduce impact to the species?
	The monitoring methods were established in 2002 by a University research group who were investigating eastern sand darter population trends at this location. We have simply replicated these methods to provide comparable data through the remaining pre-construction phase, and the construction phase of the GS expansion project. This year we are starting the post-construction phase of the monitoring project, and wish to continue with the existing sampling regime to provide comparable post-construction data. *The only alternative method for collecting eastern sand darters is seining, or possibly some kind of trap, however, electrofishing has not been shown to significantly harm the eastern sand darters being studied. A change in methods at this point in the monitoring program would compromise the integrity of data collected.*
B	What mitigation measures have been included, and how do they minimize the potential impacts on listed species and/or habitats? What mitigation measures have been considered and not included, and for what reasons were they rejected?
	Exposure time to the electric field is minimized. If an individual fish is not stunned and netted immediately (e.g. if it evades capture by diving into a pile of rocks and is not extricated within a couple of seconds) we break-off pursuit so that we do not unduly stress it. *All fish will be released alive.*
C	Will the sampling program jeopardize survival or recovery of the species, in light of responses to 4A and 4B above? If not, why not?
	No. Eastern sand darters remain common in the study area, and the sampling area is only a small portion of the potential habitat for this species. Furthermore, the electrofishing method used has not been shown to be detrimental to this species' survival.

Please send your completed application to the relevant DFO Regional office:
http://www.dfo-mpo.gc.ca/species-especes/permits/saraapplication_e.asp

APPENDIX C. EXAMPLE OF A FIELD COLLECTION FORM

Collection of Fishes Data Sheet
Fish Species at Risk, GLLFAS, DFO, Burlington, ON

Project Name			
Project Number		**Site Number**	

Project Resample: Yes ☐ No ☐ Historical Resample: Yes ☐ No ☐

Date:		Waterbody Name:	
Arrival Time:	____:____ (24hr)	Departure Time:	____:____ (24hr)

Collectors:	**Recorder:**

Narrative Locality:

Sampling Start Time ____ : ____ (24hr)	Sampling Stop Time ____ : ____ (24hr)

Start Location		Stop Location	
Latitude	(dd.ddddd°)	Latitude	(dd.ddddd°)
Longitude	- (dd.ddddd°)	Longitude	- (dd.ddddd°)

Sampling Method

Gear Type:	Effort: (hours / min / sec)
Electrofishing Settings	

Site Characteristics

Water Quality

Air Temp: (°C)	Water Temp: (°C)	Conductivity: (µS/cm)
D.O. (ppm)	pH:	Secchi Disc: (0.00m)

Substrate Components (Total = 100%)

Type	%	Type	%	Type	%	Type	%

Site Dimensions

Stream Width (m)	MAX Stream Depth (m)	Distance from Shore (m)	MAX Depth Sampled (m)
Riffle / Run / Pool		None / Slow / Medium / Fast	

Biological Components

	Type 1	%	Type 2	%	Type 3	%	Type 4	%
Aquatic Vegetation								
Riparian Vegetation								
Aquatic Animals								

Floodplain Components

Floodplain Use:	Bank (% slope):	Channel Cover (%):

Weather Conditions

				Wind Speed	Wind Direction
Weather Notes:					

Final Notes:

Voucher Label Completed: Yes ☐ No ☐ Field Sheet Completed: Yes ☐ No ☐

SITE MAP

Voucher Label

Latitude (dd.ddddd°)	Longitude: - (dd.ddddd°)	
Project Name:		Project Code:
Description:		Collectors:

Species	Min (mm)	Max (mm)	# Captured	# Kept

Notes: