Chapter 6
Recovery of Freshwater Aquatic Macrophytes After Exposure to Herbicides and the Implications for Ecological Risk Assessment

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Abstract Ecosystem recovery following natural disturbances is a ubiquitous and well-understood process. Freshwater macrophytes are able to colonize areas in which they have been extirpated through a number of mechanisms. Herbicides, which are widely used in agriculture globally, may pose a threat to non-target freshwater plants and result in individual-, population-, or community-level impairment of plant structure and function. The same mechanisms that allow for recovery of plants from non-anthropogenic stressors apply to impacts as a result of exposure to herbicides. Current ecological risk assessment (ERAs) frameworks for herbicide registration focus primarily on characterizing toxicity, and do not explicitly require data that allow for the understanding of potential recovery of plants following effect. There is disagreement on how recovery should be incorporated into ERA's for pesticides, and currently, there are no regulatory guidelines that provide standardized methods for plants. Numerous studies have characterized the effects of herbicides and the ability of macrophytes to recover following the cessation of exposure to plant protection products. A critical review of the peer-reviewed literature on the availability and quality of evidence for recovery of macrophytes exposed to herbicides was performed. A total of 25 recovery studies published between 1986 and 2019 were assessed. The relevance of endpoint and strength of methods for the recovery studies were evaluated with a scoring rubric based on three main categories: (1) test substance; (2) test organism and experimental system; and (3) test design, statistics, and results. Ecological relevance of endpoints was based on the association of reported endpoint to the population and community levels of effect. A total of 21 test species had been evaluated for 33 different herbicides. The most tested herbicide group was photosystem II inhibitors at 38% of studies. In total, 86% of studies reported clear evidence of recovery after transfer to clean media. Around 36% and 44% of tests from exposure and recovery phases, respectively, scored >50%

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on both strength of methods and endpoint relevance scores, which was the threshold for advising a study be used in ecological risk assessment. Laboratory studies in general may underestimate the potential for recovery as external mechanisms are fully excluded. Overall, we recommend that standard laboratory guidelines for the assessment of recovery in macrophytes be developed to improve the strength of methods and encourage improved reporting of toxicity data, and ultimately more formal inclusion in ecological risk assessment.

6.1 Introduction

Recovery of ecosystem structure and function can occur following natural disturbances, such as fires, flooding, and drought, and reflects the innate capacity of ecological systems to return through succession to previous or new stable states. The underlying mechanisms and processes driving ecosystem recovery will be the same for anthropogenic stressors, including chemical contaminants such as pesticides. The primary differences between natural disturbances and those driven by pesticides are typically the degree of impairment and the selectivity of that impairment. For example, fires tend to extirpate all extant species from the area in which the event occurs, while with pesticides, the removal of all non-target species off-field as a result application is unlikely, and typically only certain species classes are impacted due to compound mode of action.

Of the pesticides, herbicides are the most widely used class of pesticide globally in agriculture, have been commonly observed in surface waters following translocation off-field, and have modes of action that target plants explicitly, including aquatic macrophytes. Therefore, as herbicide exposure in freshwater ecosystems may cause impacts on macrophyte populations and communities, understanding how and if recovery can occur following such changes is important in characterizing fully the risk posed by plant protection products. The inability to recover from herbicide exposure represents a greater risk overall relative to scenarios where recovery is possible. This chapter will outline the concept of recovery in ecotoxicology, including:

- what recovery means for macrophytes;
- approaches by which macrophyte recovery can be assessed;
- inclusion of macrophyte recovery in ecological risk assessment; and
- a review of the current state of knowledge and evidence for recovery in macrophytes exposed to herbicides.

Finally, we will make recommendations for more effective inclusion of recovery for macrophytes in the ecological risk assessment of herbicides.

6.2 Concept of Recovery and Ecotoxicology

Recovery from natural disturbances as an ecological concept has been examined extensively, and the possible mechanisms by which an individual species may initially colonize or recolonize a habitat have been well characterized (Niemi et al. 1990). Ecological recovery extends beyond structural attributes, such as species abundance and richness, to functional elements such as overall biomass or nutrient cycling. The process of recovery, whether in terms of species structure or function, is limited to a few main drivers, most of which are heavily influenced by basic life history traits (e.g., reproductive strategies and fecundity), as well as inherent mobility and capacity for dispersal, coupled with the degree of isolation of the impacted ecosystem from unimpaired populations. Species have evolved a variety of strategies to survive transiently in unfavorable conditions such as temperature changes, shading, oxygen depletion, resource bottlenecks, and droughts (Ellis 1989). In turn, ecosystems can typically exist in several alternative stable states whereby each is characterized by different structural and functional parameters of the species that are found there at any one moment in time. When an impact occurs, the shift in structure and function can be ephemeral and followed by a return to the original state (O'Neill 1998). However, recovery may occur but to a "different" ecosystem, one that is permanently displaced, with a different structural and functional attributes, and reflect a new steady-state (Holling 1973; Scheffer et al. 2003).

As noted above, species and ecosystems all have some innate capacity to withstand and recover from disturbances, whether periodic (e.g., regional seasonal changes) or stochastic (e.g., burn events, flooding, droughts, pest and disease outbreaks). The concept of functional redundancy helps explain the stability of ecosystem processes in the face of stressors and, in part, why recovery of populations following a disturbance is possible. Functional redundancy states that a decrease in biodiversity (e.g., the loss of species) can be endured to a threshold, as long as key species and their functions are not adversely affected. Most ecosystems exhibit functional redundancy, where multiple species are able to perform and contribute to some functional attribute of the system as a whole (Walker 1992, 1995). For example, manipulation of plant communities in grassland ecosystems showed that community function, such as nutrient cycling, was stable despite the loss of significant numbers (>50%) of species (Tilman 1996). This is possible because of the redundancy in roles and functions provided by surviving species in the impacted ecosystem, allowing key biotic and abiotic needs to remain available (e.g., soil nutrients, structure, moisture) for extirpated species to successfully recolonize (Lawton 1994). These observations in support of the concept of functional redundancy underpin the idea in ecotoxicological risk assessment that some effects at the organism and population level can be allowed, provided that these effects are constrained temporally and spatially (Barnthouse 2004).

It is important to distinguish at which level of biological organization recovery is being investigated (e.g., from the molecular and physiological to the ecosystem level). From an ecological context, most studies have focused on populations and communities, as well as functional attributes, while for ecotoxicology, recovery can be and has been a phenomenon examined and observed from the molecular level (e.g., binding site) and upwards (Brock et al. 2015, 2018). Recovery of a population or community from contaminant exposure will adhere to the same mechanisms as for a natural stressor. These can be broadly categorized as internal or external mechanisms (e.g., from within or outside the disturbed ecosystem) (Caquet et al. 2007; Hanson et al. 2007). For example, internal and external recovery can be through recuperation of impaired organisms after exposure, or immigration of new individuals from other uncontaminated areas, respectively (Barnthouse 2004; Brock et al. 2018). The degree and time course for recovery will be highly context-dependent, varying by species, life stage, severity and duration of effect or exposure, time between or frequency of events, the type of impairment, and the degree of ecological isolation (Barnthouse 2004). Recovery tends to be most rapid at the lower levels of biological organization, where repair and a return to normal function can occur on the order of seconds to minutes (e.g., gene expression, enzyme activity), relative to ecosystem process. Effects that are spatially and temporally confined may be viewed as ecologically unimportant and/or fall within the natural variability of impacted populations (Domsch et al. 1983). In ecotoxicology, the definition of recovery has typically remained fairly straightforward, in that recovery, regardless of the level of biological organization, is said to have occurred once the element under question is no longer statistically different from an undisturbed or previous state (Brock et al. 2015, 2018; Hanson et al. 2007; Caquet et al. 2007). As well, it is important to note the difference between actual recovery to a pre-disturbance state and the potential to recover once the contaminant exposure has declined to a level that direct effects are no longer possible and recovery could occur (see EFSA 2016).

Regardless of the stressor type, there will be a threshold of intensity to which a stressor should be limited to prevent long-term adverse impacts on ecosystem structure and functions (e.g., beyond the inherent functional redundancy capacity). From an ecotoxicological perspective, the potential for recovery following the cessation of exposure is predicated on the biological level at which the effect is observed and upon the effect itself not being permanent (e.g., malformations in an individual) or continuing to worsen to the point where recovery is simply not possible (e.g., failure to reach sexual maturity, or outright mortality). The phenomenon of latency in ecotoxicology (i.e., when effects are observed relative to exposure) helps frame our understanding of the potential for recovery by an individual or a population and will be both contaminant- and species-specific. For example, Zhao and Newman (2006) showed that contaminants that do not cause cumulative damage and or/are cleared readily from an organism were unlikely to cause continuing mortality in amphipods (*Hyalella azteca*) upon the cessation of exposure, and therefore, surviving

individuals have the theoretical capacity to recover. Ultimately, individuals, populations, communities of macrophytes, and ecosystems have the capacity to recover following a stressor, whether anthropogenic or non-anthropogenic, assuming the putative stressor is no longer present, and the impaired ecosystem has the underlying biotic and abiotic conditions to support recolonization, internally or externally.

6.3 Recovery and Macrophytes

Aquatic macrophytes are annual and perennial plants that can be found in both standing or flowing water and are physically large (i.e., individuals are visible to the naked eye) relative to phytoplankton or periphyton (Wetzel 1975). They are frequently classified by growth form and/or basis of attachment to substrates, such as non-rooted free-floating (e.g., duckweeds *Lemna* spp.), non-rooted submerged (e.g., coontail; *Ceratophyllum* spp.), rooted submerged (e.g., milfoils; *Myriophyllum* spp.), rooted with floating leaves (lily pads; *Nymphaea* spp.), and rooted emergent (e.g., cattails; *Typha* spp.) (Hanson 2013; Wetzel 1975). Their community composition, abundance, and biomass are subject to seasonal shifts, and are therefore relatively dynamic (Henry et al. 1996). Macrophytes have a sometimes-underappreciated ecological role in freshwater ecosystems from both a structural and functional perspective. They provide food, shelter, and nurseries to waterfowl, fish, and invertebrates, nutrient cycling and sequestering, oxygen production, and stability to organic sediments and other substrates from wave action and flooding. As such, there is considerable value in characterizing both the response and recovery to anthropogenic and non-anthropogenic stressors (Carpenter and Lodge 1986; Crowder and Painter 1991; Hanson 2013).

Freshwater macrophytes have specific attributes related to their life histories and physical architecture that influence how recovery occurs following a disturbance, as well as the speed and the degree to which recovery is possible (Henry et al. 1996). In terms of recolonization of habitat from which a species has been extirpated, macrophytes employ tactics that are shared by all plants. These include seed dispersal and seedbanks, plant fragments (e.g., stems), expansion from intact parent plants (e.g., lateral growth), rhizomes, and resting or overwintering phases (e.g., turions). Species traits (e.g., those related to recolonization, such vegetative or sexual dissemination) can significantly influence the degree and likelihood of macrophyte recovery. Henry et al. (1996) examined recovery following frequent flooding events over multiple years on the Rhône River, France. The authors assessed, in part, the contributions of vegetative dissemination of plants by lateral spread without dispersion (including by extension of the root system); from stem fragments; and by specialized resting phases (e.g., turions), as well as the frequency of flowering of the species in question. They found that recovery was relatively rapid overall, with most species returning within a year, and typically early recovery was by those able to produce turions or other vegetative organs, followed by recovery via lateral spread and stem fragments. Dispersal mechanisms from un-impacted to impacted patches will include physical transport

by currents, wave action, or flow, as well as by birds (e.g., migratory waterfowl). The efficiency of dispersal is driven in part by the degree of connectivity and geographic isolation between systems (e.g., small agricultural pond versus downstream on a river). Sand-Jensen et al. (2000) examined the shifts in macrophyte composition and abundance in 13 Danish streams over the period of a century. Connectivity of systems likely explained both local abundances as well as number of occupied sites by species in streams that were subject to frequent disturbance (Sand-Jensen et al. 2000).

Coastal wetlands are subject to regular storm and flooding events of varying severity that can lead to pulses of salinity that can impair plant growth, so these ecoystems lend themselves to understanding the propensity for macrophyte recovery. Howard and Mendelssohn (1999) conducted a four-month greenhouse experiments with monocultures of four perennial emergent macrophytes species (*Eleocharis palustris*, *Panicum hemitomon*, *Sagittaria lancifolia*, and *Schoenoplectus americanus*) as potted monocultures at two levels of salinity (6 or 12 g/L), rate to reach exposure (3 days or 3 weeks), and duration of exposure (1, 2, or 3 months). Transfer to freshwater followed each exposure to allow for a 1-, 2-, or 3-month period of recovery depending on initial exposure duration. Both effect and recovery were species-dependent. Mortality (nonviable aboveground tissue) for all treatments and durations combined was 17.8% for *P. hemitomon*, 6.7% for *S. lancifolia* 2.2% for *E. palustris*, and 0% for *S. americanus*. Within a species, salinity level and duration of exposure were the main factors that influenced the degree and rate of recovery, and the degree of recovery was correlated to the severity of the initial impact, with *P. hemitomon* exhibiting the least capacity for recovery, *S. lancifolia* and *E. palustris* moderate recovery, and *S. americanus* full recovery across all treatments. Howard and Mendelssohn (1999) theorized that the capacity to recover was related to the growth strategies of each tested species. Specifically, the plants with the ability to produce rhizomes that could outlast the exposure conditions provided a mechanism for recolonization once favorable growth conditions returned.

The rate of recovery following the loss of species can be influenced in part by patch dynamics and the community composition of the borders surrounding the immediately impacted area. Barrat-Segretain and Amoros (1996) experimentally cleared macrophytes from 9 m^2 patches (subdivided into 144 plots) of a river channel and tracked recovery over a period of greater than three months. Within three weeks of removal, most plots had new macrophyte growth of several species, and by the end of the study, most plots had multiple species (5–6) and dense coverage, illustrating the relatively rapid recovery that is possible for macrophytes, especially when colonizing populations are adjacent and actively growing. Barrat-Segretain and Amoros (1996) concluded that recolonization by macrophytes in their study was driven mainly by vegetative propagation. Specifically, parent plants expanded into the disturbed system from the edges of the plots ("peripheric propagation") as an intact entity (e.g., through spreading rhizomes), or they had ramets that would break off from the parent plant and move some distance away from the edges to colonize patches from a distance. They also reported that plants could exhibit both strategies simultaneously, such as *Elodea canadensis*, while others were limited to one mechanism (e.g., *Potamogeton natans*

for intact plant expansion from the edges; *Potamogeton pusillus* recolonization by fragments or propagules).

Eutrophication of freshwater ecosystems is another common stressor that can result in the loss of macrophyte species through enhanced turbidity (typically via algal blooms) and subsequent loss of light penetration to support early plant growth. With improvements in wastewater treatment and enhanced efforts to reduce nutrient movement into surface waters generally, the process of recovery by macrophyte communities can be assessed. Baastrup-Spohr et al. (2017) took data from 1990 and 2010 and examined the relationship between changes in eutrophication status and species richness and community composition of aquatic macrophytes in 56 lakes in Denmark. Overall, they found species richness increased over the 20 years with improved water quality, and that lake species richness was significantly positively related to a decline in concentrations of chlorophyll-a and improved water transparency. In terms of species composition, there was a shift to biotic homogenization, whereby the similarity between systems increased significantly through the acquisition across lakes of the same new species. In this case, macrophyte community recovery was deemed to be ongoing, and likely lagging, in part due to lack of connectivity with un-impacted systems to facilitate recolonization.

6.4 Herbicides, Macrophytes, Recovery, and Ecological Risk Assessment

Currently, the majority of herbicides are used in agriculture for crop protection, but herbicides are also registered for forestry, invasive species control, and home uses (USEPA 1998; Gettys et al. 2014). Herbicides have a variety of modes of action that target different plant physiologies. The majority of herbicides interrupt plant-unique biological mechanisms by binding at specific sites of action. In general, there are two categories of herbicides, non-selective and selective, which has implications for assessing recovery. Early herbicides tended to be non-selective, with more selective herbicides being invented following World War II (Vats 2015). Non-selective herbicides, such as glyphosate, do not have specific targets (e.g., species or classes of plants) and are able to control many types of plants (Ross and Childs 1996). In contrast, selective herbicides are more toxic to certain plant species, typically due to the mode of action that is unique to the target (De Carvalho et al. 2009). For example, dicamba is a selective herbicide that mimics plant growth hormones and mainly targets eudicots like broadleaf weeds (Ross and Childs 1996). 2,4-dichlorophenoxyacetic acid (2,4-D) is another selective herbicide used to target broadleaf dicotyledonous weeds (Song 2014). It is a pre-emergent and post-emergent herbicide that mimics growth-regulating auxins, affecting cell division and elongation (Grossmann 2010). In both these cases, monocots would be significantly less

sensitive to the herbicide, so direct effects are minimal, and the need to assess recovery in these types of plants is not necessary.

The application period of an herbicide depends on the crop, its targets, mode of action, the geographic region, and regulatory restrictions. There are three general categories of application period for herbicides: pre-plant, pre-emergence, and post-emergence (Vats 2015). Pre-plant herbicides are applied before crops are planted or seeded to clear fields of weed species. Pre-emergence herbicides are those sprayed after planting and before seed crop germination, which do not affect the seed but will impact growing weeds. Post-emergence herbicides are sprayed after seeds have germinated and emerged and are typically selective for certain species or groups, other than the crop. These patterns of applications mean that herbicides that have migrated off fields through spray drift or runoff into surface waters are not constant or consistent through space and time, but are rather pulsed in nature, with periods of relatively high exposures, followed by declines and periods of low to no exposure (Smith et al. 2021). For example, concentrations of atrazine in United States Midwestern streams near agricultural lands with intensive atrazine application tend to occur as pulses in the streams, with mean daily concentrations below 10 μg/L (Andrus et al. 2013). After rainfall, runoff concentrations were observed to increase up to 200 μg/L, but would return to under 10 μg/L in a short period of time (Andrus et al. 2013, 2015). As a result, herbicides can present a risk to macrophyte communities where they are applied and on multi-occurrences annually, and so characterizing recovery potential helps to understand to risks from possible cumulative effects.

Concentrations of herbicides in the tissues of aquatic plants tend to track those in the surrounding water (King et al. 2016). As such, with the cessation of exposure, internal concentrations should decline and aquatic macrophytes can potentially recover, at least physiologically. This rapid response has been observed in algae where a study investigating the recovery of *Pseudokirchneriella subcapitata*, *Anabaena flos-aquae*, and *Navicula pelliculosa* found that PSII quantum yields were not significantly different from the control almost instantaneously following transfer to clean media (Brain et al. 2012a). This coupled with modes of action that target plant-specific biochemical or physiological processes (e.g., inhibit chlorophyll functioning) to impair growth in general, but rarely result in direct mortality of plants below recommended application rates means less concern around possible latent effects. From a risk assessment perspective, the ability to recover following herbicide exposure reduces the risk of sustained adverse effects on macrophyte communities as a whole, which in turn is important for preventing indirect effects on organisms that rely on macrophytes for food and/or habitat (USEPA 1998).

Current risk assessments and data registration requirements for herbicides in North America do not require recovery data for macrophytes, though an evaluation of adversity may include the potential for recovery (USEPA 1998). Typical regulatory requirements at the initial tier for the registration of herbicides include the submission of toxicity data from the free-floating macrophyte *Lemna* sp., commonly known as duckweed (Arts et al. 2010; Hanson 2013). Duckweed guidelines allow for the

characterization of both toxicity and recovery under laboratory conditions and in a reasonable time frame using fairly straightforward techniques (Brain and Solomon 2007). However, concerns have been raised about *Lemna* sp. being used as a surrogate for all macrophytes (Hanson 2013; Rentz and Hanson 2009; Wang et al. 2010). As they are monocots that lack stems, true leaves, and a sediment-interacting root system, their predictive capabilities may be limited for eudicots, rooted, and/or submerged macrophytes (Hanson and Arts 2007). Despite this, duckweed as a model organism lends itself readily to the assessment of recovery in the laboratory (see Sect. 5) in part because of the ease by which one can transfer plants to fresh media to mimic the removal of the stressor.

6.5 Review of the Current State and Quality of Evidence for Macrophyte Recovery Following Exposure to Herbicides

Previous studies have expressed concerns around the quality of ecotoxicology studies and recommended criteria to determine the reliability and relevance of data for risk assessment (Ågerstrand et al. 2014; Hanson et al. 2017, 2019; Harris et al. 2014). Previous work using objective scoring rubrics to assess the quality of toxicity tests for atrazine and primary producers reported that a large number of studies had experimental data fitting basic inclusion criteria, but only a small proportion provided sufficient details on the test substance, test organism, and test results to be considered of satisfactory quality for use in decision-making (Hanson et al. 2019). As part of this chapter, we set out to critically review the availability, reliability, and ecological relevance of macrophyte recovery data following exposure to herbicides in the peer-reviewed literature. We also examined the evidence from these studies that recovery can occur in macrophytes following exposure to herbicides. This was done, in part, to identify the data gaps and common methodological issues in order to improve the quality of future recovery studies. With sufficiently high-quality recovery data, policy makers could use the information to establish more credible guidelines and regulations, as well as to assay the overall risk posed by these compounds to macrophytes.

6.5.1 Materials and Methods

We assessed both the strength of methods and ecological relevance of endpoints from peer-reviewed recovery studies performed on primary producers exposed to herbicides. For the purposes of this exercise, we defined ‘recovery’ as measured endpoints not statistically different from control(s) at the end of a herbicide-free exposure period following a herbicide exposure phase. Scoring rubrics for strength

and relevance were developed and applied to each published study that met our inclusion criteria. The rubrics were modified from the scoring criteria of Hanson et al. (2019).

6.5.1.1 Literature Search

The search for relevant literature was performed using databases available through The University of Manitoba libraries, including Scopus. Published studies that could be acquired through the University of Manitoba libraries database were reviewed. The search terms employed were combinations of "herbicide", "recovery", "exposure", "aquatic primary producer", and "macrophytes". References in the scored papers were also reviewed for possible literature to be assessed. The inclusion criteria for scoring of studies required papers to have exposure and recovery periods (e.g., a pulse exposure period) for a single herbicide (no mixtures), reported effects on aquatic macrophytes, be written in English, and published in a peer-reviewed journal. The final search for published articles was performed on September 30th, 2019.

6.5.1.2 Strength of Methods Scoring

A strength of methods score (i.e., reliability) was assigned for each paper based on the information provided in the article and any associated supplementary files. The rubric for scoring strength was modified from the one Hanson et al. (2019) developed for primary producer toxicity tests. The rubric was used to evaluate all studies, regardless of species, herbicide, or reported endpoint. The criteria were divided into three main groups (Group 1: Test Substances—six criteria; Group 2: Test Organisms and Test System—four criteria; and Group 3: Test Design, Statistics, and Results—five criteria). The rubric and justifications for the scoring categories can be seen in Table 6.1. Performing and reporting for each criterion resulted in a score of 1, otherwise a score of zero was assigned.

The score for ecological relevance of exposures is one criterion that will be highly context-dependent (e.g., compound, time of year, geographic location). This criterion reflects the proximity of the recovery studies to a "real-world" situation and consequent relevance for the purposes of ecological risk assessment. In risk assessment, demonstrating toxicity and recovery, or the lack thereof, at ecologically relevant concentrations helps reduce uncertainty. For this review, a score of 1 was given when at least one of the tested concentrations in the recovery testing was equal to or less than 20 μg/L (i.e., an "environmentally relevant concentration"), regardless of the herbicide. The level of 20 μg/L was chosen based on available data for herbicides in surface water (in general and for the compounds tested), which indicates that most environmental exposures will be at or below this level for these compounds. There are numerous monitoring programs reporting herbicide concentrations in surface water that support using 20 μg/L as a cut-off. Schuler and Rand (2008) summarized the herbicide concentrations in South Florida's surface water from 1990–2006.

Table 6.1 Scoring criteria for method strength of peer-reviewed recovery studies of aquatic macrophytes after herbicide exposure. Modified from Hanson et al., (2019).

Assessment criteria [a]		Brief explanation	Rationale	Detailed Explanations
Group A - Test substance				
1	> 95% pure	Source and purity of test substance reported	The purity of test chemical should be high to increase the confidence of study's result by concluding the test substance was the one who caused effect on test species, instead of other impurity (e.g., adjuvants, formulants) in the test substance.	1 - If the percentage and source of test substance were reported with the percentage was greater than 95%; 0 - if purity and/or source of test source were not reported, or the purity was less than or equal to 95%
2	Measured concentrations reported – any [b]	Any analytical confirmation and reporting of tested concentrations	Confirmation of the test concentration would increase the accuracy of the reported toxicity value and ensure the toxicity effect on the test species was caused by the test chemical.	1 - If the analysis of stock/test concentration solution were reported at any stage of the experiment; 0 - if the analysis of stock/test concentration was/were not reported
3	Measured concentrations reported - individual initial	Initial concentrations in individual test units reported (pooled or separate)	Measuring the initial concentration would decrease the errors (e.g. wrong dilution, incorrect test chemical) in the result. With the initial and final concentration, time-average concentration could be calculated and apply to calculations.	1 - If initial tested/stock concentrations were reported; 0 - if initial tested/stock concentration were not reported
4	Measured concentrations reported - individual final	Final concentrations in individual test units reported (pooled or separate)	Measuring the final concentration would decrease the errors (e.g. wrong dilution, incorrect test chemical) in result. With the initial and final concentration, time - average concentration could be calculated and applied to calculations.	1 - If the test concentration(s) were measured and reported at the end of experiment; 0 - if the test concentration were not reported
5	No. of concentrations tested (exclude control) [b]	3 or more concentrations, plus control	Having at least 3 concentrations would assist the determination of concentration - response model.	1 - If number of tested concentration treatments (exclude control) was 3 or greater; 0 - If number of test concentrations treatments were fewer than 3
6	Ecological relevance	At least 1 exposure concentration at 20 µg/L or less	Based on the previous studies , the herbicide concentration in surface water in surface water often are often at or lower than 20 µg/L (e.g. Andrus et al., 2013, Environment Canada, 2011). Therefore, 20 µg/L set as environmentally relevant concentration.	1 - If at least one of the tested concentration was less than or equal to environmental concentration (e.g. 20 µg/L); 0 - if none of t he tested concentration was less than environmental concentration (e.g. 20 µg/L)

(continued)

Table 6.1 (continued)

Assessment criteria [a]		Brief explanation	Rationale	Detailed Explanations
Group B - Test organism and test system				
7	Strain/source identified	Provenance of test organism (e.g. wild - collected location; laboratory strain ID)	With the information of collect location and the test species, it would help to understand if the test species were exposed to test chemical previously. and which species were being tested.	1 - If the source of the test organisms was provided ; 0 - if no mention of the source of test organisms
8	Initial test organism characteristics described	As relevant, size, density, mass, feeding protocols	With the initial size, density, mass of test species were reported, it can be used to compare to the exposed test species to access the toxicity effect.	1 - If initial front number or cells counts were repor ted; 0 - if no information on the initial front number, cells counts, or mass of the organisms
9	Standard protocol followed	e.g., EPA, OECD, ASTM; deviations acceptable if described	The testing were standardized by followed a standard protocol and the res ult could be used to compare with other studies.	1 - If test procedures followed standard pro tocol; 0 - if the procedures were based on previous studies and/or not followed any standard protocol
10	Test conditions	Temp, light, pH; TOC if sediment	Reportin g the test condition was essential because the condition of test environment (e.g., temperature, light) could affect the growth rate and toxicity effect on primary producers.	1 - If temperature and/or light intensity, and media type were reported, and the error values of light intensity and/ or temperature was reported; 0 - if temperature, light intensity, and media were not reported without error values of temperature and /or light intensity was not reported
Group C - Test design, statistics, and results				
11	Replication [b]	Minimum of 3 replicates per exposure	The testing should be repeated to confirm the toxicity effect by the test chemical is consistent.	1 - Replicates ≥ 3; 0 - Replicates < 3
12	Statistical methods described [b]	Appropriate test reported and employed for NOEC, LOEC, and EC/LCx; appropriate controls employed	Proper statistic approach should be used to prevent incorrect result and misleading result for risk assessment.	1 - If the statistic method to calculate NOEC/LOEC/ECx/LCx or other endpoints were described and reported; 0 - if the statistic method to calculate NOEC/LOEC/ECx/LCx were not described and/or reported
13	Concentration - response	Concentration - response model and parameters provided	Providing concentration - response model to evaluate the toxicity effect and response.	1 - If concentration-response model and parameters was showed or reported in the format of formula, or graphs with the concentration -response model equation information ; 0 - If equation of concentration -response model and parameters were not showed or reported

(continued)

Table 6.1 (continued)

Assessment criteria [a]		Brief explanation	Rationale	Detailed Explanations
14	Measured response (raw) values	Key measured raw values (e.g., not % of control) reported (all or avg. with error; graph or table)	Raw values (data without transformation) should be reported to access the performance of the testing and toxicity effect of the test chemical.	1 - If raw values (average raw values is accepted but not % of control) were reported in t ables/figures; 0 - If only the % of control was reported in graph/figure and none of the raw values (includes a verage) were reported or plotted in figures
15 [b]	Control performance	Control values reported and performance criteria met	Control needed to be reported to ensure the culture met the standard performance and was healthy in the testing, and if it is showing the toxicity effect appropriately.	1 - If control values (included solvent control if solvent was used to prepare the test solution) were reported and the reported values showed to meet the control performance requirements; 0 - if control values were not reported and not mention on control performance requirement

[a] Full mark is fifteen and one mark would be given for meeting each criterion; if not met, a score of zero was assigned
[b] Total score multiplied by 0.5 if the criterion is zero

The authors reported that most herbicides in ten counties of South Florida had a maximum concentration that was lower than 20 μg/L, with a range of 0.003–18 μg/L and 90th percentile ranging from 0.003–1.91 μg/L. The exception was diuron in Hendry County, which had a maximum of 76 μg/L and 90th percentile of 0.15 μg/L (Schuler and Rand 2008). The monitoring program from Environment and Climate Change Canada in 2003–2005 showed that the majority of herbicide concentrations in surface waters across Canada were below 14.9 μg/L (ECCC 2011). Based on the reported values from these studies, it is reasonable to conclude that concentrations of herbicide in North America's surface waters are usually below 20 μg/L. Therefore, 20 μg/L was set as a generic environmentally relevant concentration in the strength of methods rubric.

There were also critical criteria that were considered integral to identifying a strong study. The critical criteria were analytical confirmation and number of tested concentrations, replication, and use of appropriate statistical methods. The critical criteria are highlighted in red in Table 6.1. To better evaluate the strength of the study, total scores were reduced if a critical criterion was not met. When criterion 2, 5, 11, and/or 12 (Table 6.1) was not met, the total score would be multiplied by 0.5 for each missed criterion. If two critical criteria were not met, the total score would be multiplied by 0.25. The total score would be multiplied by an additional factor of 0.5 if expert judgment deemed there were additional study flaws that were not captured in the standard rubric criteria. However, no further fundamental errors were found among reviewed papers, so no scores were reduced based on the judgment of the reviewer.

6.5.1.3 Ecological Relevance of Endpoints Scoring

The endpoints monitored in the studies were used to assign a score to the data for its relevance to ecological risk assessment. We worked from the assumption that the endpoints that are associated with higher levels of biological or ecological organization (i.e., population or community level) are most useful for risk assessment (Hanson et al. 2019). For each study, each reported response was assigned a relevance score. The scores for relevance for each endpoint were between 0 and 5 (Table 6.2). The greater the relevance score for the endpoint, the more the response was conceptually and objectively linked to population and community-level responses that best inform ecological risk assessment.

Table 6.2 Scoring criteria for the ecological relevance of endpoints from peer-reviewed recovery studies of aquatic macrophyte after herbicide exposure

Score	Macrophytes	Detailed explanation
0	No known linkage to survival, development, growth, and/or reproduction	If a response has no real or hypothetical linkage to higher-level effects, then it has little to no value in elucidating ecological risk
1	Biomarker response that has limited linkage to higher-level effects (e.g., genomic, proteomic, metabolomic)	While informative from a mechanistic perspective, the relevance of these responses to population, community, and ecosystem-level effects is considered very low. In many cases, the responses characterized are regular processes to detoxify or adapt to a transient stressor, which in and of themselves are not adverse
2	Biomarker responses such as enzymatic changes (e.g., nitrogenase activity) or general physiology (e.g., PSII quantum yield, chlorophyll-a concentrations) or functional responses, (e.g., rate of oxygen production)	While informative from a mechanistic perspective, the relevance of these responses to population, community, and ecosystem-level effects is considered very low. In many cases, the responses characterized are regular processes to detoxify or adapt to a transient stressor, which in and of themselves are not adverse
3	Changes in growth and development, such as biomass and growth rates	These responses are typically highly relevant to the success or sustaining populations and communities in an ecological context
4	Changes in reproduction, such as seed production, seed viability, flower production, frond number, plant number, and related metrics (e.g., growth rates)	These responses are typically highly relevant to the success or sustaining populations and communities in an ecological context
5	Mortality and/or community-level changes such as shifts in species diversity/composition	Loss of individuals is highly relevant to the success or sustaining populations and communities in an ecological context

6.5.1.4 Review QC/QA

Once the strength and relevance scores were assigned, the resulting data spreadsheet underwent a quality control and quality assurance (QA/QC) exercise. The spreadsheets (and papers) were reviewed again by a separate individual with experience in ecotoxicology studies to help ensure accuracy of interpretation and reporting.

6.5.1.5 Data Analysis

Graphs (e.g., scatter plot, bar graphs, bubble plots, and box plots) were generated with the use of R-studio (R Development Core Team 2019). Descriptive statistical analyses were performed in R studio (R Development Core Team 2019).

6.5.2 Results

6.5.2.1 Summary of Reviewed Studies

A total of 25 studies published between 1986 and 2019 met the inclusion criteria and were reviewed (Table 6.3). The number of recovery studies has steadily increased over years (Fig. 6.1).

Table 6.3 Summary of 25 peer-reviewed studies published between 1986 and 2019 on aquatic macrophytes exposed to herbicide and followed by a recovery period

Organism type	Number of peer-reviewed papers	Number of experiments	Number of species tested	Number of herbicides tested	Number of endpoints reported
Duckweed	14	44	3	27	25
Others	14	32	18	10	39
Total	**25**	**76**	**21**	**33**	**58**

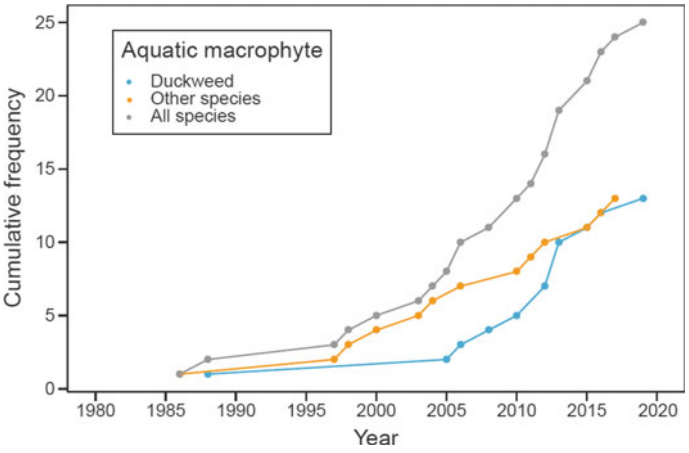

Fig. 6.1 The cumulative number of published peer-reviewed recovery studies on aquatic macrophytes (duckweed, other species, total) exposed to herbicides between 1986 and 2019. The total number of papers found was 25

Test Organism Class and Species

There were 76 unique experiments with macrophytes for 33 different herbicides and 58 distinct endpoints (Tables 6.3 and 6.4). The most commonly tested species by unique experiments were *Lemna* sp. ($n = 17$), *Lemna minor* ($n = 15$), and *Lemna gibba* ($n = 12$).

Test Substances

Figure 6.2 shows the herbicide groups tested over time. In general, there was increasing diversity of herbicide groups included in the recovery studies over time. It increased from a single herbicide group in the 1980s to eight herbicide groups in the 2010s. A total of 33 herbicides were tested in the reviewed studies, and some studies examined recoveries after multiple types of single herbicide exposure (Table 6.4). Photosystem II inhibitors were the most studied herbicide group ($n = 29$ experiments). The second most commonly tested herbicide group for macrophytes was acetolactate synthase (ALS) inhibitors ($n = 21$ experiments). The most commonly tested herbicides were atrazine (9 papers), diuron (4 papers), and metsulfuron-methyl (4 papers).

Test Duration

The mean and median exposure durations for macrophytes were 18 and 7 days, respectively ($n = 296$) (Fig. 6.3). The mean and median duration for the recovery period were 13 and 7 days for macrophytes, respectively ($n = 359$). For duckweed, the mean and median were 9 and 7 days for exposure ($n = 168$), and 8 and 7 days for recovery ($n = 176$). The mean and median for others aquatic macrophyte were 29 and 13 for exposure ($n = 128$), and 17 and 14 days for recovery, respectively ($n = 183$).

6.5.3 Strength of Method Scores

The mean and median of strength of method scores for macrophytes were 6.0 and 5.0, respectively ($n = 76$; Fig. 6.4). The mean and median of strength of method scores were 5.5 and 5 for duckweed ($n = 44$) and 6.6 and 8.0 for other species ($n = 32$). The percentage of individual tests that received a score greater than 7.5 out of 15 (i.e., > 50%) were 37% for total macrophytes ($n = 76$), 25% for duckweed ($n = 44$), and 53% for others macrophyte species ($n = 32$). The highest strength scores by each herbicide are found in Table 6.5.

Table 6.4 Summary of tested herbicides from twenty-five reviewed studies on aquatic macrophyte (duckweed, others, and total) that was published between 1986 and 2019

Herbicide group (mode of action)	Number of papers in group[a]	Number of herbicides tested	Number of duckweed experiment tested	Number of others macrophyte experiment tested	Number of total macrophyte experiment tested	Number of papers tested by herbicide	Number of experiment by species
Photosynthesis II inhibitor	16	8	14	15	29	atrazine (9), diuron (4), isoproturon (3), bromacil (1), linuron (1), simazine (1), simetryn (1), terbuthylazine (1)	*Lemna minor* (6), *Lemna gibba* (5), *Lemna* sp. (3), *Zostera capricorni* (3), *Vallisneria americana Michx* (2), *Ceratophyllum demersum* (1), *Chara globularis* (1), *Cymodocea serrulata* (1), *Elodea nuttallii* (1), *Halophila ovalis* (1), *Myriophyllum aquaticum* (1), *Myriophyllum spicatum L.* (1), *Potamogeton crispus L.* (1), *Potamogeton perfoliatus L.* (1), *Ranunculus circinatus Sibth.* (1)

(continued)

Table 6.4 (continued)

Herbicide group (mode of action)	Number of papers in group[a]	Number of herbicides tested	Number of duckweed experiment tested	Number of others macrophyte experiment tested	Number of total macrophyte experiment tested	Number of papers tested by herbicide	Number of experiment by species
Acetolactate synthase (ALS) inhibitor	8	13	18	3	21	metsulfuron-methyl (4), cyclosulfamuron (3), bensulfuron-methyl (2), thifensulfuron-methyl (2), ethoxysulfuron (1), flazasulfuron (1), flupysulfuron-methyl (1), imazamox (1), imazosulfuron (1), iofensulfuron-sodium (1), nicosulfuron (1), pyrazosulfuron-ethyl (1), rimsulfuron (1)	*Lemna* sp. (10), *Lemna gibba* (5), *Lemna minor* (3), *Myriophyllum spicatum* (2), *Elodea canadensis* (1)
Photosynthesis I inhibitor	5	3	3	2	5	Irgarol (2), paraquat (2), diquat (1)	*Zostera capricorni* (2), *Lemna gibba* (1), *Lemna minor* (1), *Lemna* sp. (1)
Acetyl coenzyme A carboxylase (ACCase) inhibitor	2	2	2	0	2	cyhalofop-butyl (1), thiobencarb (1)	*Lemna* sp. (2)

(continued)

Table 6.4 (continued)

Herbicide group (mode of action)	Number of papers in group[a]	Number of herbicides tested	Number of duckweed experiment tested	Number of others macrophyte experiment tested	Number of total macrophyte experiment tested	Number of papers tested by herbicide	Number of experiment by species
Carotenoid biosynthesis inhibitor	2	1	0	11	11	fluridone (2)	*Hydrilla verticillata* (4), *Chara* spp. and *Najas guadalupensis Magnus* (1), *Elodea canadensis Michaux* (1), *Myriophyllum spicatum* L (1), *Potamogeton nodosus Poiret* (1), *Potamogeton pectinatus L* (1), total plant community (1), *Vallisneria americana Michaux* (1)
Pigment synthesis inhibitor	2	1	1	1	2	norflurazon (2)	*Lemna minor* (1), *Vallisneria americana* (1)
Root-growth inhibitor	2	3	3	0	3	pendimethalin (1), propyzamide (1), trifluralin (1)	*Lemna minor* (3)

(continued)

Table 6.4 (continued)

Herbicide group (mode of action)	Number of papers in group[a]	Number of herbicides tested	Number of duckweed experiment tested	Number of others macrophyte experiment tested	Number of total macrophyte experiment tested	Number of papers tested by herbicide	Number of experiment by species
Shoot-growth inhibitor	2	1	2	0	2	alachlor (2)	*Lemna gibba* (1), *Lemna* sp. (1)
Mitochondrial ATP-ase activity inhibitor	1	1	1	0	1	pentachlorophenol (1)	*Lemna minor* (1)

[a]More than one herbicide was tested in some studies

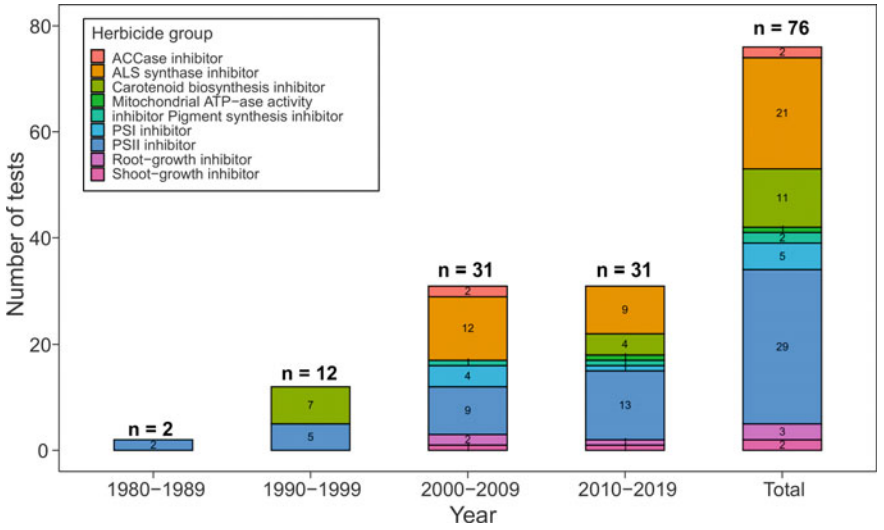

Fig. 6.2 The number of aquatic macrophyte herbicide recovery studies by herbicide group between 1986 and 2019. The *n* above each bar was the total number of unique tests in each year group, and the number in each stacked bar was the number of tests in each herbicide group in the corresponding year group

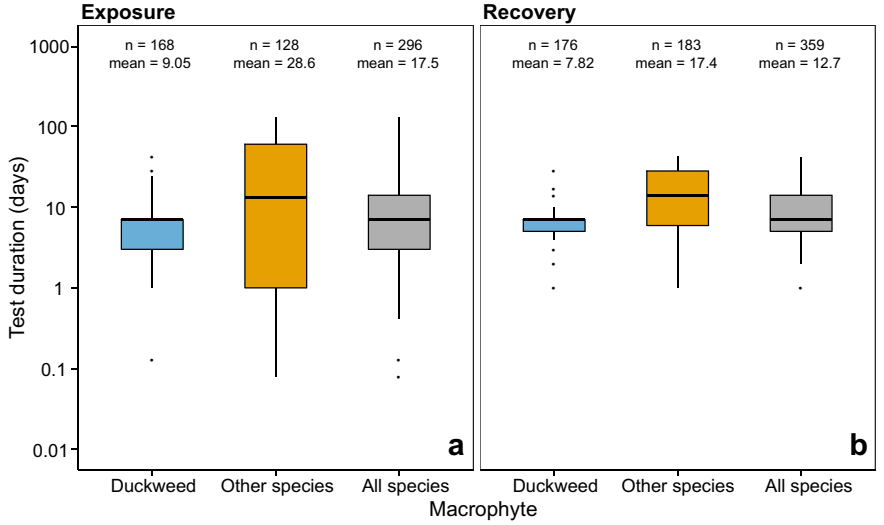

Fig. 6.3 Boxplot with (A) exposure and (B) recovery test durations on macrophytes (duckweed, others, and total) herbicide exposure recovery studies. The *n* and mean listed at the top of the boxplot were the total number and mean unique responses by time point reported

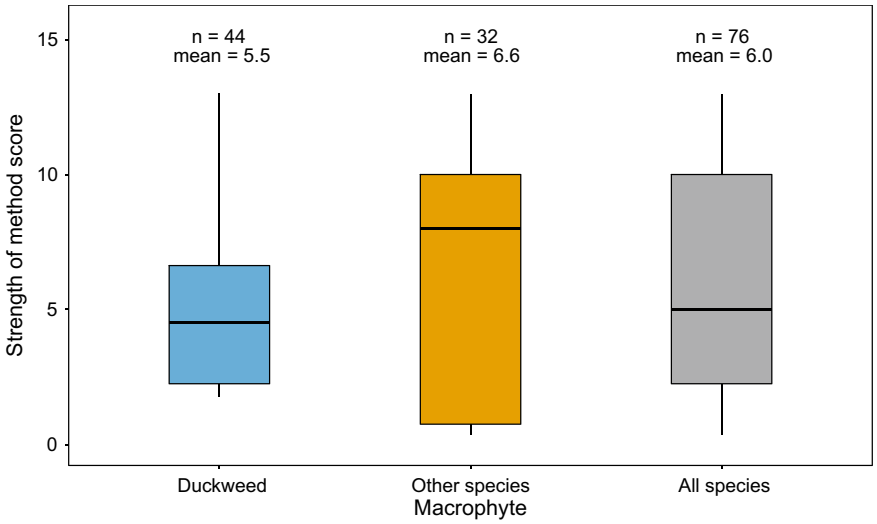

Fig. 6.4 Boxplot with strength of method score for each aquatic macrophytes (duckweed, others, and total). The *n* and mean listed above each boxplot were the total number and mean of individual test species in the studies

The scores as percentages for each criterion are found in Fig. 6.5. Criterion 8 (The initial test organism characteristics were described) was met most commonly for macrophytes (92%, $n = 76$), followed by Criterion 7 (Test organisms strain/source were identified) with 91% ($n = 76$). The criterion least likely to be met was Criterion 15 (Control criteria and performance) for macrophyte studies (18%, $n = 76$).

For critical criteria, Criterion 2 (measured concentrations) was met in 43% of macrophyte tests ($n = 76$). Criterion 5 ($\geq$ three test concentrations, excluding control) was met by 87% of macrophyte studies. Criterion 11 ($\geq$ 3 replicate in each concentration) was met by 83% of macrophytes studies. For Criterion 12 (Appropriate test statistics for NOEC, LOEC, ECx), 78% of macrophyte studies met this requirement.

6.5.4 Ecological Relevance of Endpoint Scores

The majority of endpoint relevance scores for macrophytes from exposure and recovery phases ranged between 2 and 4 (Fig. 6.6). The most common endpoint assessed was reproduction (46%, $n = 130$ for exposure; 46%, $n = 183$ for recovery). For duckweed specifically, the most commonly tested endpoint was reproduction for exposure (70%, $n = 81$) and recovery (73%, $n = 89$). The most commonly tested endpoint class for other macrophyte species was physiological for exposure (53%, $n = 49$) and recovery (43%, $n = 94$).

Table 6.5 List of reviewed studies with the highest strength of method score for macrophytes by tested herbicide group. The studies with a strength of method score (full mark of 15) greater than 7.5 marks are bolded

Herbicide group	Study	Herbicide	Test species	Endpoints	Overall score
ACCase inhibitor	Mohammad et al. 2008	Cyhalofop-butyl, Thiobencarb	*Lemna* sp.	Daughter fronds production (relative growth rate)	1.75*
ALS synthase inhibitor	Wieczorek et al. (2017)	**Iofensulfuron-sodium**	***Myriophyllum spicatum, Elodea canadensis***	**Dry weight of total shoots (growth rate), main shoot length (growth rate), side shoot length (growth rate), and total shoot length (growth rate)**	**12**
Carotenoid biosynthesis inhibitor	**Netherland** (2015)	**Fluridone**	***Hydrilla verticillata***	**Biomass, chlorophyll florescence yield, and shoot biomass**	**11**
Mitochondrial ATP-ase activity inhibitor	Boxall et al. (2013)	**Pentachlorophenol**	***Lemna minor***	**Growth rate and total frond area**	**10**
Pigment synthesis inhibitor	**Wilson and Koch** (2013)	**Norflurazon**	***Lemna minor***	**Frond production**	**12**

(continued)

Table 6.5 (continued)

Herbicide group	Study	Herbicide	Test species	Endpoints	Overall score
PSI inhibitor	Mohammad et al. (2010)	Paraquat	*Lemna gibba*	Frond number, frond number (relative growth rate)	5
PSII inhibitor	Brain et al. (2012b)	**Atrazine**	***Lemna gibba***	**Average growth rate, biomass, and frond number**	**13**
	Burns et al. (2015)	**Diuron**	***Lemna gibba, Lemna minor***	**Plant number, frond number, fresh weight, dry weight, plant number (relative growth rate), frond number (relative growth rate), fresh weight (relative growth rate), and dry weight (relative growth rate)**	**13**
	King et al. (2016)	**Atrazine**	***Ceratophyllum demersum***	**Dry mass**	**13**
Root-growth inhibitor	Knežević et al. (2016)	**Trifluralin**	***Lemna minor***	**Frond number (relative growth rate) and fresh weight (relative growth rate)**	**12**
Shoot-growth inhibitor	Mohammad et al. (2010)	Alachlor	*Lemna gibba*	Frond number, frond number (relative growth rate)	5

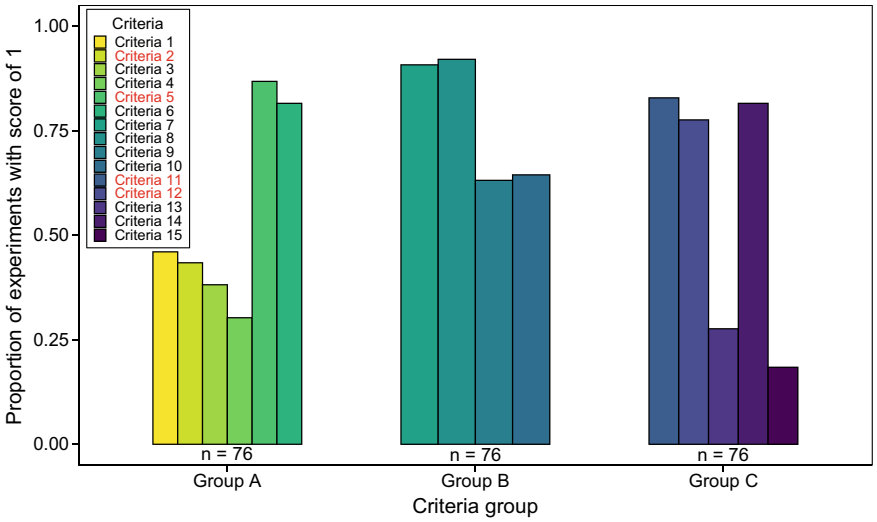

Fig. 6.5 The proportion of experiments with score of 1 for Criteria 1—15 from Group A (test substances), B (test organism and test system) and C (test design, statistics, and results) by aquatic macrophytes ($n = 76$). Criteria 2, 5, 11, and 12 were critical criteria and highlighted in red. The n is the total number of unique tests in each criteria group

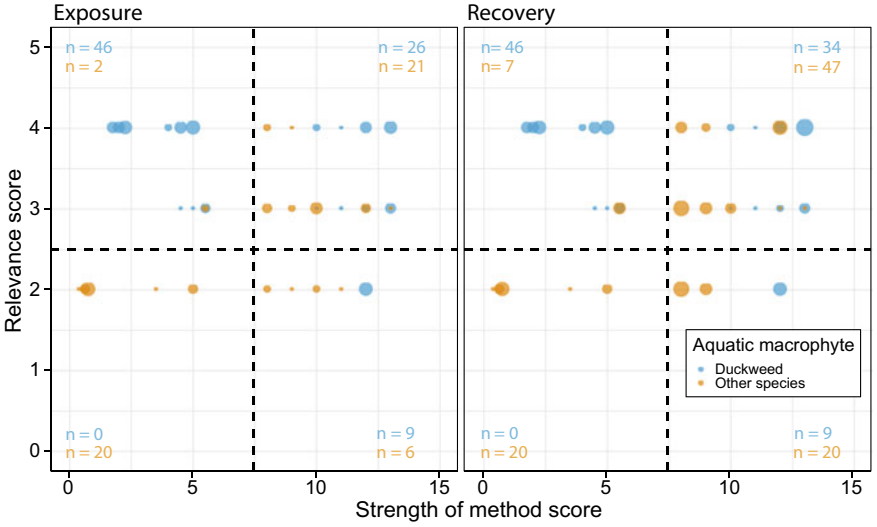

Fig. 6.6 Bubble plot with relevance score versus strength of method score from (A) exposure ($n = 130$ of total number of individual endpoint in exposure) and (B) recovery ($n = 183$ of total number of individual endpoint in recovery) period for aquatic macrophytes (duckweed and other species). The n in each corner is the total number of individual endpoints in each quadrat

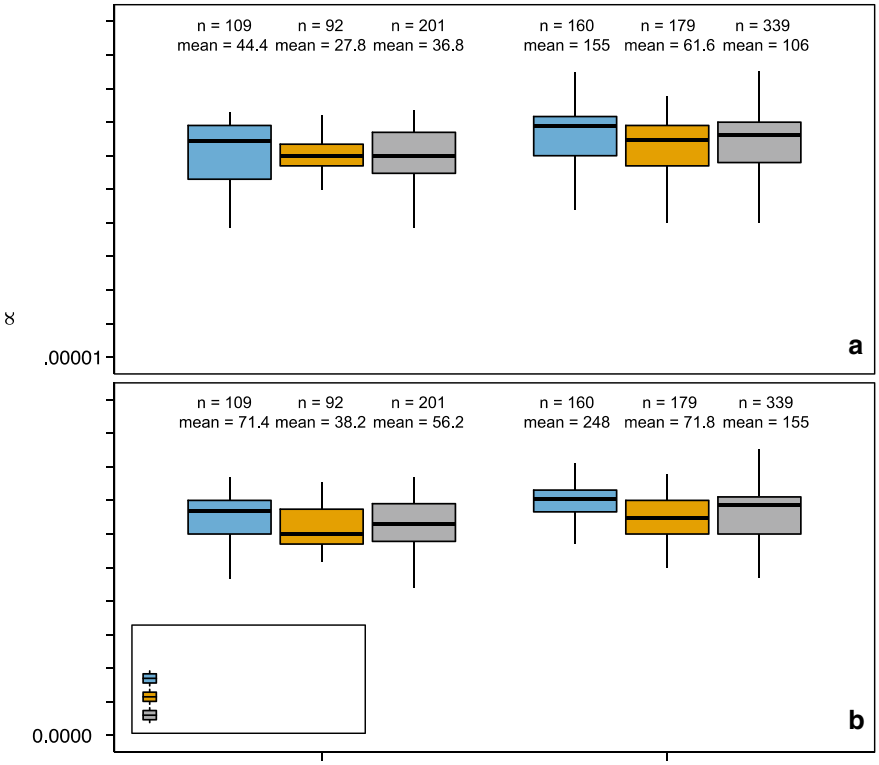

Fig. 6.7 Boxplot of (A) NOECs (no observed effect concentration) and (B) LOECs (lowest observed effect concentration) for macrophytes (duckweed, other species, all species) from the exposure and recovery periods for herbicides. The n and mean at the top of the boxplot were the total number and mean of reported NOECs and LOECs in the reviewed studies

6.6 How to Improve the Assessment of Macrophyte Recovery for Ecological Risk Assessment

Overall, recovery after herbicide exposure was observed consistently across plant species and herbicides tested as indicated by the changes in NOECs and LOECs for exposure and recovery phases, as well as a review of the statements and conclusions of the papers themselves (Fig. 6.7). We did find that many studies would fail to meet our quality threshold to be recommended for inclusion in risk assessment (see Table 6.5 for highest scoring studies). To increase the relevance and reliability of data for risk assessment, we recommend development of a guideline for recovery test procedures and encourage the use of reporting criterion to improve the reliability and relevance of recovery studies in future. The majority of tests were performed with duckweed, which is understandable considering its dominance as an organism for assessing direct toxic effects of contaminants in general, as well as the relative ease by which

recovery can be assessed with this group of plants. Despite this, there is no defined methodology for assessing recovery, which hinders cross compound and species comparisons (ECCC 2007). Standardization of methodologies is important because aspects of the protocol (e.g., the size of test chambers, amounts of nutrients, and duration of the study) can affect growth rates and result in plants reaching the carrying capacity of the test system before the study ends. For example, with duckweed tests, the rates of change in growth endpoints are the most reliable endpoints because affected fronds may have a delayed response (i.e., latency), but after a few days in clean media show normal growth rates comparable to controls. However, since they are delayed in their onset of recovery, they will not catch up to the controls in frond number or biomass as the controls had a "head start". Using rates of change over time gives a clearer and more realistic interpretation of recovery (e.g., Brain et al. 2012b).

To effectively incorporate recovery into ecological risk assessment, the decision-maker may consider the recovery of function or structural attributes via internal and external recovery mechanisms (Barnthouse 2004; Brock et al. 2018). The recovery studies in this review mainly evaluated recovery after herbicide exposure in the lab under controlled conditions. Recovery was observed in both monocot (e.g., *L. minor*) and dicot (e.g., *M. spicatum*) plants and showed that both types of physiologies have the potential to recover after exposure to herbicides. It is rare for laboratory studies to evaluate the toxicity and recovery effect with the interaction of various species from the same (e.g., intra-competition) or different (e.g., grazing pressure) trophic levels (Barnthouse 2004).

Given that laboratory tests do not allow for assessment of external recovery or other internal mechanisms of recovery (e.g., different life stages, seed banks), the true potential to recover following effects related to herbicides is likely underestimated in the available peer-reviewed literature.

Our review found less than half of individual tests provided sufficient information to achieve a score > 7.5 for strength of methods (i.e., be recommended for inclusion in risk assessment). The criterion where most of the studies lost marks was the reporting of control performance. This is consistent with Hanson et al. (2019), where many atrazine primary producer exposure studies lacked information on control performance. Reporting the control value is important for demonstrating that test organisms were healthy and meeting the requirement from standard guidelines (i.e., $\geq$ 8 times increase of *L. minor* frond number within a week), which increases the reliability of the study (ECCC 2007; Hanson et al. 2019). To further increase the number of reliable and relevant studies, journals should provide to researchers strict guidance for reporting and conducting basic toxicity studies.

The need to incorporate realistic exposure concentrations to assess recovery is highlighted in studies where unrealistic concentrations are used to cause toxicity, and no subsequent recovery is observed. In this case, the potential for recovery is not captured, nor can the responses reported be easily extrapolated to the field to make predictions related to actual herbicide exposure. In the case where toxicity occurs, but recovery also occurs at unrealistic concentrations, the uncertainty about recovery at lesser concentrations has in fact been resolved (i.e., it can occur) for risk assessment.

6.7 Conclusions and Recommendations

Macrophytes play important ecological roles in freshwater ecosystems, and have the capacity to recovery from natural stressors through a variety of mechanisms in a relatively short period of time (days to weeks) if conditions are appropriate. Herbicides present a possible risk to these organisms, so understanding the potential for recovery from an herbicide-driven effect is important for reducing uncertainty in ecological risk assessments. Most published studies on recovery by macrophytes from herbicides are lab-based and conducted with *Lemna* spp., and many have data reporting and methodological deficiencies that limit their full incorporation into risk assessments. Moving forward, we recommend that: (1) ecotoxicologists performing response and recovery tests review and implement best practices to reduce uncertainty and improve data quality and reporting (e.g., control performance) for risk assessment overall; (2) test guidelines for duckweed recovery be developed and validated in the lab as well as the field; and (3) the data on the recovery of aquatic plants be incorporated formally into the lower tiers of ecological risk assessment of herbicides where a non-continuous exposure is expected.

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