Chapter 5
Wild Rice (Zizania spp.) as a Model Macrophyte Toxicity Test Species for Ecotoxicological Risk Assessment

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Abstract This chapter outlines the life history of wild rice (*Zizania* spp.), assesses their ecological, sociocultural, and economic relevance, reviews the current state of knowledge around their use as a test species, and makes recommendations around their possible inclusion in ecological risk assessments. Northern wild rice (*Zizania palustris*) holds significant importance to North American Indigenous communities, is an integral aspect of wetland structure and function, and is rising in commercial demand and value due to their high nutritional content and long shelf-life. While *Z. palustris* has been used as a species in toxicity assessments, a standard test protocol has not yet been established. We performed a review to assess the utility and identify gaps in the available peer-reviewed literature for wild rice toxicity studies pertaining to methodology and experimental design. We found 11 articles reporting 22 studies that specifically examined the responses of *Z. palustris* to contaminants under controlled conditions (laboratory or mesocosm studies). The studies were evaluated for methodological reporting in five categories: (1) test organism; (2) test conditions; (3) test media; (4) experimental design; and (5) test performance. The conditions for stratification and control performance, both crucial for experimental replication and credibility, were under-reported in the literature (only 45% and 14% of studies, respectively). It was also found that conditions for seed storage were highly ambiguous or were not included at all. There were few consistent approaches between different research groups when conducting wild rice toxicity studies. We recommend that wild rice toxicity test reports incorporate experimental conditions in detail to ensure both transparency as well as to facilitate the ability of others to adopt

Throughout this chapter, wild rice is referred to as "they/them". In Anishinaabemowin (the shared language of the Algonquin, Mississauga, Nipissing, Odawa, Ojibwe, Potawatomi, and Saulteaux North American Indigenous peoples), northern wild rice, or manoomin, is grammatically referred to as "him/her/them", as opposed to "it", since they are not viewed as inanimate "resources" by the Anishinaabeg (Vizenor 2008). This important distinction in translation highlights the need for Western societies to recognize the rights of all organisms, not just humans and animals.

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Z. palustris as a toxicity test species. Overall, wild rice has potential as a macrophyte toxicity test species, but significant work is required to validate methods to ensure repeatable and reproducible data across various life stages.

5.1 Introduction

As outlined in Chap. 1, macrophytes are an essential component of nontarget toxicity characterization when assessing the risk to aquatic ecosystems; however, their widespread use in ecotoxicological testing is still relatively lacking, with most studies focusing on a narrow range of species. Many ecological risk assessments rely on a single macrophyte test species to extrapolate responses to population, community, or ecosystem-level effects for this class of organisms. This has led to concerns about the predictive capabilities of these assessments, especially under varying exposure scenarios (e.g., sediment, water column, or aerial exposure). As it pertains to primary producers, standardized algae and duckweed tests offer advantages for characterizing the effects of contaminants present in the water column (e.g., cost-effective, quick, and simple to conduct); however, they may lack ecological relevance for sediment-bound toxicants. To reduce uncertainty when characterizing the risk to nontarget organisms, representation of macrophytes with different morphologies and exposure pathways (e.g., rooted emergent species) are needed in the standard regulatory risk assessment process. As such, wild rice (*Zizania* spp.) may be a suitable candidate for inclusion into the battery of test species when assessing the risk to wetland ecosystems.

Wild rice species (*Zizania* spp.) are rooted emergent aquatic macrophytes that are indigenous to North America (except for Manchurian wild rice, *Z. latifolia*), resulting in potential exposure to toxicants bound to sediment, suspended in the water column, or deposited aerially. Additionally, studies have found that wild rice is sensitive under both laboratory and field conditions, predominantly conducted using the species *Zizania palustris* (Durkee Walker et al. 2006, 2010; Fort et al. 2014, 2017, 2020; Johnson et al. 2019; LaFond-Hudson et al. 2018; Malvick and Percich 1993; Nimmo et al. 2003; Pastor et al. 2017; Sims et al. 2012). Overall, wild rice presents the opportunity to address some of the identified gaps in macrophyte testing within North American ecotoxicological risk assessments.

To extrapolate meaningful and relevant results from toxicity tests, six criteria should be considered when selecting an appropriate test organism: (1) a group of species representing a broad range of sensitivities should be used whenever possible, as sensitivities vary among species; (2) species that are widely abundant and available should be considered; (3) species that are indigenous to or representative of the ecosystem of interest should be studied whenever possible; (4) species of ecological, cultural, or commercial importance should be included; (5) species should be amenable to routine maintenance, with techniques available for culturing and rearing in the laboratory to facilitate both acute and chronic tests; and (6) species with adequate background information (e.g., their genetics, physiology, and behavior) may allow for test results to be more easily interpreted, and should be considered (Rand et al. 1995). In this chapter, we examine how wild rice would meet these expectations, as well as reviewing the current state of knowledge and making recommendations to promote their inclusion as an alternative test species in ecological risk assessment.

5.2 Wild Rice Life History

Wild rice species (*Zizania* spp.) are emergent aquatic macrophytes that grow in dense (often monotypic) stands, typically in freshwater riparian and littoral zones (Ahmad et al. 2018; Crow and Hellquist 2006; LaFond-Hudson et al. 2018; Myrbo et al. 2017; Pastor et al. 2017; Wetzel 1975). They are monocotyledonous flowering grasses of the Family Poaceae (Aiken et al. 1988; Crow and Hellquist 2006; Pastor et al. 2017; Terrell et al. 1997). They have also been classified as part of the Tribe Oryzeae, as there is extensive genetic colinearity and synteny between wild rice (*Zizania* spp.) and domesticated rice (*Oryza sativa*), with differences primarily occurring in the number of chromosomes (e.g., wild rice has 15 pairs, while domesticated rice has 12) and total DNA content (e.g., wild rice has two times more than domesticated rice) (Grombacher et al. 1996; Hass et al. 2003; Kennard et al. 2000; Porter 2019). There are four recognized species of wild rice within the genus *Zizania* L.; two of which are annual species, *Z. palustris* L. (northern wild rice) and *Z. aquatica* L. (southern wild rice), and the other two are perennial species, *Z. latifolia* (Griseb.) Turcz. ex Stapf (Manchurian wild rice), and *Z. texana* Hitchc. (Texas wild rice) (Ahmad et al. 2018; Aiken et al. 1988; Archibold 2003; Crow and Hellquist 2006; Duvall and Biesboer 1988; Porter 2019; Terrell et al. 1997). In this chapter, we primarily focus on northern wild rice (*Z. palustris*) and discuss the other species for context and contrasting.

Northern wild rice (*Z. palustris*) is the most prevalent of the four species, and due to their larger seed size, they have been traditionally and commercially harvested as a food source (Archibold 2003; Porter 2019). They are predominantly found in freshwater wetlands, slow-moving rivers and streams, and the shallow waters of lakes within the Great Lakes and Boreal Forest regions of Canada and the United States, as seen in Fig. 5.1 (Ahmad et al. 2018; Aiken et al. 1988; Archibold 2003; Crow and Hellquist 2006; Duquette and Kimball 2020; Fort et al. 2014; LaFond-Hudson et al. 2018; Malvick and Percich 1993; Pastor et al. 2017; Porter 2019). Southern wild rice (*Z. aquatica*) can be found along the Atlantic coastal plains of Canada and the United States, with one variety (*Z. aquatica* var. *brevis* Fassett) found in the tidal waters and tributaries of the St. Lawrence River in Quebec (Aiken et al. 1988; Crow and Hellquist 2006; Terrell et al. 1997). Manchurian wild rice (*Z. latifolia*) is widely grown in southeastern Asia, primarily as a cultivated crop (Surendiran et al. 2014; Terrell et al. 1997; Xu et al. 2010). Texas wild rice (*Z. texana*) is an endangered species that is native to a small portion of the upper San Marcos River in Texas (Porter 2019; Surendiran et al. 2014; Xu et al. 2010).

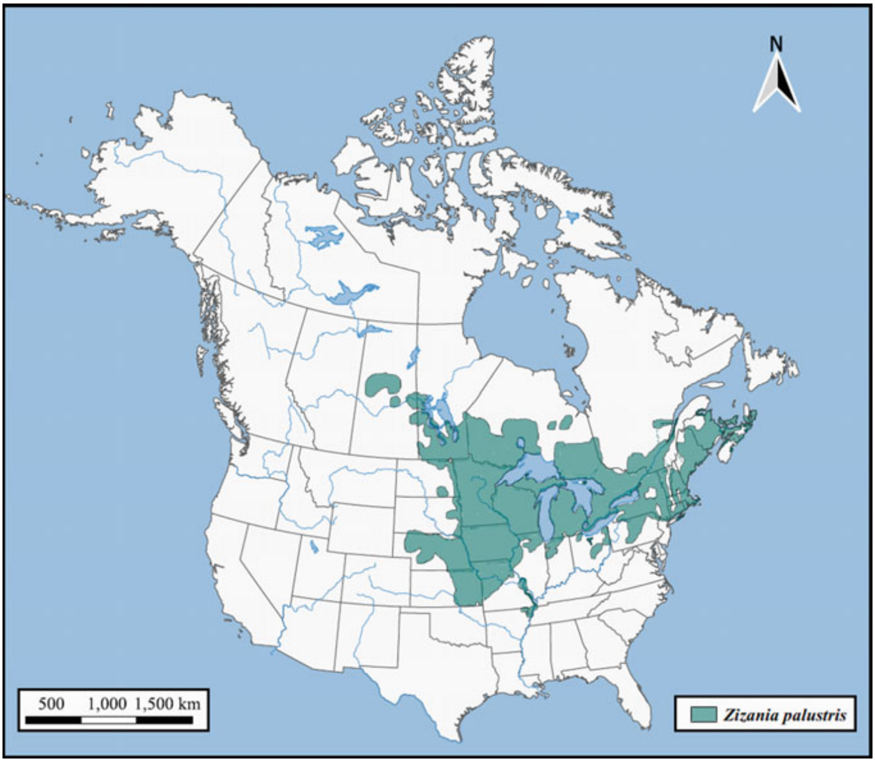

Fig. 5.1 Map of the distribution of northern wild rice (*Zizania palustris*) across Canada and the United States, excluding artificial paddies for commercial harvesting. The natural ranges of both *Z. palustris* var *palustris* and *Z. palustris* var *interior* were included and adapted from maps by Barkworth et al. (2007) and Porter (2019)

Aside from distribution and life cycle duration (annual versus perennial), spikelet anatomy is a reliable characteristic for distinguishing between the species, as the morphology of the pistillate lemmas and paleas of *Z. palustris* are coriaceous (i.e., leathery), whereas the intercostal species (e.g., *Z. aquatica*, *Z. latifolia*, and *Z. texana*) are chartaceous (i.e., papery), as described by Crow and Hellquist (2006), Duvall and Biesboer (1988), and Porter (2019). The two varieties of *Zizania palustris* are *Z. palustris* var. *palustris* and *Z. palustris* var. *interior*, both of which are commonly referred to as northern wild rice (Ahmad et al. 2018; Archibold 2003; Crow and Hellquist 2006). These can be distinguished based on height, leaf width, ligule length, and number of spikelets on the lower pistillate branches, as *Z. palustris* var. *palustris* has a height of about 0.7–1.5 m, 3–15 mm wide leaves, 3–5 mm long ligules, and 2–8 spikelets, while *Z. palustris* var. *interior* has a height of 0.9–3 m, 20–40 mm wide leaves, 10–15 mm long ligules, and 9–30 spikelets (Ahmad et al. 2018; Crow and Hellquist 2006). Wild rice (hereafter referring to the annual species *Z. palustris* and *Z. aquatica* collectively) are heterophyllus, with submerged and floating leaves preceding mature aerial leaves and the production of aerial reproductive organs (Wetzel 1975). They typically have short roots, long, narrow blade-like leaves, hollow, cylindrical stems, a panicle at the apex for the type of inflorescence, with spikelets of the upper inflorescence branches pistillate (female), and spikelets of the lower branches staminate (male) (Ahmad et al. 2018; Crow and Hellquist 2006; Surendiran et al. 2014). With their roots only extending into the shallow depths of the sediment, there is an increased risk of exposure to sediment-bound contaminants (i.e., those that form residues near the top of the sediments) in comparison to deeper rooting macrophytes. In addition, these short roots are easily pulled up, which can be ideal when examining root and shoot endpoints directly. As annual macrophytes, they must undergo all allocation processes required to complete their life cycle within the same year as their germination, often resulting in trade-offs between seed, leaf, stem, and root development if carbon or nutrients are limited (Sims et al. 2012). Wild rice has been found to respond plastically to environmental conditions, as the morphology of wild rice typically varies between natural stands and years. However, when seeds are grown in similar conditions, the variation significantly decreases (Archibold et al. 1989; Durkee Walker et al. 2010; Sims et al. 2012). Therefore, it is important to maintain appropriate water levels (ideally 0.75–1 m) when establishing wild rice stands, as greater water depths produce plants with longer, thinner stems and fewer seed heads (Archibold et al. 1989; Archibold 2003).

With the male and female flowers separate from each other on the same stalk, wild rice cross-pollinates to reproduce, and with clusters of receptive female florets emerging prior to the male florets, the chances of self-pollination are low, as females are often pollinated before the males emerge and shed pollen (Duquette and Kimball 2020). As seeds mature, they will shatter from the panicle, falling to the bottom of the water column to overwinter in the sediments in a dormant state. However, during commercial production, seeds are harvested and stored in near freezing water to mimic overwintering in controlled settings (Duquette and Kimball 2020; Grombacher et al. 1996). Early growth stages are susceptible to being uprooted or drowned by wave action if there is too much wind or the water depth is greater than 2.5 m (Aiken et al. 1988; Archibold 2003; Porter 2019). Wild rice does require water for growth, but does not grow well in saline, alkaline, or acidic water that is low in essential nutrients, as optimal alkalinity values range from 40 to 80 mg/L and optimal pH values range from 6.9 to 7.4 (Archibold 2003). Optimal wild rice habitats have long, cold winters, as seeds will germinate at low rates if the winter is too short or too warm (Ahmad et al. 2018; Myrbo et al. 2017). Additionally, transparent surface waters in the spring and summer are ideal, as low water clarity can inhibit photosynthesis prior to emergence (Ahmad et al. 2018; Myrbo et al. 2017).

In more northern latitudes, wild rice commence their annual life cycle with seed germination in the spring (i.e., May), followed by emergence from the sediment and water column typically in June, continuing their vegetative growth throughout the summer, with flowering and seed production usually beginning in August, and then the seeds begin to shed in autumn (Fig. 5.2); the plant dies as temperatures drop at the end of the season and seeds overwinter in the sediment until the cycle begins again the following spring (Grava and Raisanen 1978; LaFond-Hudson et al.

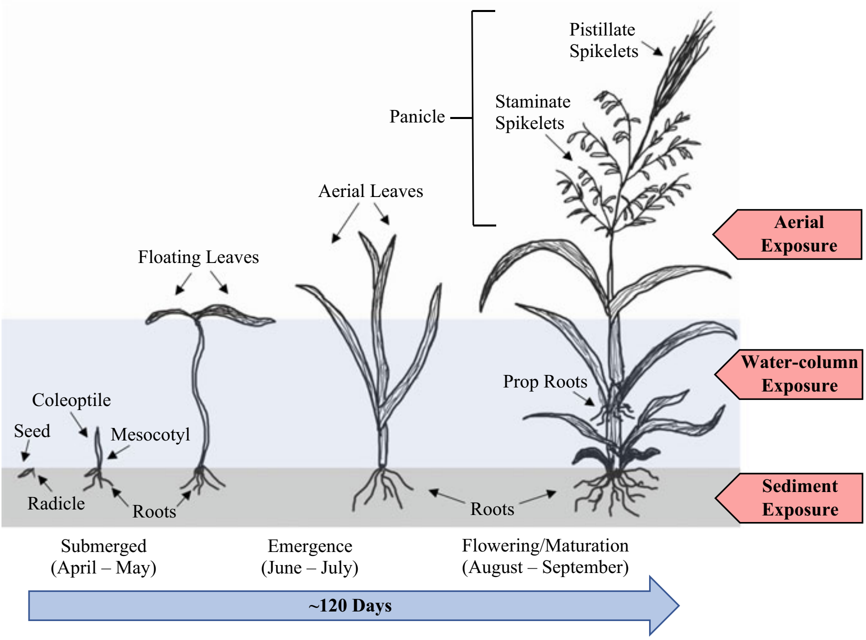

Fig. 5.2 The life cycle of northern wild rice (*Zizania palustris*). Seeds germinate in the sediment, with the development of the primary root (radicle). The mesocotyl then emerges from the sediment and elevates the coleoptile, which sheathes the emerging shoot and first leaf. This is followed by the floating-leaf stage, with further development of the roots. Next is the aerial leaf stage, where the plant begins to emerge out of the water. Then, at the mature plant stage, prop roots arise from the stem to provide additional support, and there is the development of the panicle, comprised of staminate spikelets (male florets) and pistillate spikelets (female florets). Potential contaminant exposure pathways are highlighted in red

2018; Sims et al. 2012). The plant requires approximately 120 days to reach maturity from germination (Archibold 2003). Seeds will penetrate the upper 2–5 cm of the sediment after falling through the water column, and this is where seed germination and early seedling growth (e.g., development of primary root and shoot) occurs the following spring (Pastor et al. 2017). Young plants are submerged for their first 3 to 4 weeks of growth and then long, thin leaves reach the top of the water column as they enter the floating-leaf stage (Archibold 2003). The floating leaves fix carbon into carbohydrates for root production and subsequently nutrient (e.g., nitrogen and phosphorous) uptake (Pastor et al. 2017). They will then progress to the aerial stage, with the stem emerging out of the water, and then as they enter the reproductive cycle, the panicle emerges, the stem elongates, the flowers develop for pollination, and the seeds begin to mature prior to senescence (Duquette and Kimball 2020).

5.3 Wild Rice Ecological Relevance

Wild rice plays an integral role in the structure and function of freshwater ecosystems. Emergent angiosperms (such as wild rice) are highly productive macrophytes, due to the abundance of available water and nutrients in sediments compared to floating macrophytes, and the greater availability of atmospheric carbon dioxide and oxygen compared to submerged macrophytes (Wetzel 1975). Emergent macrophytes are often found within the littoral region of small and shallow lakes, and as such are a major source of organic matter synthesis, contributing significantly to the productivity and metabolism regulation of the whole lake ecosystem (Wetzel 1975). By converting carbon dioxide and solar energy to organic matter via primary production, they provide food and habitat resources for herbivores, omnivores, and detritivores in aquatic ecosystems (Arts et al. 2008, 2010; Fairchild et al. 1998; Wetzel 1975). Wild rice is a vital food source for waterfowl, muskrats, beavers, moose, and other wildlife (Aagaard et al. 2019; Archibold 2003; Crow and Hellquist 2006; Fort et al. 2014; Myrbo et al. 2017; Pastor et al. 2017). Wild rice also provides habitat and shelter for both aquatic and terrestrial organisms, as the dense monotypic stands hide them from predators (Lewis 1995; Myrbo et al. 2017; Pastor et al. 2017). Wild rice stands are especially valuable resources for migrating waterfowl and other wetland birds, as they provide direct (e.g., consumption of seeds, flowers, young shoots, leaves, and mature stems) and indirect forage (e.g., consumption of nearby invertebrates), roosting habitat during migration, and nesting habitat for breeding (Aagaard et al. 2019). For instance, wild rice is a primary dietary constituent of mute swans (*Cygnus olor*), Canada geese (*Branta canadensis*), and red-winged blackbirds (*Agelaius phoeniceus*) (Bailey et al. 2008; Haramis and Kearns 2007; Meanley 1961), and the preferred food of soras (*Porzana carolina*), with wild rice comprising up to 94% of their fall diet (Webster 1964).

In addition to primary productivity, emergent macrophytes, such as wild rice, contribute to the biogeochemical cycling and structural complexity of aquatic ecosystems (Carpenter and Lodge 1986; Lemly et al. 1999; Lewis and Thursby 2018). The emergent leaves of wild rice reduce light availability to submerged macrophytes and algae, reduce water column circulation, and the shading provided by the leaves may reduce water temperatures (Carpenter and Lodge 1986; Lemly et al. 1999). The roots and shoots of wild rice stabilize sediments, introduce structural components (e.g., cellulose and lignin) to the detrital pool, and may enhance or reduce mineral uptake and release into aquatic ecosystems (Carpenter and Lodge 1986; Diepens et al. 2017; Fairchild et al. 1998; Lemly et al. 1999; Lewis 1995; Wetzel 1975). The roots of wild rice create a redox interface, which cycles nitrogen, sulfur, iron, and other metals (LaFond-Hudson et al. 2018). As oxygen is transported from the atmosphere to the roots, an aerobic rhizosphere develops from the radial oxygen loss of the roots, which may result in the sequestration of heavy metals due to the high adsorption capacity of iron hydroxides that may form as iron plaque on the root (Jorgenson et al. 2013).

These iron root plaques may also function to sequester nutrients, such as phosphorus, which has implications on bioremediation efforts in eutrophic systems (Jorgenson et al. 2013). Wild rice plays clear structural and functional roles, as well as in the suppling of essential ecosystem services.

5.4 Wild Rice Socio-Cultural and Economic Importance

Northern wild rice, or manoomin as it is called by the Anishinaabe First Peoples of North America, is an important food resource for Indigenous communities that has been traditionally harvested across North America for thousands of years (Ahmad et al. 2018; Aiken et al. 1988; Archibold 2003; Crow and Hellquist 2006; Fort et al. 2014; Porter 2019). Manoomin is often translated as "the good fruit" or "the good berry" in Anishinaabemowin or Ojibwemowin (David et al. 2019). They are a nutritious staple that is high in carbohydrates, proteins, vitamins (e.g., riboflavin), minerals (e.g., potassium and zinc), antioxidants, and dietary fiber, while also having a low-fat profile (Ahmad et al. 2018; Aiken et al. 1988; Fort et al. 2014; Surendiran et al. 2014). Within the traditional diet, manoomin was overall more nutritious than any other food available, and despite the labor-intensive process of harvesting and finishing, grains were seasonally abundant, and could be preserved for extensive periods of time (e.g., over the winter, when other foods are scarce) (David et al. 2019; Vennum Jr 1988). Manoomin is an integral part of the lives of the Anishinaabeg, and is often the first food given to children and the last food given to elders (David et al. 2019; Vennum Jr 1988; Vizenor 2008).

Manoomin holds strong spiritual and cultural significance and remains part of many ceremonies (as both a sacred food and medicine) and legends (Archibold 2003; David et al. 2019; Vennum Jr 1988). According to the sacred migration story of the Anishinaabeg, a prophet long ago beheld a vision from the Creator calling the Anishinaabeg to move west until they found the place "where food grows on the water" (Vizenor 2008). This journey led them to find the wild rice stands of the Great Lakes region. For generations, the Anishinaabeg of the western Great Lakes and upper Mississippi region have understood their connection to Anishinaabe Akiing (the land of the people) and the significance of manoomin as a gift from the Creator (Vizenor 2008). In the words of White Earth Tribal Historian Andy Favorite (as told by Erma Vizenor, former Chairwoman of the White Earth Nation), "Wild rice is part of our prophecy, our process of being human, our process of being Anishinaabe … we are here because of the wild rice. We are living a prophecy fulfilled" (Vizenor 2008).

Northern wild rice is connected to the identity, culture, religion, and livelihood of the Anishinaabeg (Vizenor 2008). Wild rice is still harvested using traditional methods, with one person poling a canoe through the dense aquatic stands, while another knocks ripe seeds from the stems using ricing sticks, with many seeds also intentionally knocked into the water to ensure re-seeding for the following year (Archibold 2003; Grombacher et al. 1996; Porter 2019). Other Indigenous groups, such as the Cree and Dene, have actively managed natural and planted stands of wild rice as their livelihoods (Grombacher et al. 1996). Though, by the end of the nineteenth century, wild rice as a commodity was of interest to non-Indigenous groups. Initially, brokers sought control of processing and sales, and then farmers and other planters attempted to gain control of the industry (Archibold 2003). After repeated attempts of Indigenous communities highlighting the significance of wild rice during treaty negotiations, several federal and state laws in the United States and legislation in Canada were passed, specifying the amount that can be commercially harvested, the type of equipment used, and Indigenous involvement in wild rice production (Archibold 2003). Though, due to high commodity prices and increased commercial demand in the 1970s, artificial paddies were rapidly established to enhance production (Archibold 2003).

The majority of wild rice production now occurs in artificial paddies, and with recent interest in their health-promoting properties (e.g., high in nutrients, with antioxidant and cholesterol-lowering effects), the commercial harvesting industry holds significant economic values (Fort et al. 2014; Surendiran et al. 2014). Wild rice has been cultivated in paddies since the early 1950s, and is still undergoing domestication as a crop (Porter 2019). Wild rice was initially cultivated in Minnesota, but with the recent commercial exploitation, production of the crop has extended beyond their natural range to California, Oregon, Saskatchewan, and has been established outside of North America in Australia, Finland, and Hungary (Ahmad et al. 2018; Archibold 2003; Malvick and Percich 1993; Porter 2019). Globally, the production and demand for wild rice is continuing to rise, likely due to their unique properties, such as their nutritional values, long shelf-life, versatility in food dishes, food-processing potential (e.g., wild rice blended with precooked meat has reduced cook times and enhance nutritional properties), use of presently discarded hulls (e.g., in the adhesive, paper, and textile industries), and the ability to re-seed themselves once established, unlike other commercial crops (Ahmad et al. 2018; Archibold 2003; Porter 2019; Surendiran et al. 2014).

5.5 Review of the Current State of Northern Wild Rice Ecotoxicology

5.5.1 Background

Toxicology test methods used in studies must be reported with sufficient detail for the experimental setup and procedures to be replicated effectively by other researchers. As well, inadequate reporting in peer-reviewed literature could result in the exclusion of data from formal ecological risk assessments due to uncertainty related to data quality. These concerns surrounding reliability and completeness of methodological reporting in the ecotoxicology literature are not unusual or limited to macrophytes (Ågerstrand et al. 2011; Hanson et al. 2017). Therefore, the need for direct, precise, and transparent methodology reporting must be a priority if northern wild rice is to be more widely adopted as a test species. Currently and to our knowledge, no standard test method exists for wild rice in toxicology studies. Therefore, contrasting between wild rice studies, and wild rice with other species, is difficult or not possible without clear methodological reporting, and ideally consistent methods across tests in general.

This section collates and summarizes current toxicity test methods for northern wild rice (as of 2021). The totality of the peer-reviewed scientific literature was systematically evaluated to outline similarities and differences in basic methodological techniques and reporting. Gaps were identified and direction is given on how to approach the growth and maintenance of this species for future testing. The effects that test compounds have on the wild rice were beyond the scope of this review. The aim was to identify areas in need of further research and standardization to effectively allow the use of *Z. palustris* in ecotoxicology.

5.5.2 Methods

5.5.2.1 Literature Search

Our focus was on studies of northern wild rice toxicity tests that were conducted in a laboratory, an indoor area (such as a greenhouse), or outdoor mesocosms (e.g., simulated wetland enclosures). The databases Google Scholar, Scopus, Web of Science, and University of Manitoba Library Services were utilized to search for articles related to wild rice ecotoxicology. Search queries commenced with "Wild rice OR Zizania palustris", and then became more specific including, "wild rice toxicology testing" and "wild rice stratification". Additional articles were also found by reviewing references in relevant wild rice articles. Alerts were set up on Google Scholar and Web of Science using key words such as "Wild Rice", "Zizania palustris", and "Wild Rice Toxicity Testing". The search was completed by April 2021. The selection criteria for the inclusion of articles in this review were:

1. Must use northern wild rice (*Z. palustris*) as test organism
2. Toxicity test conducted in a laboratory, indoor area (greenhouse), or a mesocosm
3. Written in English language only
4. Peer-reviewed article published in a scientific journal by a recognized database

For the purposes of this chapter, single published papers within a scientific journal are referred to as an article, while separate experiments conducted within an article are referred to as studies, as an article may contain multiple types of studies. The criteria used to distinguish between an article and a study revolved around if the experiments in question were: (1) conducted at separate times; (2) independent control organisms were used; and (3) if any component of the study design was changed.

5.5.2.2 Methodology Assessments

The review was organized as a list of questions in five categories: (1) test organism; (2) test conditions; (3) test media; (4) experimental design; (5) and test performance (Fig. 5.3). These categories pertained directly to elements that would allow for effective replication and data quality assessment of any experiment. Metadata were extracted from each study, covering contributing authors, the scientific journal, test compounds with their accompanying concentration, and whether the experiment was laboratory, greenhouse, or outdoor mesocosm based. Questions were generated with direction from the ASTM (American Society for Testing and Materials) International E1841-04 Standard Guide for conducting renewal phytotoxicity tests with freshwater emergent macrophytes (ASTM 2012), as well as previous reviews of data reliability for primary producer toxicity literature (Hanson et al. 2019). The guide provided key details that "must be met", and requirements that were relevant to wild rice toxicity testing design or methods were considered and incorporated. For instance, the ASTM guide requires that plant test organisms used must be the same age and collected from the same source.

Laboratory and mesocosm studies were addressed separately for certain aspects (e.g., growth chamber settings) that were not applicable across study types. The test organism section first identified the source of wild rice seeds or plants either by collection location or by supplier to satisfy the ASTM requirements (ASTM 2012). Depending upon if seeds were purchased from a supplier or harvested, storage conditions prior to and post-purchasing were collected to assess viability (Kovach and Bradford 1992). The remainder of the section focused largely on stratification techniques. Stratification is a crucial component for the germination of wild rice, as it is a process used to simulate the natural overwintering conditions necessary to break seed dormancy (Baskin and Baskin 2014).

Test conditions pertained to such elements as growth chambers and vessels used to house the plants. The photoperiod and temperature are fundamental conditions for replication of the experiment. In-depth questions on vessel structure, size, and rooting substrates were included, as the ASTM requirements outlined that the vessel should be large enough to prevent the plant from becoming root bound (ASTM 2012). Test media looked specifically at the composition of nutrient solutions used to support adequate plant growth, and what type of water source was used for dilution. A subsection was also created in the case that a nutrient solution was not used, common with mesocosm experiments, in which only water conditions were addressed.

Experimental design was related to setup procedures, such as numbers of test organisms per replicate and replicate numbers. Maintenance of the test conditions, such as if the test organisms spent time outside the growth chamber, were also covered as exposure to different surroundings can influence growth; ASTM requirements stress the importance of consistency within an experiment (ASTM 2012). The test performance category was solely focused on controls and potential contamination of the system throughout the duration of the study. Test performance criteria for

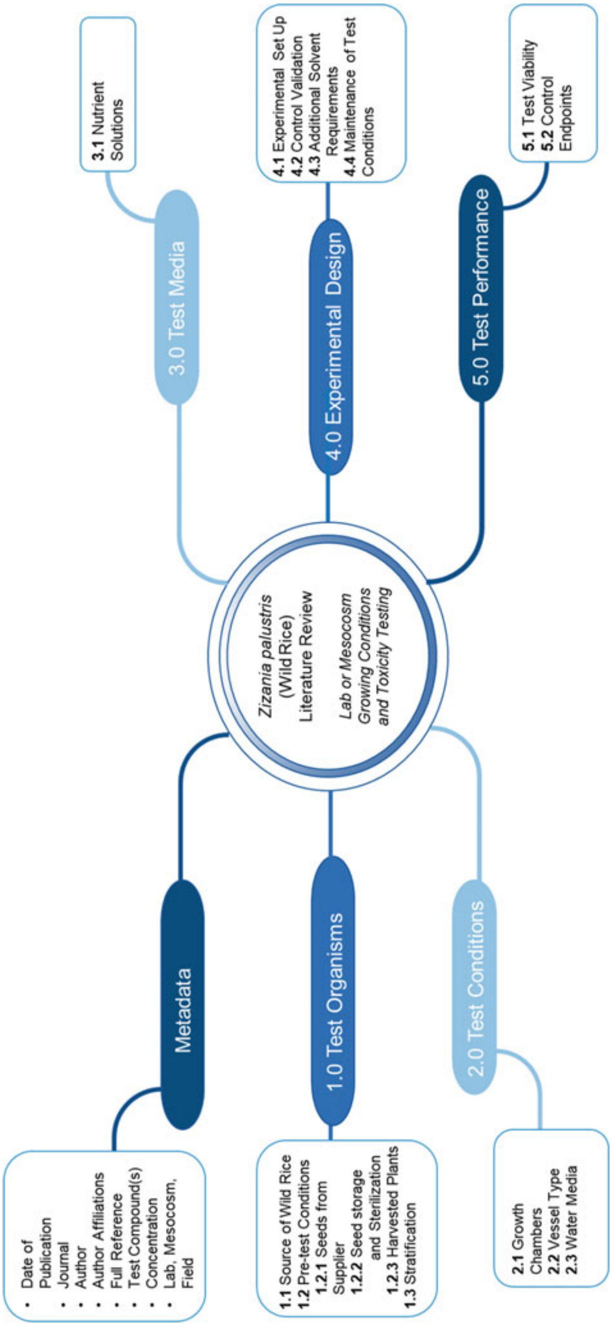

Fig. 5.3 Conceptual model of review categories and subcategories for peer-reviewed wild rice toxicity tests

controls were recorded as either qualitatively and/or quantitatively as applicable, as the success of controls provides an indicator of method viability. Specific questions concerning wild rice toxicity outcomes in response to tested compounds were not included, as this was beyond the scope of the review.

5.5.3 Results

5.5.3.1 Overview of Articles and Studies Reviewed

The search returned 11 published articles that met the inclusion criteria, and of those, 22 unique studies were identified and individually assessed. The majority of articles were published in the last decade (2011–2021). Overall, there were six outdoor mesocosm and 16 laboratory or indoor studies conducted within the total articles collected; therefore, laboratory or indoor experiments were the dominant type of experiment. Due to the nature of control in these types of experiments (laboratory or indoor vs. mesocosm), they were compared separately for certain components of study design, and the breakdown of results was presented independently.

5.5.3.2 Summary of Seed Harvest and Preparation

Half of the studies ($n = 11$) utilized northern wild rice seeds as the initial test organism, with the remaining half using seedlings or mature plants (Fig. 5.4). All studies conducted in mesocosms ($n = 6$) used seeds, and then allowed the plant to complete successive life cycles, which produced seeds fueling the successive generations. Durkee Walker et al. (2006) was the only mesocosm study to use both seeds and seedlings. The source of seeds (e.g., harvested or purchased) was not reported in all studies ($n = 3$ did not report), but of the studies that did report source, all were obtained by harvesting from natural stands ($n = 19$). Locations for the harvesting of wild rice seeds were all within the native growing range of the species, but at times vaguely stated (e.g., Central Minnesota by Malvick and Percich [1993]). None of the studies reported using commercial suppliers.

Only 27% of the studies ($n = 6$) provided information on seed sterilization or debris removal techniques. Fort et al. (2014, 2017) used a sieve with mesh to remove unwanted debris and the four studies within Nimmo et al. (2003) used deionized water to rinse harvested seeds. No indoor or laboratory studies indicated use of any sterilization techniques on the seeds to remove potential pathogens prior to experimental use.

Seed storage conditions were generally inadequately reported. Explicit information on storage conditions or time frames were limited. For example, Pastor et al. (2017) stated that storage of seeds occurred until needed for experiments, but did not include information such as temperature, light, or humidity, leading to questions about possible decline in seed viability over time. Fort et al. (2014, 2017, 2020),

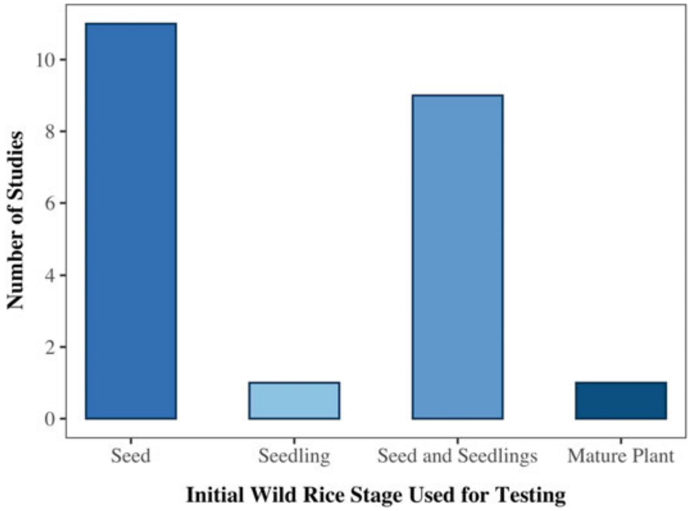

Fig. 5.4 Initial growth stage of northern wild rice (*Zizania palustris*) utilized for laboratory or indoor and mesocosm experiments from 22 ecotoxicology studies

stored seeds at 4 °C in the dark, but did not include details on storage duration, vessel type, or if water media was used in the three studies. LaFond-Hudson et al. (2018) indicated a one-year storage period, but did not include storage conditions. Storage of seed is not an experimental condition of the toxicity test itself; however, the literature should acknowledge this step, especially over long durations of times (months to years), to ensure the viability of test organisms. Ultimately, it is akin to the culturing of test organisms (along with stratification discussed below), which typically have detailed protocols that are followed. We concluded that all available studies provided insufficient information on all three of the following categories: seed acquisition, cleaning, and storage conditions.

Stratification

The conditions under which studies reported performing stratification of their northern wild rice seed is found in Table 5.1. The use of stratification was stated in 45% of the studies ($n = 10$). Of these 10 studies, 60% ($n = 6$) provided limited information, meaning they mentioned at least one component, such as duration of stratification, but failed to further expand on other necessary details to allow for replication. The four laboratory Nimmo et al. (2003) studies, which accounts for the remaining 40%, had sufficient information to replicate the process. They completed stratification by submerging a burlap sack into a lake; however, all were from the same article, and therefore, only one stratification approach was undertaken to germinate all the seeds used. Durkee Walker et al. (2006) and Sims et al. (2012) were the

Table 5.1 Summary of stratification data for the 22 studies conducted with *Zizania palustris*. Letter designations present the breakdown of studies within the article. N/R (Not Reported)

Study	Stratification performed	Temperature	Duration	Stratification vessel	Seed density or mass	Stratification location
Durkee Walker 2006*	Yes	2–4 °C	26 weeks	N/R	N/R	N/R
Durkee Walker 2010a*	N/R	N/R	N/R	N/R	N/R	N/R
Durkee Walker 2010b	N/R	N/R	N/R	N/R	N/R	N/R
Fort 2014	N/R	N/R	N/R	N/R	N/R	N/R
Fort 2017	N/R	N/R	N/R	N/R	N/R	N/R
Fort 2020	N/R	N/R	N/R	N/R	N/R	N/R
Johnson 2019*	N/R	N/R	N/R	N/R	N/R	N/R
LaFond-Hudson 2018*	N/R	N/R	N/R	N/R	N/R	N/R
Malvick 1993a	Yes	3.5 °C	12 weeks	N/R	N/R	N/R
Malvick 1993b	Yes	3.5 °C	12 weeks	N/R	N/R	N/R
Malvick 1993c	Yes	3.5 °C	12 weeks	N/R	N/R	N/R
Malvick 1993d	Yes	3.5 °C	12 weeks	N/R	N/R	N/R
Nimmo 2003a	Yes	N/R	8.5 weeks	Burlap sack	50 kg	Submerged 1.5 m from lake surface
Nimmo 2003b	Yes	N/R	8.5 weeks	Burlap sack	50 kg	Submerged 1.5 m from lake surface
Nimmo 2003c	Yes	N/R	8.5 weeks	Burlap sack	50 kg	Submerged 1.5 m from lake surface
Nimmo 2003d	Yes	N/R	8.5 weeks	Burlap sack	50 kg	Submerged 1.5 m from lake surface
Pastor 2017a	N/R	N/R	N/R	N/R	N/R	N/R
Pastor 2017b	N/R	N/R	N/R	N/R	N/R	N/R
Pastor 2017c	N/R	N/R	N/R	N/R	N/R	N/R
Pastor 2017d	N/R	N/R	N/R	N/R	N/R	N/R
Pastor 2017e*	N/R	N/R	N/R	N/R	N/R	N/R
Sims 2012*	Yes	4 °C	34.7 weeks	N/R	N/R	N/R

*Indicates a mesocosm study

only two studies conducted in mesocosms that report that the seeds were stratified before being added into the system. The remaining mesocosm studies state adding their seeds to the mesocosms in springtime without mention of stratification. Without the inclusion of information on seed harvesting (and therefore potential for natural stratification if done in early spring), nor information on stratification techniques, seeds used in a replication of this experiment may not reach sufficient temperature to break dormancy, and thus would be unsuccessful.

Stratification durations (Table 5.1) were highly variable and ranged from 60 days to approximately eight months. The maximum duration value reported, eight months, is approximate as Sims et al. (2012) indicated a date range of Fall 2008 to Spring 2009, with the sowing of seeds the following June 2009. Overall, stratification conditions were poorly reported. The average stratification temperature across the ten studies was 3.5 °C, but it was not indicated whether this was water or air temperature in six of the studies. Stratification conditions, such as vessel type, photoperiod, seed density, and media use, were also not reported in these six studies.

5.5.3.3 Test Conditions

All studies described the growth environment and housing vessels used in their experiments; the types of chambers and vessel data identified in each study are described in Table 5.2. ASTM guidelines for photoperiod with freshwater emergent macrophytes in growth chambers or greenhouses is 16 h of light (ASTM 2012). While all laboratory or indoor studies reported photoperiod values, consistent durations or justifications were not provided. Nimmo et al. (2003) used 12 h in three studies, and one from Durkee Walker et al. (2010) used a range of 10, 14, and then natural light exposure durations since they were contained in a greenhouse. All mesocosm studies were conducted outdoors, using natural light sources, but none reported use of light meters to confirm light levels.

The specifications surrounding types of test vessels, their measurements, and material type were well reported across all studies, with either the volume or dimensions of the test vessel(s) provided. Studies using sediment as substrate in mesocosm studies, as seen in Table 5.2, were all obtained from location of the water source where the wild rice seeds were harvested; however, in some of these studies, additional sand was added. It was not clear if this sand was naturally obtained or purchased from a commercial supplier. None of the laboratory or indoor experiments that used artificial substrates ($n = 4$) reported the substrate brand names or other characteristics of the materials. It should be noted that 44% ($n = 7$) of laboratory or indoor experiments failed to indicate whether a substrate was used.

Table 5.2 Summary of study design data for initial setup of all 22 studies conducted with *Zizania palustris*. Letter designations are added beside the year of an article to present the breakdown of studies within the article. N/R (Not Reported)

Study	Growth environment	Test vessels	Volume (L)	Substrate	Photoperiod	Test duration	Full life cycle	Number of test organisms/ replicate	Number of replicates/ treatments	Test compounds
Durkee Walker 2006*	Mesocosm	Stock tanks	378	Sediment & sand	Natural photoperiod	N/R	Yes	30	9	Straw litter
Durkee Walker 2010a*	Mesocosm	Stock tanks	378	Sediment & sand	Natural photoperiod	4 years	Yes	30 (thinned), N/R (not thinned)	N/R	Wild rice root and shoot litter
Durkee Walker 2010b	Greenhouse	Pots housed in pails	5 & 20	Sediment	10 h, 14 & natural photoperiod	16 weeks	Yes	1	4	Nitrogen & phosphorus
Fort 2014	Hydroponic chamber	Baskets housed in aquaria	N/R & 10	Inert mesh	16 light & 8 dark	21 days	No	60	4	Sulfate & chloride
Fort 2017	Exposed in laboratory	Baskets housed in aquaria	N/R & 10	Inert mesh	16 light & 8 dark	21 days	No	80	4	Sulfide
Fort 2020	Hydroponic tent	Baskets housed in aquaria	N/R & 10	Inert mesh	16 light & 8 dark	21 days	No	80	4	Sulfide
Johnson 2019*	Mesocosm	Stock tanks	457.5	Sediment	Natural photoperiod	4 years	Yes	N/R	6	Sulfate

(continued)

Table 5.2 (continued)

Study	Growth environment	Test vessels	Volume (L)	Substrate	Photoperiod	Test duration	Full life cycle	Number of test organisms/replicate	Number of replicates/treatments	Test compounds
LaFond-Hudson 2018*	Mesocosm	Pails housed in bucket	4 & 20	Sediment	Natural photoperiod	265 days	Yes	2	40	Sulfate
Malvick 1993a	Growth chamber	Pots with mesh bottom housed in buckets	0.025 & 6	Acetyl beads	16.5 light & 7.5 dark	6 weeks	N/R	27	2	Nutrient solutions
Malvick 1993b	Growth chamber	Buckets	6	N/R	16.5 light & 7.5 dark	8–9 weeks	N/R	7	5	Nutrient solutions
Study	Growth environment	Test vessels	Volume (L)	Substrate	Photoperiod	Test duration	Full life cycle	Number of test organisms/replicate	Number of replicates/treatments	Test compounds
Malvick 1993c	Growth chamber	Buckets	6	N/R	16.5 light & 7.5 dark	7–8 weeks	N/R	7	4, 6	Silicon
Malvick 1993d	Growth chamber	Buckets	6	N/R	16.5 light & 7.5 dark	5–6 weeks	N/R	7	5	Silicon & pathogen *B. oryzae*
Nimmo 2003a	Growth chamber	Petri dish	0.015	No substrate	12 light & 12 dark	10 days	No	1	3	Copper

(continued)

Table 5.2 (continued)

Study	Growth environment	Test vessels	Volume (L)	Substrate	Photoperiod	Test duration	Full life cycle	Number of test organisms/replicate	Number of replicates/treatments	Test compounds
Nimmo 2003b	Growth chamber	Petri dish	0.015	No substrate	12 light & 12 dark	14 days	No	1	8	Copper
Nimmo 2003c	Growth chamber	Petri dish	0.015	No substrate	12 light & 12 dark	14 days	No	1	12	Copper
Nimmo 2003d	Growth chamber	Petri dish	0.015	No substrate	12 light & 12 dark	14 days	No	1	8	Copper
Pastor 2017a	Growth chamber	Mason jars	0.473	N/R	All dark	11 days	No	50	3	Sulfate
Pastor 2017b	Growth chamber	Bottles	0.7	N/R	All dark	11 days	No	50	3	Sulfide
Pastor 2017c	Growth chamber	Kimax tubes	0.07	N/R	16 light & 8 dark	10 days	No	1	20	Sulfate
Pastor 2017d	Growth chamber	Bottles	0.125	N/R	16 light & 8 dark	10 days	No	7	3	Sulfide
Pastor 2017e*	Mesocosm	Stock tanks	400	Sediment & sand	Natural photoperiod	5 years	Yes	30	6	Sulfate
Sims 2012*	Mesocosm	Gusseted bags and stock tanks	30.5 & 1693	Sediment & sand	Natural photoperiod	131 days	Yes	2	N/R	Nitrogen & phosphorus

*Indicates a mesocosm study

Nutrient Solutions and Other Media

In total, 11 studies (50% of all; 69% of laboratory or indoor studies) reported using a standardized nutrient solution in their experimental procedures. Modified Hoagland's solution was the only standardized nutrient solution used among laboratory or indoor studies. All mesocosm studies indicated reliance of natural sediment from northern wild rice stands to provide nutrients instead. While each study using the modified Hoagland's solution reported it as such, the modifications (e.g., concentrations and recipes) differed between studies. For example, four of the Pastor et al. (2017) studies indicated a 1/5 strength Hoagland's solution, while Fort et al. (2014, 2017, 2020) used a modified Hoagland's solution with 25% ammonium (molar basis) in a mixture of ammonium and nitrate.

The laboratory or indoor experiments that did not use a standardized solution either had a short test duration (ten days in the case of studies a-d in Nimmo et al. 2003) or had nitrogen and phosphorus as the test compound (Durkee Walker et al. 2006) and, therefore, did not require additions to prevent nutrient deficiency. No studies autoclaved their nutrient solutions and none used an additional solvent to add a test compound, other than water. As seen in Table 5.3, pH and type of water diluent were not reported in various laboratory or indoor studies utilizing a standardized solution. These types of inconsistencies between studies of the same article were not uncommon. Mesocosm studies used either groundwater or well water for the filling of system; however, none of the studies stated if a characterization for nutrients occurred. Water volume levels used in the mesocosms were all reported.

5.5.3.4 Experimental Design and Performance

General experimental design weaknesses in the overall dataset were the lack of clarity on replicate and treatment numbers, endpoint rationales, and control performance. Three studies were missing information in regard to the number of test organisms per replicate or the replicates per treatment. Of these three studies, either the number of test organisms per replicate, or the number of replicates per treatment were indicated, but not both. All six mesocosm studies allowed their northern wild rice to complete a full life cycle and used seed production as a test endpoint.

Control Validation and Standards

Overall, 90% ($n = 20$) of the studies reported use of controls; however, of these 20 studies, only three had clearly stated control standards (i.e., expectations around performance). Reported control standards were 95% seed activation, 30% mesocotyl emergence, 90% control survival, and boron control >80% phytotoxicity (Fort et al. 2014, 2017, 2020); standards were met in all three studies. Of the 22 studies, only the same three (14%) Fort et al. (2014, 2017, 2020) experiments used a positive control, boron from boric acid, for the purpose of validating the experimental procedure and were all laboratory or indoor experiments. No citation was provided to support the

Table 5.3 Summary of laboratory or indoor study data that used standardized test solutions. Letter designations are added beside the year of an article to present the breakdown of studies within the article. N/R (Not Reported)

Study	Standardized test solution	pH	Type of water dilutant	Dissolved oxygen measured
Fort 2014	Modified Hoagland	6.1–7.2	Deionized	Yes
Fort 2017	Modified Hoagland	6.0–7.5 ± 0.5	Deionized	Yes
Fort 2020	Modified Hoagland	6.3–7.4 ± 0.3	Deionized	Yes
Malvick 1993a	Modified Hoagland	N/R	Deionized	N/R
Malvick 1993b	Modified Hoagland	N/R	Deionized	N/R
Malvick 1993c	Modified Hoagland	5	Distilled	N/R
Malvick 1993d	Modified Hoagland	5	Distilled	N/R
Pastor 2017a	1/5 Hoagland	6.8 ± 0.3	N/R	Yes
Pastor 2017b	1/5 Hoagland	6.8 ± 0.3	N/R	No
Pastor 2017c	1/5 Hoagland	6.8 ± 0.3	N/R	N/R
Pastor 2017d	1/5 Hoagland	6.8 ± 0.3	N/R	N/R

use of boron as a positive control, but its known plant toxicant properties were stated in Fort et al. (2014, 2017); though, in the 2020 study, the author's previous two experiments were cited as rationale for its use.

5.5.4 Discussion

This review was performed to assess key procedures and design gaps related to ecotoxicological experiments on northern wild rice (*Z. palustris*). In doing so, we hope to improve scientific reporting and direct future research. While relatively few articles were found in the peer-reviewed literature ($n = 11$), it is clear that key methodological components were missing across all articles for these experiments. This highlights the significant data reliability and replication issues within the field, and hinders the adoption of the species more widely within ecotoxicology.

Ideally, test methods should focus on sensitive and ecologically relevant endpoints that allow for sufficient and conservative extrapolations to the field, which may include expanding the range of standard test endpoints beyond growth and biomass measures (Hanson and Arts 2007). Growth measurements are relatively easy to quantify, have been widely applied under both laboratory and field conditions, and are useful for integrating overall effects of toxicants on macrophytes; however, they lack specificity (Lemly et al. 1999). Responses such as reduced growth rates, or growth inhibition, do not indicate which specific sites or mechanisms are being affected by a particular toxicant. This is particularly notable for rooted macrophytes, where it can be difficult to assess if toxic responses are occurring due to sediment or water column exposure. Other common test methods include measurements of biomass (dry and wet), chlorophyll-α concentrations, chloroplast morphology, photosynthetic rate, enzyme activity, reproduction, seed germination, seedling growth, and root growth (Hanson 2013; Lemly et al. 1999). There are ranges of variability, sensitivity, and relevance within macrophyte toxicity testing endpoints, though root endpoints have been found to be among the most sensitive (Arts et al. 2008). This further highlights the need for macrophyte toxicity tests to encompass an array of endpoints to maximize protection when assessing the risk to nontarget organisms. We suggest that laboratory studies and test development with northern wild rice (*Z. palustris*) should focus on seed germination assays, as well as root and shoot endpoints as a first possible step toward a standardized toxicity test.

5.5.4.1 Major Weaknesses in Studies

Overall, the extent of stratification data was weak. If studies did report information, it was limited in terms of its completeness. With greater than 50% of the studies failing to indicate a stratification process, it prevents full replication of the designated experiment as readers could be unaware that stratification is a required process. The feasibility of the outdoor Nimmo et al. (2003) stratification technique is also a concern, and other means of this process should be still determined. While some studies alluded to the fact, the range of limited data supports the idea that no consensus of laboratory stratification procedures exists. Storage conditions, and use of a storage period, were also poorly reported, and we detected ambiguity in the entirety of the test organism information reported.

Another key methodology weakness in the overall dataset was the lack of any control performance standards. Few studies set criteria for control performance, making assay reliability highly uncertain. This is also concerning as control standards are needed to eliminate possible background effects. Therefore, none of the studies contained sufficient information to fully replicate the experiments, as either stratification, storage conditions, or control standards were absent or limited.

5.5.4.2 Recommendations for Improving the Reporting and Testing Within Northern Wild Rice Literature

To address this, we recommend those performing wild rice tests to:

1. Explicitly describe critical factors related to seed source, stratification, and seed storage.

In terms of wild rice, these two factors are crucial for seed viability and germination. The stratification technique used is particularly important to include, as no perceived standard method currently exists. The performance of various approaches will need to be assessed and contrasted to ensure selection of best practices regardless of the lab where a test is performed.

2. State control performance and whether requirements were met.
   Control performance helps to validate a study and excluding this information results in significant uncertainty in the data. Therefore, any control information in regards to experimental design should also be explicitly stated. Expectations around control performance need to be determined in order to ensure adequate test conduction.
3. Report all basic experimental conditions and design elements.
   An experimental conditions summary table, as seen in Fort et al. (2014, 2017, 2020), would be useful to readers for understanding how the study was performed. Checklists are an effective means for authors to confirm all essential information for replication is included in the paper.

5.6 Summary and Conclusions

Wild rice (*Zizania* spp.) presents themselves as a suitable candidate for inclusion into the battery of test species for risk assessment. They meet all six criteria of an appropriate test organism to varying degrees (Rand et al. 1995), as they are: (i) sensitive to a range of exposure types and contaminants; (ii) abundant and available in their natural range; (iii) indigenous to impacted ecosystems within North America; (iv) ecologically, culturally, and economically important; (v) amenable to routine maintenance in the laboratory for both acute and chronic toxicity tests; and (vi) have adequate background information on their physiology and life history.

We feel that risk assessments with wild rice will be most useful in North American contexts within their natural range and in situations where Indigenous concerns are paramount. Still, a significant amount of work is needed to advance wild rice toxicity testing by improving methods and reliability prior to wider adoption for ecological risk assessment, as noted by the results of this literature review.

References

Aagaard K, Eash J, Ford W, Heglund PJ, McDowell M, Thogmartin WE (2019) Modeling the relationship between water level, wild rice abundance, and waterfowl abundance at a central North American wetland. Wetlands 39:149–160. https://doi.org/10.1007/s13157-018-1025-6

Ågerstrand M, Breitholtz M, Rudén C (2011) Comparison of four different methods for reliability evaluation of ecotoxicity data: a case study of non-standard test data used in environmental risk assessments of pharmaceutical substances. Environ Sci Eur 23:17. https://doi.org/10.1186/2190-4715-23-17

Ahmad F, Hameed M, Ahmad MSA (2018) Exploring potential of minor/underutilized grasses for remote areas facing food scarcity. In: Ozturk M, Hakeem KR, Ashraf M, Ahmad MSA (eds) Global perspectives on underutilized crops. Springer International, New York, NY, USA, pp 189–206. https://doi.org/10.1007/978-3-319-77776-4_7

Aiken SG, Lee PF, Punter D, Stewart JM (1988) Wild rice in Canada. Agriculture Canada. Publication 1830. NRC Press, Toronto, ON, Canada, p 130

Archibold OW (2003) Wild rice. In: Caballero B, Trugo L, Finglas PM (eds) Encyclopedia of food sciences and nutrition, 2nd edn. Elsevier Academic Press, Amsterdam, The Netherlands, pp 6183–6189. https://doi.org/10.1016/B0-12-227055-X/01294-3

Archibold OW, Sutherland JM, Good AG (1989) Annual variation in wild rice (*Zizania palustris* L.) growth and potential yield in Saskatchewan. Can J Plant Sci 69:653–665. https://doi.org/10.4141/cjps89-081

Arts GHP, Belgers JDM, Hoekzema CH, Thissen JTNM (2008) Sensitivity of submersed freshwater macrophytes and endpoints in laboratory toxicity tests. Environ Pollut 153:199–206. https://doi.org/10.1016/j.envpol.2007.07.019

Arts GHP, Davies J, Dobbs M, Ebke P, Hanson M, Hommen U, Knauer K, Loutseti S, Maltby L, Mohr S, Poovey A, Poulsen V (2010) AMEG: the new SETAC advisory group on aquatic macrophyte ecotoxicology. Environ Sci Pollut Res 17:820–823. https://doi.org/10.1007/s11356-010-0309-z

ASTM (2012) Standard guide for conducting renewal phytotoxicity tests with freshwater emergent macrophytes. ASTM Standard E1841-04. American Society for Testing and Materials, Philadelphia, PA, USA.

Bailey M, Petrie SA, Badzinski SS (2008) Diet of mute swans in lower Great Lakes coastal marshes. J Wildl Manag 72:726–732. https://doi.org/10.2193/2007-133

Barkworth M, Capels K, Long S, Anderton L, Piep M (2007) Magnoliophyta: Commelinidae (in part): Poaceae, part 1: North of Mexico. Flora of North America. Oxford University Press, New York, NY, USA, p 48

Baskin CC, Baskin JM (2014) Seeds: ecology, biogeography, and evolution of dormancy and germination, 2nd edn. Elsevier Academic Press, San Diego, CA, USA

Carpenter SR, Lodge DM (1986) Effects of submersed macrophytes on ecosystem processes. Aquat Bot 26:341–370. https://doi.org/10.1016/0304-3770(86)90031-8

Crow GE, Hellquist CB (2006) Aquatic and wetland plants of northeastern North America, volume II: angiosperms: monocotyledons: a revised and enlarged edition of Norman C. Fassett's *A Manual of Aquatic Plants*. University of Wisconsin Press, Madison, WI, USA, p 400

David P, David L, Kiiwetinepinesiik Stark H, Stark KJ, Niso-Asin Fahrlander S, Manidoonoodin Schlender J (2019) Manoomin. Version 1.0. Great Lakes Indian Fish and Wildlife Commission, p 158

Diepens NJ, Koelmans AA, Baveco H, van den Brink PJ, van den Heuvel-Greve MJ, Brock TCM (2017) Prospective environmental risk assessment for sediment-bound organic chemicals: a proposal for tiered effect assessment. In: de Voogt P (ed) Reviews of environmental contamination and toxicology, vol 239. Springer International, New York, NY, USA, pp 1–77. https://doi.org/10.1007/398_2015_5004

Duquette J, Kimball JA (2020) Phenological stages of cultivated northern wild rice according to the BBCH scale. Ann Appl Biol 176:350–356. https://doi.org/10.1111/aab.12588

Durkee Walker R, Pastor J, Dewey BW (2006) Effects of wild rice (*Zizania palustris*) straw on biomass and seed production in northern Minnesota. Can J Bot 84:1019–1024. https://doi.org/10.1139/b06-058

Durkee Walker R, Pastor J, Dewey BW (2010) Litter quantity and nitrogen immobilization cause oscillations in productivity of wild rice (*Zizania palustris* L.) in northern Minnesota. Ecosystems 13:485–498. https://doi.org/10.1007/s10021-010-9333-6

Duvall MR, Biesboer DD (1988) Anatomical distinctions between the pistillate spikelets of the species of wild-rice (*Zizania*, Poaceae). Am J Bot 75:157–159. https://doi.org/10.1002/j.1537-2197.1988.tb12170.x

Fairchild JF, Ruessler DS, Carlson AR (1998) Comparative sensitivity of five species of macrophytes and six species of algae to atrazine, metribuzin, alachlor, and metolachlor. Environ Toxicol Chem 17:1830–1834. https://doi.org/10.1002/etc.5620170924

Fort DJ, Mathis MB, Anderson K, Beranek R (2020) Impact of hydroponic oxygen control in sulfide toxicity to early life stages of wild rice (*Zizania palustris*). Environ Toxicol Chem 39:659–666. https://doi.org/10.1002/etc.4653

Fort DJ, Mathis MB, Walker R, Tuominen LK, Hansel M, Hall S, Richards R, Grattan SR, Anderson K (2014) Toxicity of sulfate and chloride to early life stages of wild rice (*Zizania palustris*). Environ Toxicol Chem 33:2802–2809. https://doi.org/10.1002/etc.2744

Fort DJ, Todhunter K, Fort TD, Mathis MB, Walker R, Hansel M, Hall S, Richards R, Anderson K (2017) Toxicity of sulfide to early life stages of wild rice (*Zizania palustris*). Environ Toxicol Chem 36:2217–2226. https://doi.org/10.1002/etc.3759

Grava J, Raisanen KA (1978) Growth and nutrient accumulation and distribution in wild rice. Agron J 70:1077–1081. https://doi.org/10.2134/agronj1978.00021962007000060044x

Grombacher AW, Porter RA, Everett LA (1996) Breeding wild rice. In: Janick J (ed) Plant breeding reviews, pp 237–265. https://doi.org/10.1002/9780470650073.ch8

Hanson ML (2013) Aquatic macrophytes in ecotoxicology. In: Férard JF, Blaise C (eds) Encyclopedia of aquatic ecotoxicology. Springer, Dordrecht, Netherlands, pp 89–98. https://doi.org/10.1007/978-94-007-5704-2_9

Hanson ML, Arts GHP (2007) Improving regulatory risk assessment—using aquatic macrophytes. Integr Environ Assess Manag 3:466–467. https://doi.org/10.1002/ieam.5630030321

Hanson ML, Baxter L, Anderson J, Solomon K, Brain R (2019) Strength of methods assessment for aquatic primary producer toxicity data: a critical review of atrazine studies from the peer-reviewed literature. Sci Total Environ 685:1221–1239. https://doi.org/10.1016/j.scitotenv.2019.04.336

Hanson ML, Wolff BA, Green JW, Kivi M, Panter GH, Warne MSJ, Ågerstrand M, Sumpter JP (2017) How we can make ecotoxicology more valuable to environmental protection. Sci Total Environ 578:228–235. https://doi.org/10.1016/j.scitotenv.2016.07.160

Haramis GM, Kearns GD (2007) Herbivory by resident geese: the loss and recovery of wild rice along the tidal Patuxent River. J Wildl Manag 71:788–794. https://doi.org/10.2193/2006-350

Hass BL, Pires JC, Porter R, Phillips RL, Jackson SA (2003) Comparative genetics at the gene and chromosome levels between rice (*Oryza sativa*) and wildrice (*Zizania palustris*). Theor Appl Genet 107:773–782. https://doi.org/10.1007/s00122-003-1273-5

Johnson NW, Pastor J, Swain EB (2019) Cumulative sulfate loads shift porewater to sulfidic conditions in freshwater wetland sediment. Environ Toxicol Chem 38:1231–1244. https://doi.org/10.1002/etc.4410

Jorgenson KD, Lee PF, Kanavillil N (2013) Ecological relationships of wild rice, *Zizania* spp. 11. Electron microscopy study of iron plaques on the roots of northern wild rice (*Zizania palustris*). Botany 91:189–201. https://doi.org/10.1139/cjb-2012-0198

Kennard WC, Phillips RL, Porter RA, Grombacher AW (2000) A comparative map of wild rice (*Zizania palustris* L. 2n=2x=30). Theor Appl Genet 101:677–684. https://doi.org/10.1007/s001220051530

Kovach DA, Bradford KJ (1992) Temperature dependence of viability and dormancy of *Zizania palustris* var. *interior* seeds stored at high moisture contents. Ann Bot 69:297–301. https://doi.org/10.1093/oxfordjournals.aob.a088345

LaFond-Hudson S, Johnson NW, Pastor J, Dewey B (2018) Iron sulfide formation on root surfaces controlled by the life cycle of wild rice (*Zizania palustris*). Biogeochemistry 141:95–106. https://doi.org/10.1007/s10533-018-0491-5

Lemly AD, Best GR, Crumpton WG, Henry MG, Hook DD, Linder G, Masscheleyn PH, Peterson HG, Salt T, Stahl RG Jr (1999) Workgroup II synopsis: contaminant fate and effects in freshwater wetlands. In: Lewis MA, Mayer FL, Powell RL, Nelson MK, Klaine SJ, Henry MG, Dickson GW (eds) Ecotoxicology and risk assessment for wetlands: Pellston workshop on ecotoxicology and risk assessment for wetlands (1995: Fairmont Hot Springs, Anaconda, Montana) Society of Environmental Toxicology and Chemistry (SETAC). SETAC Press, Pensacola, FL, USA, pp 69–152

Lewis MA (1995) Algae and vascular plant tests. In: Rand GM (ed) Fundamentals of aquatic toxicology: effects, environmental fate and risk assessment, 2nd edn. CRC Press Taylor and Francis Group, Boca Raton, FL, USA, pp 135–169

Lewis MA, Thursby G (2018) Aquatic plants: test species sensitivity and minimum data requirement evaluations for chemical risk assessments and aquatic life criteria development for the USA. Environ Pollut 238:270–280. https://doi.org/10.1016/j.envpol.2018.03.003

Malvick DK, Percich JA (1993) Hydroponic culture of wild rice (*Zizania palustris* L.) and its application to studies of silicon nutrition and fungal brown spot disease. Can J Plant Sci 73:969–975. https://doi.org/10.4141/cjps93-127

Meanley B (1961) Late-summer food of red-winged blackbirds in a fresh tidal-river marsh. The Wilson Bulletin 73:36–40

Myrbo A, Swain EB, Engstrom DR, Coleman Wasik J, Brenner J, Dykhuizen Shore M, Peters EB, Blaha G (2017) Sulfide generated by sulfate reduction is a primary controller of the occurrence of wild rice (*Zizania palustris*) in shallow aquatic ecosystems. J Geophys Res Biogeosci 122:2736–2753. https://doi.org/10.1002/2017JG003787

Nimmo DWR, Preul MA, Castle CJ, Self JR, Pillsbury RW, Bergey EA (2003) Effects of excess copper on growth of wild rice (*Zizania palustris*) seedlings tested in reconstituted and natural waters. Environ Manage 32:466–475. https://doi.org/10.1007/s00267-003-2899-4

Pastor J, Dewey B, Johnson NW, Swain EB, Monson P, Peters EB, Myrbo A (2017) Effects of sulfate and sulfide on the life cycle of *Zizania palustris* in hydroponic and mesocosm experiments. Ecol Appl 27:321–336. https://doi.org/10.1002/eap.1452

Porter R (2019) Wildrice (*Zizania* L.) in North America: genetic resources, conservation, and use. In: Greene SL (ed) North American crop wild relatives: important species. Springer Nature, Switzerland, pp 83–97. https://doi.org/10.1007/978-3-319-97121-6_3

Rand GM, Wells PG, McCarty LS (1995) Introduction to aquatic toxicology. In: Rand GM (ed) Fundamentals of aquatic toxicology: effects, environmental fate and risk assessment, 2nd edn. CRC Press, Taylor and Francis Group, Boca Raton, FL, USA, pp 3–67. https://doi.org/10.1201/9781003075363

Sims L, Pastor J, Lee T, Dewey B (2012) Nitrogen, phosphorus and light effects on growth and allocation of biomass and nutrients in wild rice. Oecologia 170:65–76. https://doi.org/10.1007/s00442-012-2296-x

Surendiran G, Alsaif M, Kapourchali FR, Moghadasian MH (2014) Nutritional constituents and health benefits of wild rice (*Zizania* spp.). Nutr Rev 72:227–236. https://doi.org/10.1111/nure.12101

Terrell EE, Peterson PM, Reveal JL, Duvall MR (1997) Taxonomy of North American species of *Zizania* (Poacea). SIDA, Contrib Bot 17:533–549

Vennum T Jr (1988) Wild rice and the Ojibway people. Minnesota Historical Society Press, St. Paul MN, p 358

Vizenor E (2008, February 8) As citied in natural wild rice in Minnesota: a wild rice study document submitted to the Minnesota legislature by the MNDNR

Webster CG (1964) Fall foods of Soras from two habitats in Connecticut. J Wildl Manag 28:163–165. https://doi.org/10.2307/3797946

Wetzel RG (1975) The littoral zone. In: Wetzel RG (ed) Limnology. W. B. Saunders Company, Philadelphia, PA, USA, pp 355–418

Xu X, Walters C, Antolin MF, Alexander ML, Lutz S, Ge S, Wen J (2010) Phylogeny and biogeography of the eastern Asian-North American disjunct wild-rice genus (Zizania L., Poaceae). Mol Phylogenet Evol 55:1008–1017. https://doi.org/10.1016/j.ympev.2009.11.018