Appendix A

Harmonia^{+PL} – procedure for negative impact risk assessment for invasive alien species and potentially invasive alien species in Poland

QUESTIONNAIRE

A0 | Context

Questions from this module identify the assessor and the biological, geographical & social context of the assessment.

a01. Name(s) of the assessor(s):

first name and family name

1. Ewa Szczęśniak
2. Monika Myśliwy – external expert
3. Zygmunt Dajdok

acomm01. Comments:

	degree	affiliation	assessment date
(1)	dr	Department of Botany, Institute of Environmental Biology, University of Wrocław	26-01-2018
(2)	dr	Department of Plant Taxonomy and Phytogeography, Faculty of Biology, University of Szczecin	24-01-2018
(3)	dr	Department of Botany, Institute of Environmental Biology, University of Wrocław	31-01-2018

a02. Name(s) of ***the species*** under assessment:

Polish name: Azolla drobna (Azolla karolińska)

Latin name: ***Azolla filiculoides*** Lam.

English name: Water Fern

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acomm02. Comments:

The taxonomy of the genus *Azolla* is difficult due to the small dimensions of the plant, morphological variations and the different features assumed to differentiate the species. The number of distinguished species also differs. Some botanists have synonymized *Azolla filiculoides* and *Azolla caroliniana* (inter alia, Valentine and Moore 1993 – P), which attitude was also accepted in the paper "Flowering plants and pteridophytes of Poland checklist" (Mirek et al. 2002 – P), where Polish name 'Azolla karolińska' was given for the species found in Poland. Simultaneously some researchers considered those two species as separate taxa which differed in anatomical and ecological aspects (inter alia, Lumpkin 1993 – P). Nowadays *Azolla caroliniana* has been included in the *Azolla cristata* and separated from *Azolla filiculoides* (Evrard and van Hove 2004 – P), thus the Polish name proposed in the paper by Mirek is incorrect and should not be used – it refers to another species. The *Azolla* species we find in Poland is *Azolla filiculoides*.

Polish name (synonym I) Azolla karolińska	Polish name (synonym II) Azolla paprotkowa
Latin name (synonym I) *Azolla caroliniana*	Latin name (synonym II) –
English name (synonym I) Red water fern	English name (synonym II) Mosquito fern

a03. **Area** under assessment:

Poland

acomm03. Comments:

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a04. **Status** of *the species* in Poland. *The species* is:

	native to Poland
	alien, absent from Poland
	alien, present in Poland only in cultivation or captivity
X	alien, present in Poland in the environment, not established
	alien, present in Poland in the environment, established

aconf01. Answer provided with a

low	medium	high
	X	

level of confidence

acomm04. Comments:

The first information on the presence of *Azolla filiculoides* in Poland dated from 1927 from the region of Lower Silesia. It was found in the palace pond in Wawrzyszewo (Lorenzberg), where it wintered for some time (Schube 1928 – P). After 1945, the pond was destroyed and *Azolla* was not observed in Poland for a long time. It was again noticed at the end of the 1990s in Bielsk Podlaski but it was a one-season stand only – *Azolla* did not survive the first winter (Wołkowycki 1999 – P). At that time *Azolla* was included into the list of Polish ephemerophytes (Rostański and Sowa 1986-1987, Mirek et al. 2002 – P). Information about other stands of *Azolla* appeared after 2000. In recent years new stands of this species have been reported, and some of them have been observed for several years (Rosadziński 2008, Szczęśniak et al. 2009, Spałek 2015, Myśliwy and Szlauer-Łukaszewska 2017 – P). It has been considered to be locally established (Tokarska-Guzik et al. 2012 – P). However, further long-term observations (Szczęśniak 2007-2017 – A) indicated that many stands disappeared after 1-2 years even though *Azolla* was able to overwinter (the longest-lived stand was observed in Wrocław, Kozanów which persisted for at least 5 years, destroyed by a summer flood; Szczęśniak – own observations). In Poland *Azolla* has not been observed to undergo the complete life cycle – it does not produce sporocarps, which were observed in, inter alia, Germany (Hussner 2010 – B). Sporocarps guarantee the durability of populations as they are more resistant to habitat conditions than the vegetative sporophytes. Moreover, the

complete life cycle would activate the process of natural selection for a specific genotype that would be better adapted to local conditions. Taking into account the long-term observations and mainly ephemeral locations, *Azolla* in Poland is in the settlement phase and it has been transforming from the ephemerophyte to the established species (Myśliwy and Szlauer-Łukaszewska 2017, Szczęśniak 2007-2017 – A). At present, the eastern borderline of geographical range of *Azolla* is considered to be in western Poland. The species shows fluctuations in distribution and in the size of the population, what is typical for peripheral populations.

a05. The impact of *the species* on major domains. *The species* may have an impact on:

X	the environmental domain
X	the cultivated plants domain
X	the domesticated animals domain
X	the human domain
X	the other domains

acomm05. Comments:

This species is considered to be harmful to the aquatic environment. It may cause problems in the EU habitat 3150 Natural eutrophic lakes with *Magnopotamion* or *Hydrocharition* vegetation type (Tokarska-Guzik et al. 2012 – P). *Azolla* can affect native aquatic and water-related plant and animal species as well as plant communities by forming a thick mat of floating vegetation, effectively competing with aquatic plants and algae, preventing light penetration and blocking oxygen diffusion (Janes et al. 1996, Gratwicke and Marshall 2001 – P), and can also deposit large quantities of biogens (due to its symbiosis with cyanobacteria *Anabaena azollae*, it uses up atmospheric oxygen and enriches settled water with that element) (van Hove and Lejeune 2002 – P) and reduces pH. In Poland, there is no tradition of water plants farming and for this reason no direct and negative impact of this species on farms has been found. But *Azolla* mats may block water flow in drainage ditches which is of local importance and may indirectly affect the water balance of fields. It should be emphasized that the symbiosis of *Azolla* with cyanobacteria (*Anabaena azollae*), fixing atmospheric nitrogen, has been applied for hundreds years as biofertiliser in rice cultivation in Asia (Wagner 1997 – P). Its impact on water bodies is particularly significant in the case of fish ponds, especially for species with high oxygen requirements (Janes et al. 1996, Janes 1998, Hill 1999, Gratwicke and Marshal 2001 – P, Hussner 2010 – B). Under optimum conditions (23-29 °C) *Azolla* can double its mass during ca. 3-5 days, and the mat may achieve a thickness of >20 cm (McConnachie et al. 2004 – P) and completely cut off the water body from sunlight and oxygen, which in combination with the decay of the intensively produced biomass results in an oxygen squeeze and a considerable drop in biodiversity: the extinction of cryptogamous and seed-bearing plants, invertebrates and vertebrates (m.in. Janes et al. 1996, Gratwicke and Marshall 2001 – P). In Poland, the thickest observed mats were about 10-14 cm (Wilkszyn; Szczęśniak et al. 2009 – P and further observations). Outside Poland, cases have been reported where farm animals drowned as a result of mistaking the water body completely covered with *Azolla* mats for grazing land. It can be also dangerous for children (Hill 1999 – P) – water bodies covered with *Azolla* mats resemble stable ground onto which children try to enter (observations made in Wilkszyn near Wrocław; Szczęśniak 2007-2017). There was one case where, in a fire-protection pond in Wilkszyn, *Azolla* formed a thick floating mat, under which there was a suspension of dead plants (2007-2017 – A), which could hinder the access to water and its use in case of fire. However, such a risk seems to be slight. In warmer regions, water flow was retarded in drainage ditches and irrigation channels were blocked (Hill and Cillers 1999, Hassan and Ricciardi 2014 – P). This species also reduced the attractiveness of water bodies to tourists because of lack of access to water, anaerobic decomposition of dead biomass in water and difficult boat sailing.

A1 | Introduction

Questions from this module assess the risk for *the species* to overcome geographical barriers and – if applicable – subsequent barriers of captivity or cultivation. This leads to *introduction*, defined as the entry of *the organism* to within the limits of *the area* and subsequently into the wild.

a06. The probability for ***the species*** to expand into Poland's natural environments, **as a result of self-propelled expansion** after its earlier introduction outside of the Polish territory is:

	low
	medium
X	high

aconf02.	Answer provided with a	low	medium	high **X**	level of confidence

acomm06. Comments:

Very effective vegetative propagation using a few-millimetres of plant parts is crucial for spreading of *Azolla*. It is dispersed in the form of zoochory – using animals, mainly birds, or hydrochory- using water, for example during freshet (Hussner and Lösch 2005 – P). *Azolla* may migrate to Poland from the adjacent areas, mainly Germany, where it is frequent, populations are stable, and where it produces sporocarps with spores (Hussner 2010 – B). The species occurs in many European countries (Hussner 2012 – P) and is mainly regarded as established and invasive (e.g. Janes 1998, Van der Velde et al. 2002, Anastasiu and Negrean 2005, Muller 2006, Garcia-Murillo et al. 2007, Pyšek et al. 2012 – P).

a07. The probability for *the species* to be introduced into Poland's natural environments by **unintentional human actions** is:

	low
	medium
X	high

aconf03.	Answer provided with a	low	medium **X**	high	level of confidence

acomm07. Comments:

The species can be transported by humans e.g. in ballast tanks of ships, in boat frames and fishing equipment (Lansdown 2015 – I). This species was probably introduced during stocking with fry of water bodies near Wrocław. *Azolla* appeared quickly in new water bodies not visited by birds, but popular among anglers (Szczęśniak 2007-2017 – A). Unfortunately, neither a person responsible for the activity nor the origin of the material were detected.

a08. The probability for *the species* to be introduced into Poland's natural environments by **intentional human actions** is:

	low
X	medium
	high

aconf04.	Answer provided with a	low	medium **X**	high	level of confidence

acomm08. Comments:

At present, the species in Poland has no positive economic value or recognised ornamental aspects. It is also not used as a green manure. It does not create a positive interest for owners and users of water bodies; on the contrary some pond owners get in touch with researchers to determine the method of eliminating it as an unwanted alien. Nevertheless, the possibility cannot be excluded that this species is introduced by human as an ornamental plant cultivated in private ponds and aquaria, especially the forms with red or

	claret hyperpigmentation (an *Azolla* reaction to too much light). Cultivated *Azolla* can "escape" to the natural environment or can "be released" – a case of *Azolla* being released to the environment by aquarists occurred in Denmark (Hussner 2010 – B), and could result in species occurrences in Poland.

A2 | Establishment

Questions from this module assess the likelihood for *the species* to overcome survival and reproduction barriers. This leads to *establishment*, defined as the growth of a population to sufficient levels such that natural extinction within *the area* becomes highly unlikely.

a09. Poland provides **climate** that is:

	non-optimal
X	sub-optimal
	optimal for establishment of *the species*

aconf05.	Answer provided with a	low	medium **X**	high	level of confidence

acomm09. Comments:

Azolla filiculoides naturally occurs in subtropical and moderate parts of North and Central America, at the west coast reaching the south-eastern part of Alaska (Hussner 2010 – B). *Azolla filiculoides* is the most frost tolerant of all *Azolla* species, but it still is thermophilic. According to the literature data, direct exposure to -4°C for longer than 10 hours is lethal for this plant (Janes 1998 – P), but its tissues could survive under a thin ice layer formed on the surface of a water body (Lumpkin 1993 – I). Despite the above facts and a several-year presence of the species in some stands, even those which are exceptionally warm for Poland (the centre of Wrocław), no sporocarps were produced by *Azolla* and the life cycle (sporophytes and gametophytes) was not completed in our country. *Azolla* can survive by means of submerged buds in a suspension of slowly decomposing plant parts. It is unable to overwinter in cases with an insufficiently cover of *Azolla* mat on the water surface. If such a mat is sufficiently thick, some buds can survive in the suspension at temperatures of -20°C (Szczęśniak et al. 2009, Szczęśniak 2009 – P). Whole plants are able to survive a short-term freezing inside the ice. So far, all cases of *Azolla* overwintering have been observed in the lowland of western Poland, mainly in the valley of the Odra River.

According to the map of climate similarities between Poland and the rest of the world contained in the "Procedure of risk assessment ....", the similarity of climatic conditions of the country and the zone of the natural spread of the species range from 0% (subtropics) to 94-100% (temperate climate). In comparison with Great Britain, the Netherlands and Belgium, where *Azolla* is more widespread, the similarity is 45-94%. When compared with Germany, where *Azolla* is classified as a naturalized species (Hussner 2010 – B) and produces sporocarps, the similarity of climatic conditions to those in Poland is as high as 94-100%. Therefore, it can be assumed that in future status of *Azolla* in Poland can become similar to that in Germany (i.e. larger populations, complete life cycle).

a10. Poland provides **habitat** that is

	non-optimal
	sub-optimal
X	optimal for establishment of *the species*

aconf06.	Answer provided with a	low	medium	high **X**	level of confidence

acomm10. Comments:

Azolla filiculoides is known to be highly tolerant of different habit conditions. It occurs in water with pH of 3.5-10, and shows tolerance for heavy metals, salinity and low

concentration of nitrogen in water (Lumpkin and Plucknett 1980 – P). The first data on habitat conditions for this species in Poland were published by Myśliwy and Szlauer-Łukaszewska (2017 – P). So far, it has been found in oxbow lakes, eutrophic and mesotrophic water bodies of anthropogenic origin (fish ponds, fire protection reservoirs and park ponds). Moreover, *Azolla* has also been observed in-between the groynes at the main Odra River bed and in slow-flowing water in channels. The great number of natural and anthropogenic water bodies makes Poland an area with very good habit conditions for *Azolla* dispersion, especially taking into account its low dependence on the nitrogen content in water. Additionally, the migration route of waterfowl passes through Poland and there are regular flood surges twice a year – both those factors are the main natural vectors for *Azolla*. Winter temperatures are the only factor limiting the species expansion effectiveness.

A3 | Spread

Questions from this module assess the risk of *the species* to overcoming dispersal barriers and (new) environmental barriers within Poland. This would lead to spread, in which vacant patches of suitable habitat become increasingly occupied from (an) already-established population(s) within Poland.

Note that spread is considered to be different from range expansions that stem from new introductions (covered by the Introduction module).

a11. The capacity of *the species* to disperse within Poland by natural means, **with no human assistance**, is:

	very low
	low
	medium
	high
X	very high

aconf07.	Answer provided with a	low	medium	high **X**	level of confidence

acomm11. Comments:

Data of A type – dispersion from a single point.The rate of spontaneous dispersion in Great Britain has been assessed as medium (Lansdown 2015 – I). In Poland, the species occurred until quite recently in south-west Poland where it gradually, but for a short period, occupied oxbow lakes (Szczęśniak 2008, 2009, Szczęśniak et al. 2009 – P, Szczęśniak 2007-2017 – A). In 2016, the species was observed in six stands in the main bed of the Odra River, in the section between Krosno Odrzańskie and Owczary. It was dispersed farther north by the current (Kobierski and Ryś 2016, Myśliwy and Szlauer-Łukaszewska 2017 – P, also own observations). River water can transport this fern over many kilometres with the current, and – during flood – also across the valley. Water fowl which migrate, or fly over lesser distances, are also considered as a vector of spread. Both means of dispersion are not controlled by humans, its distances may vary and expansion to cooler regions of Poland could be irregular. Assuming single-point dispersion as an indicator of species dispersion, that is dispersion of whole plants with the water current, the species may travel over 50 km/ year at least in the Odra River valley.

a12. The frequency of the dispersal of *the species* within Poland by **human actions** is:

	low
	medium
X	high

aconf08.	Answer provided with a	low	medium **X**	high	level of confidence

acomm12. Comments:

The Regulation of the Polish Ministry of Environment of 9 September 2011 classifies *Azolla filiculoides* as being within the list of alien plants, which in case of release can threaten native species or natural habitats. According to the Act of Environmental Protection (article 210), in Poland it is forbidden to introduce alien species, particularly those listed in the above mentioned regulation (article 210, point 2f) into the environment. However, the intentional human spreading of this species (e.g. internet sale for cultivation, as an ornamental plant, followed by "escape" or "release" into the natural environment), and especially its spreading due to unintentional human activities cannot be excluded (Hussner 2010 – B, Lansdown 2015 – I). *Azolla* is most likely to be transported by people during restocking of water bodies and when "releasing" aquarium organisms. Unfortunately, there is no direct evidence, only assumptions (sudden occurrence of a large number of *Azolla* populations in water bodies which had no shows of the species 1-2 days earlier). Such evidence is practically impossible to obtain in cases of uncontrolled restocking.

A4a | Impact on the environmental domain

Questions from this module qualify the consequences of *the species* on wild animals and plants, habitats and ecosystems.

Impacts are linked to the conservation concern of targets. Native species that are of conservation concern refer to keystone species, protected and/or threatened species. See, for example, Red Lists, protected species lists, or Annex II of the 92/43/EWG Directive. Ecosystems that are of conservation concern refer to natural systems that are the habitat of many threatened species. These include natural forests, dry grasslands, natural rock outcrops, sand dunes, heathlands, peat bogs, marshes, rivers & ponds that have natural banks, and estuaries (Annex I of the 92/43/EWG Directive).

Native species population declines are considered at a local scale: limited decline is considered as a (mere) drop in numbers; severe decline is considered as (near) extinction. Similarly, limited ecosystem change is considered as transient and easily reversible; severe change is considered as persistent and hardly reversible.

a13. The effect of *the species* on native species, through **predation, parasitism or herbivory is**:

X	inapplicable
	low
	medium
	high

aconf09. Answer provided with a

low	medium	high

level of confidence

acomm13. Comments:

Azolla filiculoides does not demonstrate such effects – it is an autotrophic plant.

a14. The effect of *the species* on native species, through **competition** is:

	low
	medium
X	high

aconf10. Answer provided with a

low	medium	high
		X

level of confidence

acomm14. Comments:

This species affects all aquatic organisms, both plants and animals. It is the species that can effectively eliminate other aquatic plants and algae – it mainly affects submerged plants preventing their photosynthesis and blocking oxygen diffusion (inter alia, Janes et al. 1996 – P). *Azolla* often forms thick monospecific mats floating on the water surface which effectively reduce the biodiversity of plant communities present (Janes et al. 1996,

Szczęśniak 2009, Szczęśniak et al. 2009, Tokarska-Guzik et al. 2012 – P). The population in new stands in the Lower Odra River is sparse (Myśliwy and Szlauer-Łukaszewska 2017 – P), but in Międzyodrze it effectively competes with the native fern *Salvinia natans* (Myśliwy and Szlauer-Łukaszewska 2017 – P). In water bodies of the Upper Odra River valley (Lower Silesia), where *Azolla* stands were recorded for a few years, a positive impact on the coastal zone species was observed. Due to eutrophication, they were growing and settling shallow parts of the water body faster (communities of reed, water pineapple, yellow flag), what may lead to limitation other plant communities. The impact of *Azolla* mats on animals is mainly observed as deteriorated oxygen conditions as deposited dead biomass uses oxygen present in water and atmospheric air cannot enter water; it may lead to oxygen-squeeze. This has a negative impact on fauna diversity (inter alia, Gratwicke and Marshall 2001 – P). This effect is particularly dangerous for oxbow lakes, which are a protected habitat under Natura 2000 (code 3150) and which are no longer formed in the Odra River valley (the effect of river regulation).

a15. The effect of *the species* on native species, through **interbreeding** is:

X	no / very low
	low
	medium
	high
	very high

aconf11.	Answer provided with a	low	medium	high **X**	level of confidence

acomm15. Comments:

In Poland, there are no native species of *Azolla*, so that possible hybridization, although is known (Van Cat et al. 1989 – P), does not pose any problem. The native flora in Poland does not contain any other species closely related to *Azolla*, so there is no risk of interbreeding. So far, it has also not been possible due to the reliance of *Azolla* in Poland on vegetative propagation (*Azolla* in Poland does not produce sporocarps and gametophytes; Szczęśniak et al. 2009 – P and further observations).

a16. The effect of *the species* on native species by **hosting pathogens or parasites** that are harmful to them is:

	very low
X	low
	medium
	high
	very high

aconf12.	Answer provided with a	low	medium **X**	high	level of confidence

acomm16. Comments:

There is no information about hosting pathogens by *Azolla* in Poland. This results currently from the very small number of *Azolla* stands in Poland and the lack of direct contact with crops. There is low significance of animals as vectors considering that there are such a low number of stands. In case of *Azolla* expansion and it's elimination the mats (biomass) of the species removed from the water surface can be used in farmlands as green manure. *Azolla* plants may be attacked, among others, by *Rhizoctonia solani*, a pathogen that causes potato rhizoctonia, but among tested species, *Azolla filiculoides* found in Poland proved to be the most resistant and quickly recovered its population (Dath and Singh 1998 – P). *Azolla* was found to be host to the aphid *Rhopalosiphum nymphaeae*, which can be particularly destructive to water gardens and is considered to be a vector for at least five viral pathogens, inter alia, causing leaves mosaic of banana, cauliflowers, cucumbers, yellow dwarf of onion and cabbage plant disease (Lansdowan 2015 – I). Aphids can be hosted by many organisms, thus *Azolla* has no particular significance for them, and moreover they

have not been observed on *Azolla* in Poland. However, aphids were found in 2010 on a species quite closely related to *Azolla* – the floating fern *Salvinia natans* (Borowiak-Sobkowiak et al. 2010 – P).

a17. The effect of *the species* on ecosystem integrity, by **affecting its abiotic properties** is:

	low
	medium
X	high

aconf13. Answer provided with a

low	medium	high	level of confidence
		X	

acomm17. Comments:

The species causes a drastic change in chemical and physical properties of water: light, oxygen and trophic aspects which have a negative impact on biodiversity of aquatic organisms (inter alia, Janes et al. 1996, Gratwicke and Marshall 2001 – P). *Azolla* significantly affects physical and chemical properties of water bodies, where there is massive occurrence. The results are: blocking sunlight penetration into deeper water layers, reducing the quantity of dissolved oxygen in water and increasing CO_2, water acidification, and slowdown of water flow (Janes 1998, Janes et al. 1996, Gratwicke and Marshall 2001 – P). At the same time, the species was found to eliminate heavy metals from water (e.g. Cohen-Shoel et al. 2002, Oren Benaroya et al. 2004 – P) and reduce nitrogen and phosphorus level in wastewater (Forni et al. 2001 – P). These could be helpful during biological treatment of water providing that *Azolla* would be eliminated from water at the end of the growing season to prevent the release of cumulated compounds and elements to the environment from decomposing dead biomass.

a18. The effect of *the species* on ecosystem integrity, by **affecting its biotic properties** is:

	low
	medium
X	high

aconf14. Answer provided with a

low	medium	high	level of confidence
		X	

acomm18. Comments:

This invasive fern is present in ponds and oxbow lakes, entering the protected habitat 3150: Natural eutrophic lakes with *Magnopotamion* or *Hydrocharition* vegetation type (Tokarska-Guzik et al. 2012 – P). During quite fast vegetative reproduction, *Azolla* creates thick mats that effectively eliminate the native vascular plants and algae, and has a negative impact on aquatic invertebrates and fish. Massive occurrence of that species may eliminate aquatic algae and vascular plants (lack of light, Janes et al. 1996 – P) and largely reduce the number of species of aquatic animals (Gratwicke and Marshall 2001 – P). Moreover, *Azolla* mats inhibit the development of mosquitoes and other insects (thus, the American common name mosquito fern), whose larvae live in water and breathe in atmospheric oxygen as they are cut off from water surface and cannot absorb oxygen. It may have a significant effect on the population of birds feeding on the adult forms of these larvae. *Azolla* may also affect the feeding base for herbivorous birds, particularly those feeding with submerged plants. There is no detailed information on *Azolla* impact on biodiversity in Central Europe – this issue requires further field tests.

A4b | Impact on the cultivated plants domain

Questions from this module qualify the consequences of *the species* for cultivated plants (e.g. crops, pastures, horticultural stock).

For the questions from this module, consequence is considered 'low' when presence of *the species* in (or on) a population of target plants is sporadic and/or causes little damage. Harm is considered 'medium' when *the organism's* development causes local yield (or plant) losses below 20%, and 'high' when losses range >20%.

a19. The effect of *the species* on cultivated plant targets through **herbivory or parasitism** is:

	inapplicable
X	very low
	low
	medium
	high
	very high

aconf15.	Answer provided with a	low	medium	high **X**	level of confidence

acomm19. Comments:

Azolla filiculoides is an autotrophic plant, it exhibits no parasitic properties.

a20. The effect of *the species* on cultivated plant targets through **competition** is:

	inapplicable
X	very low
	low
	medium
	high
	very high

aconf16.	Answer provided with a	low	medium	high **X**	level of confidence

acomm20. Comments:

It is an aquatic fern, and in Poland there are no amphibious nor water plant cultivations which could be affected by that species, thus there is no direct impact on plants in field crops as a result of interspecific breeding. *Azolla* shows no allelopathic interactions with crop plants, it is also inert to pollinators (no negative or positive impact), also due to lack of common habitats.

a21. The effect of *the species* on cultivated plant targets through **interbreeding** with related species, including the plants themselves is:

	inapplicable
X	no / very low
	low
	medium
	high
	very high

aconf17.	Answer provided with a	low	medium	high **X**	level of confidence

acomm21. Comments:

In Poland, there are no native species closely related to *Azolla*, which could lead to interbreeding. Cultivated plants mostly originate from evolutionarily distinct units (seed-bearing plants) and there are no references to any problem of interbreeding with ferns.

a22. The effect of *the species* on cultivated plant targets by **affecting the cultivation system's integrity** is:

	very low
X	low

	medium
	high
	very high

aconf18.	Answer provided with a	low	medium	high **X**	level of confidence

acomm22. Comments:

In Poland, there are no cultivations of water or amphibious plants. Even in the case of progressive climatic changes, there will be no significant agriculture change, thus we will not observe the direct impact of *Azolla* on crops. In the case of the massive occurrence of *Azolla*, it may directly affect agriculture by slowing down water flow, and clogging channels that irrigate such crops (Hill and Cillers 1999, Hassan and Ricciardi 2014 – P). Water level control system has local significance in Poland, so the impact of the species will be not significant in large scale. Massive occurrences of *Azolla* may result in using it in farmlands as a green manure, and consequently may affect soil fertility. However, there will be no direct impact of that species and it will not appear without human intentional activity. *Azolla* may occur naturally on the fields only when flood water transports its mats to flooded farmlands.

a23. The effect of *the species* on cultivated plant targets by hosting **pathogens or parasites** that are harmful to them is:

	very low
X	low
	medium
	high
	very high

aconf19.	Answer provided with a	low	medium	high **X**	level of confidence

acomm23. Comments:

There is no information about pathogen hosting by *Azolla* in Poland. Generally, *Azolla* representatives may be attacked, among others, by *Rhizoctonia solani*, a pathogen that causes potato rhizoctonia, but among tested *Azolla* species, *A. filiculoides* found in Poland proved to be the most resistant and quickly recovered its population (Dath and Singh 1998 – P). Moreover, *Azolla* was found to host aphid *Rhopalosiphum nymphaeae*, which can be particularly destructive to water gardens and is considered to be a vector for at least five viral pathogens, inter alia, causing leaves mosaic in banana, cauliflowers, cucumbers, yellow dwarf in onion and cabbage plant disease (Lansdowan 2015 – I), of which the last four have importance for Polish crops. It must be underlined that aphids can be hosted by many organisms, thus *Azolla* is an accidental host with no particular significance for aphid populations. Its presence on *Azolla* also has not been noticed in Poland.

A4c | Impact on the domesticated animals domain

Questions from this module qualify the consequences of *the organism* on domesticated animals (e.g. production animals, companion animals). It deals with both the well-being of individual animals and the productivity of animal populations.

a24. The effect of *the species* on individual animal health or animal production, through **predation or parasitism** is:

X	inapplicable
	very low
	low
	medium
	high
	very high

aconf20.	Answer provided with a	low	medium	high	level of confidence

acomm24. Comments:

Azolla is an autotrophic plant and shows no such effects.

a25. The effect of *the species* on individual animal health or animal production, by having properties that are hazardous upon **contact**, is:

	very low
X	low
	medium
	high
	very high

aconf21.	Answer provided with a	low	medium **X**	high	level of confidence

acomm25. Comments:

Azolla exhibits no harmful (chemical or physical) impact in direct contact with animals. However, there have been isolated cases of farm animal drowning (an important effect) as a result of mistaking dense *Azolla* mats, covering the whole water body, for stable grazing land (low probability). Such situations could also apply to dogs and other domestic animals (Hill 1999 – P). This species has an intensive indirect effect by changing the chemical properties of water, blocking sunlight penetration into the water body which results in the impaired vitality of fish or even their death – this is a great and real risk for fish ponds.

a26. The effect of *the species* on individual animal health or animal production, by hosting **pathogens or parasites** that are harmful to them, is:

X	inapplicable
	very low
	low
	medium
	high
	very high

aconf22.	Answer provided with a	low	medium	high	level of confidence

acomm26. Comments:

No cases of *Azolla* carrying fish pathogens have been reported.

A4d | Impact on the human domain

Questions from this module qualify the consequences of *the organism* on humans. It deals with human health, being defined as a state of complete physical, mental and social well-being and not merely the absence of disease or infirmity (definition adopted from the World Health Organization).

a27. The effect of *the species* on human health through **parasitism** is:

X	inapplicable
	very low
	low
	medium
	high
	vert high

aconf23.	Answer provided with a	low	medium	high	level of confidence

acomm27. Comments:

Azolla is an autotrophic plant.

a28. The effect of *the species* on human health, by having properties that are hazardous upon **contact**, is:

- [ ] very low
- [X] low
- [ ] medium
- [ ] high
- [ ] very high

aconf24.	Answer provided with a	low	medium	high **X**	level of confidence

acomm28. Comments:

Properties of *Azolla filiculoides* threatening the health of humans are not known.

a29. The effect of *the species* on human health, by hosting **pathogens or parasites** that are harmful to humans, is:

- [X] inapplicable
- [ ] very low
- [ ] low
- [ ] medium
- [ ] high
- [ ] very high

aconf25.	Answer provided with a	low	medium	high	level of confidence

acomm29. Comments:

Azolla does not contain any chemical substances or physical structures that can directly harm humans. The impact can be only indirect: documented in the case of drowning animals (Hill 1999 – P) (large effect) may also occur in small children, e.g. attempts to climb to the surface of a pond covered with *Azolla* were observed near Wrocław (Szczęśniak 2007-2017 – A) but the likelihood of such an event is low.

A4e | Impact on other domains

Questions from this module qualify the consequences of *the species* on targets not considered in modules A4a-d.

a30. The effect of *the species* on causing damage to **infrastructure** is:

- [ ] very low
- [ ] low
- [X] medium
- [ ] high
- [ ] very high

aconf26.	Answer provided with a	low	medium **X**	high	level of confidence

acomm30. Comments:

Azolla has no direct impact on the infrastructure, but its possible occurrence on a massive scale may hinder the operation of hydrotechnical equipment (weirs, culverts etc.). Such a situation would only refer to infrastructure related to slow flowing or still water. Indirect

	impacts of *Azolla filiculoides* on real and personal property have not been so far evaluated and require better recognition of the problem. *Azolla* occurrence on a massive scale may: make water bodies less attractive for tourists due to the lack of access to water and the anaerobic processes of dead biomass decomposition, reduce the investment value for stock-breeders of fish (by causing reduced productivity of ponds), cause difficulties in moving over the water body (problems with boat sailing), and hinder water access in open fire-protection ponds (blocked water pumps). Generally, deteriorating aesthetic qualities of an area reduce the value of properties (Lansdown 2015 – I).

A5a | Impact on ecosystem services

Questions from this module qualify the consequences of *the organism* on ecosystem services. Ecosystem services are classified according to the Common International Classification of Ecosystem Services, which also includes many examples (CICES Version 4.3). Note that the answers to these questions are not used in the calculation of the overall risk score (which deals with ecosystems in a different way), but can be considered when decisions are made about management of *the species*.

a31. The effect of *the species* on **provisioning services** is:

X	significantly negative
	moderately negative
	neutral
	moderately positive
	significantly positive

aconf27.	Answer provided with a	low	medium	high **X**	level of confidence

acomm31. Comments:

Facilities using surface water bodies as the source of potable water may notice serious deterioration of water quality as the consequence of *Azolla* occurrence on a massive scale (Lansdown 2015 – I). An invasion of *Azolla* in farm ponds will cause a collapse of freshwater fish production. Despite the very quick growth of the species (in field trials, it doubled its mass within 7-10 days; Hussner 2010 – B) the additional problem is that *Azolla* as a fern does not exhibit the typical reaction to herbicides. Additionally, such substances are forbidden to be applied in the majority of water habitats. The plant also cannot be eliminated mechanically due to the brittle and easily-detached tiny parts involved in vegetative reproduction, which are able very quickly to restore the population (Szczęśniak 2009 – P). Countries facing this issue as an important economic problem, apply a combined technique: mechanical elimination and spraying (Hill and Cilliers 1999 – P). This assessment has also taken into consideration the possible negative impact of *Azolla filiculoides* on agriculture, by clogging irrigation channels (Hill and Cillers 1999, Hassan and Ricciardi 2014 – P) and cases of drowned breeding animals (Hill 1999 – P). Using *Azolla* as green manure in farmlands may potentially have a positive effect, on condition that this species be considered for use in such a way in Poland.

a32. The effect of *the species* on **regulation and maintenance services** is:

	significantly negative
X	moderately negative
	neutral
	moderately positive
	significantly positive

aconf28.	Answer provided with a	low	medium	high **X**	level of confidence

acomm32.	Comments: *Azolla* can be used to eliminate heavy metals from polluted water, and nitrogen and phosphorus from wastewater (inter alia, Forni et al. 2001, Benicelli et al. 2004, Oren Benaroya et al. 2004 – P), which is regarded as a positive effect for polluted water bodies. If *Azolla* occurs on a massive scale in clean water bodies (which in Poland occur often and are very important for biodiversity), we can observe intensive eutrophication and a serious deterioration of water quality caused by the anaerobic decomposition of dead biomass under the floating *Azolla* mat and blocked oxygen penetration into the water body. It will also affect the quality of adjacent soil into which the polluted water penetrates. Impact of polluted waters could be far-reaching in time of flood, however effect of pollution diluted in water is weaker.

a33. The effect of *the species* on **cultural services** is:

X	significantly negative
	moderately negative
	neutral
	moderately positive
	significantly positive

aconf29.	Answer provided with a	low	medium	high **X**	level of confidence

acomm33.	Comments: This assessment included sport and recreational activities (fishing, swimming) which are hindered by *Azolla filiculoides* forming dense fern mats in water bodies and by negative impact on landscape beauty (Lansdown 2015 – I*). Azolla* occurrence on a massive scale in water bodies practically eliminates their recreational and aesthetical value – leading to an invisible water table, no access to water, its deteriorated quality, difficulties in performing water sports. Its positive effect is demonstrated on a considerably smaller scale, where its unusual form is so attractive to admirers of ponds that they start to cultivate *Azolla* which may ultimately be the cause of the introduction of *Azolla* surplus into the natural environment.

A5b | Effect of climate change on the risk assessment of the negative impact of *the species*

Below, each of the Harmonia^{+PL} modules is revisited under the premise of the future climate. The proposed time horizon is the mid-21st century. We suggest taking into account the reports of the Intergovernmental Panel on Climate Change. Specifically, the expected changes in atmospheric variables listed in its 2013 report on the physical science basis may be used for this purpose. The global temperature is expected to rise by 1 to 2°C by 2046-2065.

Note that the answers to these questions are not used in the calculation of the overall risk score, but can be but can be considered when decisions are made about management of *the species*.

a34. INTRODUCTION – Due to climate change, the probability for *the species* to overcome geographical barriers and – if applicable – subsequent barriers of captivity or cultivation in Poland will:

	decrease significantly
	decrease moderately
	not change
X	increase moderately
	increase significantly

aconf30.	Answer provided with a	low	medium **X**	high	level of confidence

acomm34.	Comments: Predicted climate changes, that is, temperature increase by 1-2 degrees, facilitate its further introduction, settling and distribution across Poland. So far its stands predominate in western and south-western regions (Myśliwy and Szlauer-Łukaszewska 2017 – P). *Azolla filiculoides* originates from moderately warm and subtropical areas in America, but *Azolla* was also present in Europe before the glacial period (e.g. O'Brien and Jones 2003, Stachnowicz-Rybka 2011 – P). Low temperatures during winter are the biggest constraints for the species in our climate (Espinar et al. 2015 – P). According to sources, the optimal temperature is 15-20(25)°C (Tung and Watanabe 1983, Sang et al. 1987 – P). Despite a higher frost resistance than is defined according to the literature data, climate in Poland is still too cold for *Azolla* (winter temperatures often prevent overwintering of the population and make stands ephemeral, or at least lasting only for a few years), and the period with temperatures above +20°C is too short for the species to produce sporocarps and go through the complete lifecycle (Szczęśniak et al. 2009 – P and further observations). In Germany, *Azolla* has a complete life cycle and produces sporocarps (Hussner 2010 – B). Production of sporocarps and the complete life cycle significantly increases the possibility of this species overwintering in Poland and the effectiveness of its spreading – sporocarps are much more effective propagules than quickly drying fragments of sporophytes: they are smaller, lighter and more resistant to habitat conditions which enable their successful transport by birds over larger distances. Moreover, the genetic variability generated by sexual reproduction allows natural selection favourable to genotypes that effectively work under given conditions, which in consequence improves their invasive potential.

a35. ESTABLISHMENT – Due to climate change, the probability for *the species* to overcome barriers that have prevented its survival and reproduction in Poland will:

	decrease significantly
	decrease moderately
	not change
	increase moderately
X	increase significantly

aconf31.	Answer provided with a	low	medium	high **X**	level of confidence

acomm35.	Comments: Winter temperatures and still insufficiently long periods with warm temperatures are the only factors limiting the effectiveness of *Azolla* invasion in Poland (Szczęśniak et al. 2009 – P). Due to global warming, the species will be able to complete its life cycle, produce spores (via sporocarps), activate a process of genotype selection, and finally to occupy suitable habitats in Poland.

a36. SPREAD – Due to climate change, the probability for *the species* to overcome barriers that have prevented its spread in Poland will:

	decrease significantly
	decrease moderately
	not change
	increase moderately
X	increase significantly

aconf32.	Answer provided with a	low	medium	high **X**	level of confidence

acomm36.	Comments: Winter temperatures are the only factors limiting the effectiveness of *Azolla* invasion in Poland and constrain its occurrence to the warmest western part of Poland (Szczęśniak et al. 2009, Myśliwy and Szlauer-Łukaszewska 2017 – P); global warming will enable its effective settlement in Poland.

a37. IMPACT ON THE ENVIRONMENTAL DOMAIN – Due to climate change, the consequences of *the species* on wild animals and plants, habitats and ecosystems in Poland will:

	decrease significantly
	decrease moderately
	not change
	increase moderately
X	increase significantly

aconf33.	Answer provided with a	low	medium	high **X**	level of confidence

acomm37. Comments:

Global warming will enable effective expansion of *Azolla* and its permanent occurrence in water bodies. Longer warm periods are favourable for longer and larger production of biomass. *Azolla* plant biomass is capable of doubling every week in the growing season. On the other hand, it will deteriorate conditions in water bodies, where *Azolla* already exists, and will drastically change those without *Azolla*. Its permanent impact (no winterkill) will cause biological degradation of such water bodies.

a38. IMPACT ON THE CULTIVATED PLANTS DOMAIN – Due to climate change, the consequences of *the species* on cultivated plants and plant domain in Poland will:

	decrease significantly
	decrease moderately
X	not change
	increase moderately
	increase significantly

aconf34.	Answer provided with a	low	medium **X**	high	level of confidence

acomm38. Comments:

Predicted climate changes may be favourable for plant development, and in effect it will cover new areas (see question a34). But its impact on crops should not significantly increase because this species does not affect plant production in Poland due to the country agriculture specificity (only terrestrial seed plants cultivation). It cannot be excluded that a greater number of *Azolla* stands will affect the distribution of plant pathogens using, among other things, aphid *Rhopalosiphum nymphaeae* (Lansdown 2015 – B) as a vector. However, *Azolla* applied as green manure, as e.g. in Asia, may also have a positive effect (Lumpkin and Plucknett 1980 – P). Impeded water flow in drainage ditches is not unlikely as a result of the spread of *Azolla* due to climate change; however this would be of only local significance in Poland.

a39. IMPACT ON THE DOMESTICATED ANIMALS DOMAIN – Due to climate change, the consequences of *the species* on domesticated animals and animal production in Poland will:

	decrease significantly
	decrease moderately
	not change
	increase moderately
X	increase significantly

aconf35.	Answer provided with a	low	medium	high **X**	level of confidence

acomm39. Comments:

Azolla occurrence on a massive scale in fish reservoirs will reduce fish production caused by a serious deterioration of living conditions (lack of light, lack of oxygen, anaerobic decomposition of dead biomass). If unenclosed water is used as the source of drinking

	water for cattle, *Azolla* may deter and even prevent their access to water of satisfactory quality.

a40. IMPACT ON THE HUMAN DOMAIN – Due to climate change, the consequences of *the species* on human in Poland will:

	decrease significantly
	decrease moderately
	not change
X	increase moderately
	increase significantly

aconf36.	Answer provided with a	low	medium	high **X**	level of confidence

acomm40. Comments:

An increasing number of water bodies with *Azolla* will increase the impact of the species on people. However it will only have an indirect effect expressed as a decreased recreational value of water bodies, deteriorated its aesthetic qualities and a limitation to the diversity of the food base (a drop in fish production). Massive occurrence of *Azolla* on water surfaces may also have a positive aspect: there would be smaller population of mosquitoes as their larvae would have no access to oxygen. This may be significant if the extent of malaria carried by those insects starts to cover larger areas (because of global warming).

a41. IMPACT ON OTHER DOMAINS – Due to climate change, the consequences of *the species* on other domains in Poland will:

	decrease significantly
	decrease moderately
	not change
	increase moderately
X	increase significantly

aconf37.	Answer provided with a	low	medium **X**	high	level of confidence

acomm41. Comments:

The impact of *Azolla* on infrastructure refers only to its impact on water bodies (access to water and its quality), ditches and their water (flow rate or no downflow). *Azolla* occurrence on a massive scale will have significant effects for water bodies in Poland.

Summary

Module	Score	Confidence
Introduction (questions: a06-a08)	0.83	0.67
Establishment (questions: a09-a10)	0.75	0.75
Spread (questions: a11-a12)	1.00	0.75
Environmental impact (questions: a13-a18)	0.65	0.90
Cultivated plants impact (questions: a19-a23)	0.10	1.00
Domesticated animals impact (questions: a24-a26)	0.25	0.50
Human impact (questions: a27-a29)	0.25	1.00
Other impact (questions: a30)	0.50	0.50

Invasion (questions: a06-a12)	0.86	0.72
Impact (questions: a13-a30)	0.65	0.78
Overall risk score	0.56	
Category of invasiveness	moderately invasive alien speciesp	

A6 | Comments

This assessment is based on information available at the time of its completion. It has to be taken into account. However, that biological invasions are, by definition, very dynamic and unpredictable. This unpredictability includes assessing the consequences of introductions of new alien species and detecting their negative impact. As a result, the assessment of the species may change in time. For this reason it is recommended that it regularly repeated.

acomm42. Comments:

In this risk assessment, the water fern *Azolla filiculoides* was judged to be a moderately invasive alien species. It was allocated a relatively high mark of 0.65 in the module: Impact on environmental domain (questions: a13-a18) and an average mark of 0.5 in question a30 (Impact on other domains). In the remaining modules, this species got slightly lower marks: Impact on cultivated plants domain (questions: a19-a23) – 0.10, Impact on human domain (questions: a27-a29) – 0.25, and Impact on domesticated animals domain (questions: a24-a26) – also 0.25.

Azolla is regarded as a species without a complete life cycle in Poland. In our climate, this fern does not produce spores. Taking into account the likely possibilities for spreading its vegetative parts with water current and by means of waterfowl, high marks were given in modules related to its expansion (questions: a06-a12) – within the range of 0.75-1.00.

The assessment was based on expertise and available data. Due to the invasive nature of *Azolla* and its strong impact on the natural environment, it is recommended to undertake actions aiming at its elimination. Due to the nature of habitat conditions, we should focus on preventive measures against using the species for aquarium and gardening purposes and ensure adequate monitoring. Also, suitable educational actions should be carried out. A lack of actions which would reduce the occurrence of the species may favour its further expansion. The risk to the native fauna and flora of aquatic ecosystems shall be regarded as an additional argument for paying special attention to this species that should be controlled, at least in the most valuable natural areas.

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