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Review

Detection and Management of Freshwater Invasive Alien Species through Environmental DNA Analysis and Geographic Information Systems: A Review

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Abstract: Freshwater invasive alien species (IAS) are non-native organisms that were intentionally or unintentionally released into local water bodies and later harmed the invaded habitat by disrupting the ecological processes. Over the last few years, environmental deoxyribonucleic acid (eDNA) analyses have been used in many studies to detect IAS, with positive results. However, with the help of geographic information systems (GIS), efforts to detect the presence of IAS can be made faster and more efficiently. In this paper, we review the background of IAS in Southeast Asia and management efforts undertaken involving the input of known habitat-specific geographical parameters into GIS mapping. Via this strategy, it is possible to identify and distinguish areas that fit IAS habitat features from those that do not. eDNA analysis can later be applied to confirm the presence of IAS in detected areas, enabling further studies and actions. The presence of IAS in certain areas can be used as an indicator to assess the environmental integrity of native waterways. This combined method is likely the first approach to be applied to the detection of freshwater IAS in local water bodies. Apart from saving energy and resources, embedding GIS and eDNA into the study of IAS not only benefits the ecosystem but also assists locals and authorities in managing and taking necessary enforcement actions to curb further spread.

Keywords: invasive alien species; freshwater; management; environmental DNA; geographic information system; sustainability

1. Introduction

Humans and nature are generally dependent on each other [1]. The relationship between humans and the environment can also be considered somewhat mutualistic. Freshwater ecosystems are already suffering from biodiversity loss and overexploitation [2]. Invasive species can be deemed as one of the most detrimental causes of the decline in native habitat populations [3] and overall freshwater ecosystem biodiversity [2,4,5]. The unnecessary introduction of invasive species into native environments will only worsen existing woes with respect to freshwater biodiversity, conservation efforts, and economies, such as biological pollution, ineffective enforcement and habitat loss, and loss of income, respectively [6–9].

In the case of non-native freshwater animal species, anthropogenic activities are the main reason why such invasive alien species (IAS) could be lingering in local ecosystems [10–15]. Such effects are especially apparent in terrestrial environments where confined geographical boundaries and natural landscapes make the introduction of non-native species more repercussive, implying dangerous ramifications for local and global ecosystems and biodiversity [16–18]. In some countries, IAS have been bred in local environments, often for reasons such as food (aquaculture industries), collection (ornamental), or recreational fishing [19,20].

The accidental or intentional release of IAS into wild habitats leads to invasion of native waterways, effectively disrupting and harming natural local ecosystems [19,21]. If minimal or no actions are taken, such alien species influence native species in the invaded ecosystem, as they fight for the same resources and benefits, such as territory, shelter, and food [20]. In the worst-case scenario, native species might be out-competed or even rendered extinct by invasive species [22]. Bellard et al. [23] added that invasive species are the major reason for species extinction globally. In the event of extinction, local ecosystems and biodiversity can collapse. Humans may suffer, especially in the economic sector and in terms of human health, eventually leading to major public concern [24–27]. Thus, early assessment is vital to predict, supervise, and prevent the impacts of IAS from inflicting unwanted damage or disruptions on native ecosystems and biodiversity [28–32]. The presence of IAS in certain areas can also be used as an indicator to assess the environmental integrity of native waterways [32–34]. The biodiversity of fishery ecosystems can be a good ecological indicator [35] to assess IAS, providing important information for further studies.

Carpio et al. [36] and Khaleel et al. [9] reported that regional breaches in biogeographical barriers have led to increased introduction of IAS in native areas. The ornamental trade is the second most significant route of IAS introduction in some countries [37]. Online auction marketplaces are deemed among the international pathways to the establishment of aquatic IAS [38]. Fishery activities, in combination with international species trading, have influenced the introduction of non-native species into local areas [39]. During the global COVID-19 pandemic, the implementation of the movement control order (MCO) likely reduced recreational fishing activities, limiting the release of IAS into other regions around the world. However, Cooke et al. [2] warned that when the COVID-19 pandemic is finally under control, human-induced IAS release into local waterways might hasten as the economy recovers.

Our freshwater ecosystem, including agriculture and freshwater food production, should always be protected; the dangers that may be posed by IAS have never been clearer. Therefore, locating and eradicating IAS are important to prevent more damage from occurring. As stated by Walton et al. [40], studies on IAS might assist in conservation efforts to protect the biodiversity of tropical environments. Together with studies on invasive species distribution in local landscapes, continuous mitigation efforts and aggressive enforcement action should be undertaken to ensure the protection of native species and their biodiversity [41].

A systematic review was conducted by Kestel et al. [42] to identify the current uses of eDNA-based monitoring in agriculture, the substrates and organisms routinely being targeted, and the geographical distribution of such studies. Invasive species may indirectly affect ecosystem functioning by altering plant variety, plant biomass, or both. Many invasive trees, including Prosopis [43], were originally introduced to increase soil stability, carbon storage, and soil fertility [44]. For example, a decline in plant diversity lowers biomass output, which, in turn, affects other functions, allowing us to distinguish between three indirect effects of invaders on functioning: those mediated by only diversity, those mediated by only biomass, and those mediated by both richness and biomass. However, we do not know the significance of changes in biomass and diversity as indirect effects of invaders because few studies have taken into account how they can propagate through ecosystems to affect associated functions [45]. Determining which invasive species are responsible for environmental change is often challenging. For instance, a disturbance could lead to more invasion and have an impact on ecosystem health [46].

Strong mitigation efforts are needed to curb the introduction of IAS. Moreover, a new method should be developed to assist in related studies pertaining to global IAS distribution and dispersal behavior. Conventional sampling methods may be considered destructive or invasive if performed frequently in a study area, which can lead to habitat destruction [32,47–49]. By using eDNA, the mere collection of water samples in targeted areas is enough for researchers to determine the presence of IAS [50].

Together with eDNA analysis, we propose that researchers also use GIS information to assist in the selection processes of suitable habitats in which IAS may reside. We suggest that such a novel method should combine the use of spatial technological data, geobiological data, and eDNA analysis to detect and predict the distribution and potential habitats [51,52] of freshwater non-native invasive species in invaded ecosystems. The spatial distribution of eDNA can not only be driven by water movement centering on the IAS activity and habitat preferences but also by other factors such as wind, precipitation, water current, and other trophic conditions [49,53–55].

The IUCN has also stated that IAS can have significant ecological effects on invaded ecosystems, as later supported by Havel et al. [56]. In their new habitats, IAS can lack natural predators, enabling their populations to rapidly increase. IAS may also bring pathogens, alter food chains, outcompete or prey on native species, consume other food resources, and even modify ecosystems [19,57–62]. IAS might replace native organisms and potentially alter ecosystems and disrupt food chains while reducing and destroying the genetic diversity of indigenous species [63]. As Dali et al. [64] mentioned, the introduction of IAS has made the study of genetic diversity vital. The presence of an invasive species, particularly in native inland waters, is considered a major challenge for the protection and preservation of local fish biodiversity [62,65].

These effects could lead to the local or global extinction and subsequent ecological and economic destruction of native species communities [66]. Moreover, IAS can affect the terrestrial fishing industry and aquaculture, disrupting the economies of the country [67]. For instance, in Malaysia, the presence of IAS (peacock bass *Cichla* spp.) in freshwater ecosystems has recently caused drastic declines in native species populations [68]. Using eDNA, early detection of IAS and their abundance may be vital for the monitoring of species distribution [69–71], in addition to aiding in successful invasion management and reducing the dangers posed by IAS to the local environment [72]. Invasive species distribution is usually unknown and can be quickly altered. Therefore, attempts should be made to monitor the expansions of IAS distributions [73]. Premature detection of invasive species can be critical in limiting the settlement and dispersal of the invasive species [74].

The main constraint of eDNA is its stability, as it can remain in the environment for a long period, together with occurrences of false positives in metabarcoding analysis [75,76]. Recent advancement in technologies such as environmental RNA (eRNA) has enabled precise ecological surveys with high positive predictions and the ability to overcome false positives [77,78]. Combining eRNA, eDNA, and GIS techniques may provide a positive solution to revolutionize ecological surveys in the future. However, studies using the eRNA method remain rare and expensive.

In this review paper, we discuss a preliminary method that can be used to assess the potential dispersal and behavior of IAS using the combination of eDNA and GIS. Identifying the patterns of IAS invasions has become increasingly important, with the goal of better comprehending their potential impacts on local environments and ecosystems [52] and identifying actions to be taken to contain said impacts. Herein, we provide an overview of IAS in conjunction with management and methods to locate and detect IAS habitat and their distribution in local landscapes. Furthermore, we discuss a preliminary protocol that can be used to assess the potential dispersal and behavior of IAS using the combination of eDNA and GIS methods.

2. Literature Review Method

Figure 1 shows all the literature and information searched, analyzed, and cited in this paper, comprising unique and reliable sources such as academic papers and/or articles (n = 234), books (n = 11), dissertations (n = 2), and Internet sources such as official websites (n = 11). Search results were filtered using predetermined keywords pertinent to the topic of this study, such as aquatic IAS, foreign freshwater species, non-native species, invasive species distribution, eDNA analysis, geographic information system (GIS) data, GIS analysis, eDNA and GIS, ecological management and conservation, Southeast Asian and Malaysian background and guidelines, and policies and laws regarding foreign and imported animals.

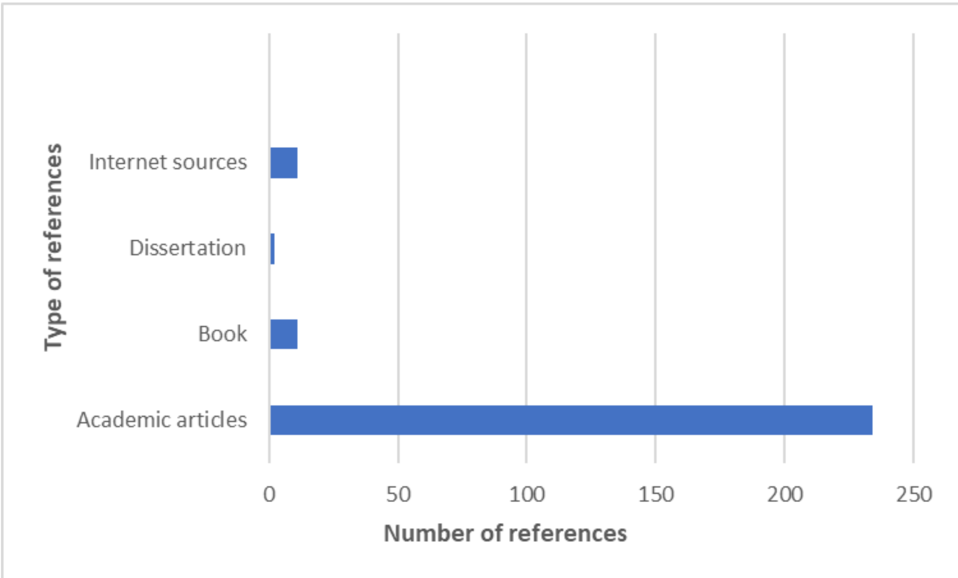

Figure 1. Literature cited in this study.

The inclusion and exclusion methods used in this review were based on those established by John et al. [79] and Sastraprawira et al. [80]. Academic papers and journal articles made up the majority (90.7%) of the cited references. Research articles from indexed databases such as Scopus, Web of Science (WoS), and/or International Scientific Indexing (ISI) were included in this literature review, making up 94.02% (n = 220) of all academic papers/articles. However, only 5.98% (n = 14) of them are other-indexed papers that were still inserted, since the information that they provide was deemed useful in this study.

Meanwhile, 4.26% of the references originated from books searched using the Google Scholar database. Dissertations were found using university databases and Google Scholar, making up 0.78% of total references. However, published dissertations were not used, since they were considered research articles. Meanwhile, Internet sources (4.26%) such as links and websites were only taken into consideration if they were associated with the official websites of known authorities or established institutions/organizations with reliable integrity.

The sources were filtered proportionally over the timespan of a decade, from the year 2011 to 2023 (as May), to maintain their relevance. Certain significant studies (n = 19) published before 2011 were also used, since not only did they match the searched keywords, but they also provided significant information that was considered apposite to the related topic of IAS.

3. Applications of Environmental DNA (eDNA) for IAS Detection

Water is an excellent medium for the deposition and transfer of intracellular or extracellular organismal DNA from organisms, rendering aquatic ecosystems appropriate for eDNA investigation [81]. For instance, many types of water bodies [49] can be designated as study areas for eDNA studies, such as rivers [81,82], ponds [83], reservoirs [84,85], lakes [54], pools [86], and marine environments [87]. Therefore, eDNA has recently risen to prominence as a cutting-edge tool for biomonitoring of freshwater biodiversity and ecosystems, as it enables the screening of entire communities [49,88,89].

Samples consisting of DNA extracted from the environment, including water, air, and soil, are referred to as eDNA-containing genetic material released by organisms that can be used to detect species types [89–91]. For instance, water samples can be analyzed and screened for traces of DNA from aquatic species using a broad approach or a targeted approach. The eDNA technique has also been used in other media, such as air and soil. Airborne eDNA can be found in the air as bioaerosols. By sequencing these bioaerosols, terrestrial biodiversity can be surveyed across all lifeforms, such as land-living vertebrates, insects, fungi, bacteria, and plants [92]. Clare et al. [93] successfully used open-environment, airborne eDNA as a biomonitoring technique to detect multiple taxa of terrestrial species in air samples. eDNA can also be used to both assess plant biodiversity and to compare the composition of eDNA collected from soil and vegetation surveys [94]. The combination of a vegetation survey and soil eDNA results in the most comprehensive plant diversity inventory. eDNA analysis is still considered a rapidly evolving method of research that can aid in the premature detection of IAS and aquatic species conservation [70,95]. Studies conducted by Hanfling et al. [96], Olds et al. [97], Pont et al. [98], and McDevitt et al. [99] have shown that using eDNA increases fish detectability compared to conventional methods.

Although eDNA has many advantages when used in detection studies, there are still some drawbacks of using this detection method. One of the limitations of using eDNA detection is the degradation. eDNA can degrade as a result of several factors, such as light (UV), pH, temperature, and bacterial activities [48,100–109]. Light in the form of UV-B was found to be among the factors that can degrade eDNA. UV-B degrades eDNA by destroying DNA pair bonds [101,107,109,110]. Another factor that influences the degradation of eDNA is pH. eDNA tends to have higher degradation rates in acidic conditions compared to more alkaline environments because of the hydrolytic processes [101,107,109,111]. Bacterial activities have also been found to contribute to eDNA degradation.

eDNA is used by bacteria as a nutrient source and a means to repair their cells by secreting DNase and breaking down the eDNA into simpler compounds [100,103,104,112]. According to Joseph et al. [112], the waste product of bacterial activities also causes eDNA to degrade, as the products destabilize the eDNA bonds, in addition to the occurrence of horizontal gene transfer, which increases eDNA degradation. Temperature is the most prominent factor contributing to eDNA degradation. eDNA was found to degrade faster in water with higher temperatures compared to colder water [100–103,105,107,113]. Temperature increment also increases bacterial activities, which can further speed up eDNA degradation [106,110,112]. Hence, precautions should be taken in the sampling and storing process for eDNA; for example, samples should be stored at cool temperatures and away from sunlight to prevent eDNA degradation.

Another factor limiting the use of eDNA is the presence of primer bias during PCR amplification. Primer biases occur owing to the use of PCR primer pairs that have low universality, which causes the DNA of some species, especially in low concentrations in extracted eDNA samples, to not be amplified [114–118]. To overcome this limitation, more than one type of primer set should be used for sample amplification. Furthermore, the eDNA detection method can cause false positives. False positive detection can be caused by the amplification of dead organisms instead of living organisms [119–122]. Therefore, DNA and RNA should be extracted, and both long and short DNA fragments should be extracted. PCR inhibitors are another drawback of using this eDNA detection method. PCR inhibitors can be sourced from extracted samples, such as tannins, humic acids, metal, or dissolved protein, which are capable of preventing the target from being amplified [123–125]. Inhibition tests of all extracted eDNA samples are recommended before amplification via PCR in order to avoid this problem.

eDNA analysis does not require the extraction of samples from the physical bodies of targeted organisms. Thus, eDNA is not an invasive technique [126]. eDNA analysis is still considered a new or alternative method to be utilized as a monitoring tool in the detection of IAS [127–129]. Sakai et al. [50] suggested eDNA analysis as a tool for local people in the role of citizen scientists. Nasir et al. [129] also concluded that using eDNA analysis for IAS detection may be a promising method to identify the species present in the environment.

In the past decade, the use of molecular genetics through eDNA analysis has attracted considerable interest from researchers for monitoring and identifying the DNA of organisms or species present in samples, as summarized in Table 1. eDNA analysis is becoming increasingly popular among researchers in general. In the context of IAS monitoring, the eDNA approach provides the opportunity to investigate the dispersion of invasive species without considerable impact on the environment, as it is a non-invasive and eco-friendly method with respect to the native environment [32,48,130,131]. According to Thomsen and Willerslev [132], eDNA is a non-invasive method that imposes no impairment on habitats or species under study. By using only water samples, species-specific fragments of DNA cam be used to improve the precision of IAS detection. DNA extracted from water samples can be utilized to deduce the existence of a given species with greater sensitivity than the traditional method [133], making it easier to detect the presence of invasive species, in addition to saving survey costs.

Table 1. The various uses of environmental DNA analysis in studies conducted from 2010 to 2023 (as of May 2023).

Title	Year	Authors
Assessing macroinvertebrate biodiversity in freshwater ecosystems: Advances and challenges in DNA-based approaches	2010	Pfrender et al. [134]
Persistence of environmental DNA in freshwater ecosystems	2011	Dejean et al. [100]
Identification of a divergent environmental DNA sequence clade using the phylogeny of gregarine parasites (Apicomplexa) from crustacean hosts	2011	Rueckert et al. [135]
Improved detection of an alien invasive species through environmental DNA barcoding: The example of the American bullfrog *Lithobates catesbeianus*	2012	Dejean et al. [127]
Surveillance of fish species composition using environmental DNA	2012	Minamoto et al. [136]
Monitoring endangered freshwater biodiversity using environmental DNA	2012	Thomsen et al. [137]
Environmental DNA as a new method for early detection of New Zealand mud snails (*Potamopyrgus antipodarum*)	2013	Goldberg et al. [138]
Endangered Proteus: Combining DNA and GIS analyses for its conservation	2014	Aljancic et al. [139]
Quantifying environmental DNA signals for aquatic invasive species across multiple detection platforms	2014	Nathan et al. [140]
Environmental DNA surveillance for invertebrate species: advantages and technical limitations to detect invasive crayfish *Procambarus clarkii* in freshwater ponds	2014	Treguier et al. [141]
Quantification of eDNA shedding rates from invasive bighead carp *Hypophthalmichthys nobilis* and silver carp *H. molitrix*	2015	Klymus et al. [142]
Environmental DNA (eDNA) detection probability is influenced by seasonal activity of organisms	2016	De Souza et al. [53]
Saving the doomed: Using eDNA to aid in detection of rare sturgeon for conservation (Acipenseridae)	2016	Pfleger et al. [143]
Monitoring of noble, signal and narrow-clawed crayfish using environmental DNA from freshwater samples	2017	Agersnap et al. [144]
Using eDNA to detect the distribution and density of invasive crayfish in the Honghe Hani rice terrace World Heritage site	2017	Cai et al. [145]
Distribution changes, genetic population structure, and a novel environmental DNA (eDNA) detection method for Darters (Subgenus Nothonotus) in the upper Ohio River watershed	2017	Honick [146]

Table 1. *Cont.*

Title	Year	Authors
Searching for a signal: Environmental DNA (eDNA) for the detection of invasive signal crayfish, *Pacifastacus leniusculus* (Dana, 1852)	2018	Harper et al. [147]
A rapid assessment method to estimate the distribution of juvenile chinook salmon (*Oncorhynchus tshawytscha*) in an Interior Alaska river basin	2018	Matter [148]
Water, water everywhere: Environmental DNA can unlock population structure in elusive marine species	2018	Parsons et al. [149]
Using environmental DNA and occupancy modelling to identify drivers of eastern hellbender (*Cryptobranchus alleganiensis alleganiensis*) extirpation	2018	Wineland et al. [150]
Refinement of eDNA as an early monitoring tool at the landscape-level: Study design considerations	2019	Mize et al. [151]
Effective detection of environmental DNA from the invasive American bullfrog	2019	Lin et al. [152]
Environmental DNA sampling reveals high occupancy rates of invasive Burmese pythons at wading bird breeding aggregations in the central Everglades	2019	Orzechowski et al. [153]
The detection of aquatic macroorganisms using environmental DNA analysis—A review of methods for collection, extraction, and detection	2019	Tsuji et al. [154]
Applications of environmental DNA (eDNA) in ecology and conservation: Opportunities, challenges and prospects	2020	Beng and Corlett [48]
Environmental DNA allows upscaling spatial patterns of biodiversity in freshwater ecosystems	2020	Carraro [155]
Large scale eDNA metabarcoding survey reveals marine biogeographic break and transitions over tropical north-western Australia	2021	West et al. [156]
eDNA metabarcoding as a biomonitoring tool for marine protected areas	2021	Gold [157]
Development and testing of an environmental DNA (eDNA) assay for endangered Atlantic sturgeon to assess its potential as a monitoring and management tool	2021	Plough [158]
Detecting spawning of threatened chum salmon *Oncorhynchus keta* over a large spatial extent using eDNA sampling: Opportunities and considerations for monitoring recovery	2021	Homel et al. [159]
GAPeDNA: Assessing and mapping global species gaps in genetic databases for eDNA metabarcoding	2021	Marques et al. [160]
Evaluating eDNA for Use within Marine Environmental Impact Assessments	2022	Hinz et al. [161]
Using eDNA techniques to find the endangered big-headed turtle (*Platysternon megacephalum*)	2022	Lam et al. [162]
Population decline of an endangered unionid, *Pronodularia japanensis*, in streams is revealed by eDNA and conventional monitoring approaches	2022	Hata et al. [163]
Mitochondrial genomes assembled from non-invasive eDNA metagenomic scat samples in the endangered Amur tiger *Panthera tigris altaica*	2022	Baeza et al. [164]
Practical eDNA sampling methods inferred from particle size distribution and comparison of capture techniques for a Critically Endangered elasmobranch	2022	Cooper et al. [165]
Aquatic eDNA can advance monitoring of a small-bodied terrestrial salamander and amphibian pathogen	2022	Kaganer et al. [166]
Playing "hide and seek" with the Mediterranean monk seal: A citizen science dataset reveals its distribution from molecular traces (eDNA)	2023	Valsecchi et al. [167]
Combining multiple markers significantly increases the sensitivity and precision of eDNA-based single-species analyses	2023	Brys et al. [168]
Quantitative monitoring of diverse fish communities on a large scale combining eDNA metabarcoding and qPCR	2023	Pont et al. [169]
Advancing DNA barcoding to elucidate Elasmobranch biodiversity in Malaysian Waters	2023	Loh et al. [170]
eDNA metabarcoding from aquatic biofilms allows studying spatial and temporal fluctuations of fish communities from Lake Geneva	2023	Rivera et al. [171]

4. Use of Geographic Information Systems (GIS) for Detection of Invasive Alien Species

A GIS is a computer-based information technology system that stores all digital data in one location, allowing for fast and efficient data retrieval and analysis in map format [172]. Using spatial and temporal data that can be obtained from various databases on the Internet, these data can be analyzed collectively to obtain different layers of information, such as elevation and climate data. Incorporating GIS in IAS studies is beneficial, as the spatial links or spatial identifiers between layers can help to reveal any hidden relationships to make inferences in such studies [172,173]. GIS is convenient tool for the creation and storage of large amounts of information at once, instantly generating results in the form of maps.

GIS mapping and eDNA analysis have also been integrated by some researchers, namely, for the study of niche modeling to map species habitats [174], the comparison of local- and regional-scale estimations of fish diversity in streams [175], the detection of large, rare river crayfish [176], the discovery of Yamato salamander [50], the evaluation of diversity across a river network [177], and the determination of the spatial distribution and dispersal pattern of redclaw crayfish in Indonesia [41], among other applications. As foreign crayfish species are versatile in terms of diet and exhibit opportunistic behavior that can alter the native ecosystem [178–180], the use of GIS data may contribute to improved understanding, as the produced maps and parameters provide a clear view of the specific locations pertaining to targeted crayfish and specific habitats and can also be used for other IAS species. eDNA can also be used to trace endangered crayfish, as reported by Atkinson et al. [181] and Troth et al. [182] for the detection of freshwater white-clawed crayfish (*Austropotamobius pallipes*).

Research conducted by Sakai et al. [50] proved that the combination of GIS analysis and eDNA analysis is fruitful. In their studies, an interesting strategy (Figure 2) for identification of the potential habitats of an endangered species of Yamato salamander (*Hynobius vandenburghi*) was established using GIS and eDNA analysis in Japan. The ability of GIS to handle large datasets and to survey large areas allowed them to analyze spatial information to predict and to locate the potential habitats of *H. vandenburghi*.

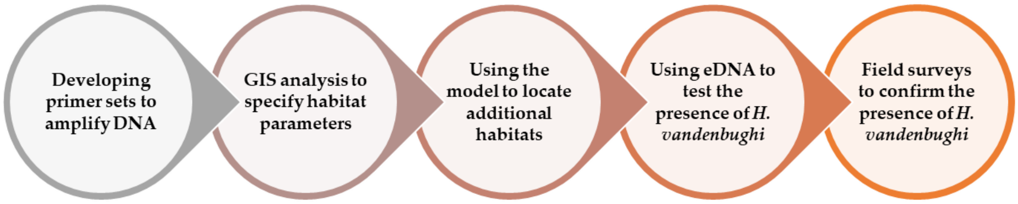

Figure 2. Strategies developed by Sakai et al. [50] to detect *H. vandenburghi*.

As shown in Figure 2, eDNA analysis was performed using samples collected based on the potential habitat map, and field surveys were conducted to confirm the presence of *H. vandenburghi* at the locations that produced positive eDNA results. Additionally, the use of eDNA allows for more efficient physical surveys; for example, Herder et al. [183] reported up to 99–100% detection probability. In their study, GIS was used to analyze parameters such as precipitation, temperature, altitude, land use, and other geographic characteristics.

Sakai et al. [50] analyzed their study area by incorporating the known environmental conditions of *H. vandenburghi*, such as elevation, slope, and vegetation coverage, through GIS analysis. By utilizing the strategy implemented in Figure 2, they effectively and rapidly identified the areas that were most likely to contain the targeted species, reducing field survey costs. They also showed that combining GIS analysis with eDNA analysis can be a very useful method that can be used by researchers and locals alike for many purposes, including in efforts to monitor IAS in Malaysia. However, this method requires specific knowledge of the environmental conditions or habitat parameters of the targeted species.

GIS data was used to observe beta diversity relative to stream distance by extracting the distances between river junctions to be added as edge weights for the assessment of different components of diversity across river networks in northwestern Switzerland using eDNA [177]. Machler et al. [177] showed that GIS data helped in their study by identifying and color-coding field sites across the river network in their study area. With the help of maps generated by GIS, eDNA samples were then collected from each site. According to their results, the local richness estimations from eDNA and sampling were similar, possibly owing to the strengthened inclusion aspects for eDNA estimates. Machler et al. [177] also concluded that eDNA can be deemed a reliable tool for detection of biodiversity patterns. However, the spatial scale of GIS analysis must be considered in order to extend biodiversity datasets to determine taxon richness.

In another study, Rice et al. [176] utilized the combined method of GIS analysis and eDNA analysis. In their paper about the detection of a large, rare river crayfish (*Faxonius eupunctus*) with little relation to local abundance, eDNA was described as an emerging surveillance technique with the potential to revolutionize the conservation and management efforts of freshwater species and ecosystems because extracted DNA can be utilized to detect the presence of species with greater sensitivity than the traditional method, as supported by Jerde et al. [133]. Moreover, Rice et al. [176] did not conduct eDNA analysis concurrently with traditional sampling in order to reduce the contamination risk.

Using GIS mapping, Rice et al. [176] managed to produce a map of The Eleven Point River drainage covering an area of approximately 1115 km^2 in the Ozark Highlands ecoregion in southern Missouri and northern Arkansas, USA. The use of GIS mapping allowed the exact locations of sampling sites to be marked and color-coded to indicate the presence of *F. eupunctus*. The probable sites were first identified within each stream based on the presence of wadeable riffle mesohabitats that were visible in satellite images with respect to the known habitat associations of *F. eupunctus*. A global model containing local *F. eupunctus* density was used as the sole predictor of occupancy. The in-stream temperature, upstream river distance, canopy closure, and local density of *F. eupunctus* were included as predictors for detection probability. The presence of *F. eupunctus* was detected at 19 out of 39 sites through the use of eDNA analysis. Local *F. eupunctus* densities were found to range between 0.08 and 7.92 individuals per meter at detected sites. The lowest densities were observed at the most downstream and upstream portions of the study area. A strong relationship was also found between the upstream river distance and the environmental DNA detection probability. These results indicated that the abundance or biomass of certain organisms correlate with the abundance of eDNA in the environment and that eDNA is reliable in representing the occupancy of species.

GIS-based modeling can also be used to map species habitats, as attempted by Rotenberry et al. [174], who reported that suitability models can be a powerful tool for researchers and environmentalists. Such models might have a direct connection with the ecology of targeted species. Modeling techniques can first be applied to identify the relative importance of variables influencing the distribution of a species. Next, a spatial assessment of habitat suitability can be conducted, and later, predictions about habitat suitability can be extended into unknown areas to search for the occurrence of a particular species. If the habitat parameters of a specific IAS are known and later provided for habitat suitability modeling/mapping, the projected probability map can be used to locate suitable habitats for IAS to develop or inhabit, and eDNA analysis can later be performed to detect their presence at the predicted areas.

Nakagawa et al. [175] also used various GIS data, namely elevation, river, lake, pond, and coastline data, to compare local- and regional-scale estimations of the diversity of stream fish using eDNA metabarcoding, as well as conventional observation methods. Existing data were projected by their environmental location, such as catchment boundaries and elevation. Because of the directional flow of river water, the authors also theorized that eDNA inferences are more consistent with fish assemblages observed upstream than downstream. The presence of fish DNA in the water column has indicated that eDNA can be utilized as a tool to detect biomass, movement activities, and spawning of fish in population genetics studies, as well as those of invasive and threatened species [66,133,175].

In a study conducted to determine the spatial distribution and dispersal pattern of *Cherax quadricarinatus* or Australian redclaw crayfish in Indonesia, Akmal et al. [41] used GIS software to help connect the database design of their study with available spatial data. Sor et al. [184] also used GIS to incorporate available geographical data to calculate the surface area and land-cover data of their study site. Akmal et al. [41] reported that different environmental conditions, such as altitude differences in Java, Indonesia, led to the adaptation of *C. quadricarinatus*, enabling them to be distinguished by their morphological variations. Therefore, the authors concluded that a high-altitude range produces the most discriminant grouping between locations. In Malaysia, the use of elevation/altitude data via GIS can also be proposed to further understand and determine the correlation between IAS dispersal patterns and morphological behaviors. Suhaila et al. [185] reported the first wild record of Australian redclaw crayfish on the east coast of Peninsular Malaysia, so the concept proposed by Akmal et al. [41] might be applicable in future *C. quadricarinatus* studies in Malaysia.

Additionally, Low et al. [186] used the ecological niche modeling (ENM) method to produce a distribution model of five well-known freshwater IAS, namely, the Australian redclaw crayfish (*C. quadricarinatus*), African sharptooth catfish (*Clarias gariepinus*), red swamp crayfish (*Procambarus clarkii*), American bullfrog (*Lithobates catesbeianus*), and Mozambique tilapia (*Oreochromis mossambicus*). ENM is a tool used to predict the suitability of habitats through the use of a species occurrence database including environmental data collected across geographical landscapes, such as climatic predictor datasets. Additional variables in terms of topographic characteristics (slope, elevation, and total upstream area) have also been used, as they appear to be informative indicators in terms of modeling freshwater species distribution.

Many methods can be used to detect, locate, study, examine, or investigate IAS distribution. Ficetola et al. [187], Mike et al. [188], Bradley [189], Elith and Leathwick [190], Smolik et al. [191], Capinha and Anastacio [192], Vaclavik and Meentemeyer [193], Guisan et al. [194], and Bae et al. [15] all used species distribution modeling (SDM) to predict species occurrence at a given location based on environmental and spatial behaviors. Ecological niche models (ENMs) have also been utilized to predict potential invasion areas based on species occurrence and environmental conditions to assist in management efforts to contain IAS [195–201]. GIS data can be manipulated in many ways for the types of research discussed herein. The combined method of GIS analysis and eDNA analysis can not only enable more efficient, resource-saving detection and eradication attempts with respect to IAS but also contribute to necessary prevention and/or control efforts.

The combination of eDNA, spatial GIS data, and other ecological modeling techniques can represent an improved method for the determination of the potential habitats of invasive alien species, leading to improved discoverability and predictability. GIS mapping can help to predict the spread of invasive species in new, undiscovered habitats. The ability of GIS to evaluate various types of spatial data makes it possible to monitor and conduct successful surveys, as proven by Sakai et al. [50] and Brown and Vasseur [202]. Potential habitats of invasive species, as well as possible new species, can be identified by covering a larger and more specific area using GIS. Fused eDNA and GIS analyses can be carried out to confirm the presence of invasive species, especially at locations that conform with habitat-specific parameters, yielding positive or negative results with respect to the presence of IAS. Because GIS is able to handle large data sets, Mitsuhashi and Kamata [203] found that surveys of targeted species were rendered more efficient using this approach.

Within this concept, research on potential habitat distributions of IAS can be conducted more effectively, cutting field survey costs, conserving manpower energy, and saving time [32]. Moreover, the confirmed presence of IAS in potential habitats can propel further management efforts to preserve ecosystem biodiversity. According to Thomsen et al. [137], conservation efforts should be conducted effectively, as freshwater ecosystems are among the most endangered habitats on the planet. Research findings can contribute to the monitoring of notorious IAS such as Australian redclaw crayfish (*C. quadricarinatus*), red swamp crayfish (*Procambarus clarkii*), Asian redtail catfish (*Hemibagrus wyckioides*), and peacock bass (*Cichla* spp.).

Therefore, eDNA and GIS technology can be combined as a novel tool to detect IAS, benefiting researchers and policy makers in monitoring the effects of IAS in Malaysia. However, there is a dearth of studies focusing on the spatial distribution and dispersal patterns of invasive species in Malaysian terrestrial waters. The presence of IAS has attracted the attention of local fishermen as they suffer many losses owing to the damage inflicted on their fishing nets and catches [185]. The situation is expected to deteriorate further for both aquaculture and aquarium industries if IAS spread is expanded and accelerated. Since the integration of eDNA and GIS for remote species detection has not been attempted before in Malaysia, this strategy can be proposed as part of fishery management methods in the future.

5. Biodiversity in Southeast Asian Ecosystems

Southeast Asia (SEA) houses some of the richest biodiversity and ecosystem regions on Earth, with a high rate of discovery of new species [204–206]. SEA countries such as Malaysia, Indonesia, and the Philippines are among the 17 mega-diverse countries globally [207]. Therefore, it is not surprising to see some aquatic IAS find their way into SEA countries. As the region is tropical with consistent climactic conditions, IAS can geographically spread and establish themselves in local environments with the help of humans, who have intentionally introduced such species for economic or leisure purposes.

Malaysia is among the most mega-diverse countries in the world, harboring a large number of flora and fauna [208,209]. Mohd Azmi [210] added that Malaysia has a variety of habitats and ecosystems suitable for a various lifeform, including fish species. Malaysia has a total surface area of inland freshwater bodies and wetlands including rivers, swamps, dams, lakes, reservoirs, and paddy fields totaling 45,459 km^2. Fish colonization is aided by vast areas of water space and diverse aquatic habitats [208]. In recent decades, Mohd Azmi [210] reported a total of 413 freshwater species in Malaysia belonging to 178 families. However, freshwater habitats accommodate the highest number of endangered or threatened fish species. A previous study recorded 32 highly threatened fish species in Malaysia, half of which are found in freshwater ecosystems [211].

6. Background on Invasive Alien Species across Southeast Asia

The non-native Australian redclaw crayfish (*C. quadricarinatus*) has not only spread to Malaysia [18] and Indonesia [41] but also to Thailand [212] and Singapore [213], mostly owing to the influences of aquaculture and ornamental trade industries. The introduction of a freshwater mollusk (*Pomacea* spp.) into Thailand and *Physa acuta* into Singapore and Malaysia was also a result of the ornamental trade [214]. The presence of *Pomacea* spp. has also been noticed in Vietnam, Laos, and Cambodia [215], making the invasiveness of apple snails significant, especially given that Cambodia is the most speciose country for unionid bivalves in SEA [214].

In addition to invasive freshwater limpets (*Ferrissia* cf. *californica*), which were accidentally introduced into Singapore through the means of aquatic plants [3], Singapore is also home to 98 non-native freshwater fish species that have dominated manmade reservoirs and canals [216]. *Cyprinidae* and *Cichlidae* account for more than 57.4% of Singapore's non-native freshwater fish species, in line with a study reported by Hui et al. [217], wherein the majority of 123 reported non-native freshwater fish species comprised *Cyprinade* and *Cichlidae*, other than *Poeciliidae* and *Osphronemidae*. Hui et al. [217] produced a distribution map concerning the density of the establishment of non-native freshwater fishes across Singapore, making it easier to estimate the spreading behavior of IAS. Nam and Pham [218] also conducted mapping to determine the distribution of invasive yellow snail species and giant spiny catfish in Ca Mau province, Vietnam. The highly adaptive *Cichlidae* has become a dominant alien fish species and is abundant in the Mount Galunggung freshwater areas of Indonesia [219]. The Philippines has also encountered freshwater IAS fish species. For example, the presence of invasive *Oreochromis niloticus* [220], *C. batrachus*, *Pterygoplichthys disjunctivus*, and *Channa striata* [221] in the Philippines suggests that studies on IAS are vital to assess the possible impacts they may impose on existing local ecosystems. However, the Philippines have also recorded invasive amphibians originating from different parts of the world that have become widespread and competed with native frog species [222,223].

According to Chan et al. [216], certain attributes can be used to determine the establishment rate of IAS in the local Singaporean ecosystem, as displayed in Table 2. In addition to the year of IAS introduction, anthropological factors can be considered to identify the purpose or the route of introduction of IAS, whether they are brought in for the ornamental trade, for angling purposes, for biological control purposes, or for aquaculture industries. With respect to the establishment of IAS in Singapore, the attributes that contribute the most are the climate matching, absolute fecundity, invasion history, trophic level, and the ornamental trade [216]. Because Singapore is geographically close, especially to Peninsular Malaysian and Indonesia, the identical climatic type and blooming ornamental trade may be partly responsible for the potential of IAS invasion into these neighboring countries. Hui et al. [217] mentioned that aquaculture freshwater fishes are mostly imported from Malaysia.

Table 2. The biological, ecological, and behavioral attributes of IAS as listed by Chan et al. [216].

Attributes	
Habitat type	Habitat generalist
Habitat salinity	Climate match
Vertical position	Climate types
Absolute fecundity	Trophic level
Maximum standard length	Reproduction mode
Parental care	Diet behavior
Air breathing	Schooling behavior

In Myanmar, Vikhrev et al. [224] used DNA barcoding to study the invasive Chinese pond mussel (*Sinanodonta woodiana*) and generated a map to record the new temperate invasive lineage of *S. woodiana*. DNA barcoding, as stated by Barman et al. [225], can be utilized to identify IAS. Furthermore, IAS such as *Pterygoplichthys multiradiatus* have seriously affected the composition of lake freshwater species, threatening Myanmar's overall freshwater fish habitat [226]. Furthermore, species such as *Hypostomus plecostomus*, *Lithobates catesbeianus*, *Pomacea canaliculata,* and *Rhinella marina* have all been categorized as IAS in Vietnam [227].

Whereas other SEA countries face major concerns with respect to aquatic IAS threats, Brunei primarily faces potential IAS threats in terms of uncontrolled ballast water exchange in their marine ports [228]. This concern is summarized in Table 3. However, the presence of marine IAS in Brunei ports has not yet been detected [229]. The ornamental and aquaculture industries are the main drivers for the spread of IAS into SEA countries. As emphasized by Gray et al. [230], SEA is defined as a region in crisis that supports more threatened species than other area worldwide; thus, strict regulations and guidelines should be imposed among SEA nations to ensure that parties translocating animals and plants are checked and permitted before entering new boundaries in order to preserve the stability of local ecosystems and endemic species. Differences in culture and governance should not be obstacles to cooperation among the SEA nations on pressing environmental issues such as the threats of IAS.

Table 3. Major concerns with respect to IAS in Southeast Asia.

Major Concerns with Respect to IAS Types in the SEA Region	
Freshwater	Marine
Malaysia, Indonesia, Thailand, Singapore, Vietnam, Myanmar, Cambodia, Philippines, and Laos	Brunei

7. Invasive Alien Species from Malaysia's Perspective

Approximately 114 years ago, most of Malaysia was covered by natural forests. In the last 63 to 64 years since the independence of Malaya, rapid changes have occurred in the country, especially to the natural ecosystems when the country began to develop as it is today. Some parts of the aforementioned natural forests were transformed into plantations, with additional land-use development, as a result of which habitat loss, booming population, pollution, and the high demand of the food industry have resulted in the endangerment of some animal and plant species.

Additional damaging effects can occur when IAS are introduced to local ecosystems, whether intentionally or unintentionally [62]. According to Talaat et al. [231], Malaysia has been widely recognized as one of the 12 most mega-rich biodiversity countries in the world. Its diversity of flora and fauna species makes it one of the richest nations in terms of biodiversity per unit area [232,233]. Therefore, IAS can easily adapt to local environmental factors, given the many varieties of available food [37,211].

On a par with the development of the country and global advancement, borderless foreign trade and the increasing tourism industry may have resulted in an increased incidence of IAS. As reported by Strecker et al. [234] and Saba et al. [37], the rapid increase in human activities has increased the rate of introduction and the dispersion of IAS. The changing climate can also play a role in the spread of IAS [235]. IAS have been recorded as present in diverse Malaysian ecosystems such as streams, lakes, rivers, swamps, reservoirs, drainage areas, and mining pools. Invasive fish species such as peacock bass (*Cichla* spp.), arapaima (*Arapaima gigas*), and tilapia (*Oreochromis* spp.) have all been intentionally introduced into local water bodies in the country [19]. Once introduced, their impacts are mostly negative [180,236–238]. However, the introduction of IAS is not always guaranteed to worsen ecological ecosystems [239–241].

Fish invasions are becoming a more difficult environmental issue, as they pose a number of significant challenges for fishery conservation and management [24,52,242]. As explained by Rahim et al. [19], the release of IAS into Malaysian waters is caused by various factors, such as recreational fishing activities and the aquaculture and aquarium industries, and as a result of natural disasters such as flooding or overflow.

Because IAS have been the topic of international agreements, regulations, conservation plans, initiatives, and strategies [243], the National Committee on Invasive Alien Species of Malaysia (NCIASM) regards IAS as the most important threat to biodiversity, as they are non-native organisms that cause environmental or economic damage [244] with inadvertent detrimental impacts on habitats, ecosystems, livestock production, the fishery industry, aquaculture [235], and human health [245]. Among the known IAS in the fisheries sector discovered in Malaysia are marbled crayfish (*Procambarus fallax* forma *virginalis*), Australian redclaw crayfish (*C. quadricarinatus*), peacock bass (*Cichla* spp.), Asian redtail catfish (*Hemibagrus wyckioides*), African catfish (*C. gariepinus*), and algae suckermouth catfish (*Hypostomus plecostomus*). The NCIASM [235] has also reported other threatening freshwater IAS in local Malaysian waterways, such as pacu fish, Alligator gar (*Actractosteus spatula*), common carp (*Cyprinus carpio*), and flowerhorn cichlids (*Cichlasoma* spp.).

Both ornamental and aquaculture species have been introduced into native freshwater ecosystems via intentional release by parties that have lost interest in the species or are unable to sustain the rearing of the species, possibly because of the insufficiency of financial support or the size of the species itself, making it unsuitable to be kept in captivity [37,245]. Ornamental and aquaculture species are also introduced into native water bodies via accidental release owing to natural disasters or human carelessness, escaping from captivity in aquaculture farms or aquariums in some cases before being established in the wild [246,247].

Another cause of the introduction of IAS into Malaysian native freshwater is sport fishing activities. Several non-native fishes, such as peacock bass, have been released into water bodies by parties hoping that the fish would increase the excitement of anglers participating in angling activities [9,19]. Such parties may introduce IAS owing to their lack of awareness or knowledge of the harmful effects of IAS. Whether the release of IAS is purposeful or accidental, it can lead to widespread distribution and establishment of alien species in local ecosystems [19].

The introduction of IAS is generally detrimental to overall ecosystem health and natural habitat environments. In this review, we discussed strategies that can be implemented for the early detection of IAS, specifically by using geospatial analytics via GIS and eDNA analysis. Malaysia ratified the Convention on Biological Diversity (CBD) in 1994, encouraging the protection of biodiversity as an important part of sustainable development in an attempt to protect local biodiversity in the face of IAS and other threats [248]. Malaysia favors a course of sustainability that advocates conservation while sustaining economic growth. Conversely, the CBD [249] states that each party should, to the greatest extent possible and as appropriate, prevent the introduction of and control or eradicate alien species that threaten ecosystems, habitats, or species.

The CBD also states that the avoidance of the introduction of IAS between and within states is much more efficient and environmentally desirable than steps taken after the introduction of IAS. If an IAS has been released, early identification and swift intervention are essential to deter its establishment, eradicating the organism as quickly as possible. Furthermore, containment and long-term monitoring mechanisms should be enforced when eradication is not possible or resources are not available. We suggest that local authorities impose heavier fines and punishment on those disobeying regulations and releasing IAS into native waters, especially repeat offenders.

Deeming the spread and effects of IAS as a serious threat, the Malaysian Ministry of Agriculture and Agro-based Industry (MOA), through its Crop Biosecurity Division, Department of Agriculture, Malaysia, prepared and published the National Action Plan for Prevention, Eradication, Containment, and Control of Invasive Alien Species (IAS) in Malaysia. The National Action Plan was drawn up to resolve concerns relating to the avoidance, early identification, containment, eradication, regulation, and supervision of IAS implementation in Malaysia. In congruence with the CBD, the National Action Plan for Prevention, Eradication, Containment, and Control of IAS includes the following overall objectives as established by MOA [250]:

i. To compile a list of invasive species in Malaysia;
ii. To investigate the cause of the invasion of alien species and their effects on biological diversity;
iii. Collecting data and conducting research to resolve the issues of IAS for the formulation of scientifically based strategies;
iv. To respond quickly to the prevention, eradication, containment, and control of ecosystem-threatening IAS;
v. To identify any gaps in current laws, rules, ordinances, regulations, protocols, and procedures to combat the introduction and establishment of alien invasive species;
vi. To promote awareness among senior officials, lawmakers, community members, industry, and the general public of IAS issues through media, education, curricula, and other means of communication;
vii. To intensify capacity building among the implementers in order to effectively enforce the National Action Plan.

Despite advocating for such a strategy for sustainability, the problems faced with respect to the conservation and preservation of Malaysia's overall biodiversity remain substantial. Under the CBD, as a signatory nation, Malaysia is committed to establishing and developing national policies, strategies, plans, or programs for the protection, conservation, and sustainable use of biological diversity resources, including research and necessary efforts to curb the spread of IAS [250].

On the other hand, the Malaysian government has also established departments and agencies to tackle the issue of IAS. Some of the laws constructed by the Malaysian authorities are listed in Table 4. The laws listed in Table 4 generally provide the objectives and guidelines for combating the direct or indirect introduction of IAS in Malaysia. To assist in enforcing the policies more effectively, locals can act as volunteers (citizen scientists) to aid researchers, the government, and other parties involved in helping eradicate IAS. Many obstacles to tackling issues regarding IAS are expected, primarily because such species are introduced intentionally because of the increasing and high demand for consumption or leisure purposes [19,37]. One way each and every one of us can commit to this cause is by spreading awareness that all parties involved should be amplified further by any communicational means, especially in this digitalization era, in order to ensure that information on IAS is received by the maximum number of people possible.

Table 4. List of laws established in Malaysia that directly or indirectly tackle IAS-related issues.

Year	Law	Background	References
1953	Animals Act	Controls the import and export of animals, preventing the spreading of diseases, and conserves and improves the general welfare of animals	[251]
1962	Animals (Importation) Order	Makes provision for the importation of animals and birds	[252]
1984	Federal Animals Quarantine Station (Management and Maintenance) By-Laws	Provides for the management and maintenance of animal quarantine stations	[253]
1985	Fisheries Act	Relating to fisheries, including the conservation, management, and development of maritime and estuarine fishing and fisheries	[254]
2007	Biosafety Act (Act No. 678)	Regulates the release and importation of living organisms to protect the environment and biological diversity	[231,251]
2010	Wildlife Conservation Act	Provides for the protection and conservation of wildlife	[255]
2011	Malaysian Quarantine and Inspection Services Act (Act 728)	Provides for integrated services relating to quarantine, inspection, and enforcement at entry points; quarantine stations and premises; and certification for the import of animals, fish, and other species	[256]

8. Conclusions

Because the integration of GIS mapping specifically for the detection of invasive alien species (IAS) in freshwater ecosystems has never been attempted before in Malaysia, we highly recommended that future studies be conducted to study this technology in depth and from other related perspectives, as well. Furthermore, utilizing GIS analysis in combination with already reliable and efficient environmental DNA (eDNA) analysis will be beneficial in terms of the study on IAS, in addition to conserving the environment, cost saving, and reducing manpower energy. Thus, this technique can be integrated into surveillance and management plans relevant to early IAS detection, as well as necessary management efforts assisting in providing data for necessary policy decisions by authorities.

In the future, we suggested that the Malaysian government double its efforts to strengthen and enforce the full implementation in accordance with related laws and policies such as the Fisheries Act 1985; the Malaysian Quarantine and Inspection Services Act 2011; and the National Action Plan for Prevention, Eradication, Containment, and Control of Invasive Alien Species. In the context of a lack of enforcement or if the present legislative regulations are ineffective, this situation will undoubtedly complicate the management of IAS and further hamper the mitigation and conservation efforts needed to secure the biodiversity of ecosystems and freshwater stocks in invaded waters.

The allocations of human and technical resources for IAS detection can likely be increased, although the utilization of GIS analysis and eDNA analysis can provide an efficient, cost-reducing, and manpower-conserving means to contribute to the success of IAS management. We also hope that this review paper and future studies on IAS will be able to serve as a reference in tackling the invasion of alien species in Malaysia. All parties, including governmental agencies and non-governmental organizations, should share their expertise and work together in planning and developing strategies for the management of IAS. Locals can also be trained to be citizen scientists to provide data and necessary assistance in efforts to eradicate IAS.

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