

MODELING, MAPPING, AND MEASURING THE RISK OF FRESHWATER INVASIVE SPECIES ACROSS ALASKA

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Freshwater Invasive Species Risk Assessment

Modeling, mapping, and measuring the risk of invasive freshwater species across Alaska

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Executive summary

Freshwater ecosystems of the Alaskan Arctic and Subarctic provide resources that are culturally, ecologically, and economically invaluable. Presently, these regions are relatively free of the impacts from invasive species compared to southern latitudes. To date, there have been relatively few verified introductions of aquatic invasive species (AIS) to freshwater ecosystems in Alaska. The expanding list and distribution of AIS has led to significant negative ecological and economic impacts (e.g., waterweed *Elodea nuttalli; E. canadensis* and northern pike *Esox Lucius* introduced outside its native range in Alaska). Escalating human activity across Alaskan lands and waters, coupled with rapidly shifting environmental conditions, increases the potential for new species introductions and subsequent establishment. Creating a proactive framework for well-informed decision-making and action can improve the effectiveness of prevention efforts and bolster decision support tools that help resource managers direct limited resources. Prioritizing AIS that may be introduced and become established, as well as the locations at highest risk of invasion, is foundational to building a proactive invasive species management framework in Alaska .

This project sought to identify and prioritize AIS known to be invasive in the contiguous United States, evaluate current and future habitat suitability for AIS in Alaska, and assess potential for AIS to be transported to habitats across Alaska, utilizing similar assessment methods as implemented for Bering Sea marine invasive species and non-native plants in Alaska. To accomplish this goal, the objectives of the project were to: 1) develop a formal ranked list of potential AIS to freshwater systems of Alaska; 2) assess the level of establishment risk for potential AIS by developing habitat suitability models for waterbodies across Alaska; and 3),

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identify potential pathways and specific vectors for high-risk AIS to invade Alaska and develop a framework for how vector analysis will be completed to understand transport risk. Overall, our goal is horizon scanning which is defined by Roy et al. (2019) as "a systematic examination of potential threats and opportunities, within a given context, and likely future developments, which are at the margin of current thinking and planning." The scans include pathway analyses and risk screening of species present at pathway origin points, with a focus on identifying species at high risk of being introduced, becoming established, spreading, and causing harm.

We refined a list of 28 AIS from a list of hundreds based on characterizations of species' invasiveness and species' proximity to Alaska (USGS 2020; GBIF 2022). Next, we evaluated the relative invasiveness of individual species to create an initial AIS ranking. We sought to characterize habitat suitability of AIS by selecting variables that were continental in scale, covering North America to include Alaska as well as the lower 48 states comparing natural discharge, sub-basin average terrain slope (degrees), average silt fraction, average organic carbon, lithological class, and human footprint in sub-basin in 2009. We estimated AIS habitat suitability across the entire state of Alaska using the physiological tolerances of the AIS (Appendix 2). We also evaluated pathways and vectors for the introduction of AIS (Appendix 2). Many pathways and vectors considered did not meet the criteria for Alaska or freshwater systems.

Of the 28 ranked species that we categorized as very high, high, and moderate levels of invasiveness; all three risk groups included fish and mollusks (Appendix 2). One commonality of the very high-invasiveness-ranked species was the availability of Ecological Risk Screening Summary documents (USFWS, 2022) produced by U.S. Fish and Wildlife Service (USFWS), except for the goldfish (*Carassius auratus*) and the New Zealand mudsnail (*Potamopyrgus antipodarum*). The Ecological Risk Screening Summary is now available for New Zealand mudsnails. In general,

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fish species often ranked very high or high in invasiveness and included sportfish and aquarium fish, suggesting the importance of pathways such as aquarium trade, fishing industry, intentional (but illegal) introductions of sportfishes and aquarium fishes for establishment. The technique we used for habitat suitability models necessitated aquatic environmental datasets that were continental in scale, which was often interpolated from very coarse resolution source data layers, particularly in Alaska. Better spatial data representing aquatic environments would likely improve this approach. While the lack of introductions in Alaska and nearby provinces and states is encouraging, the lack of occurrence data for the focal species also created complications for habitat suitability modeling. Despite the challenges, the habitat suitability models indicated limited suitability for warmwater species while some species, such as Brook trout (Salvelinus *fontinalis*), have high habitat suitability across Alaska no matter what threshold approach is taken. Some environmental predictors were more important than others. Specifically, the most important predictor variable, 'frost free days,' was critical for 15 out of 28 species as expected due to harsh winter conditions in Arctic and Subarctic regions. The second most important predictor was 'subbasin land surface runoff', a variable that indicates the amount of discharge and runoff, while the third most important predictor was 'snow cover' another indication of winter conditions.

Overall, the ability to understand the effect of future climate scenarios on the establishment of AIS was challenging. A detailed dataset of freshwater temperatures and water chemistry (e.g., pH, calcium) would greatly improve the ability to predict invasiveness of freshwater species to Alaska's ecosystems on a regional basis. Future studies may benefit from a more focused geographic scope examining a group of subbasins or a regional basin rather than the entire state. These drainages could be selected based upon the mostly likely locations of

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introduction pathways. The two most prevalent pathway risks for AIS are in-state transfer and stowaways/contaminants. Although there are examples of introductions from other pathways, the risk is somewhat mitigated by Alaska's climate and regulations. However, variable application of protocols for inspection and cleaning of fishing gear, watercraft, and other similar items while traveling into Alaska as well as transferring from waterbody to waterbody within the state creates a substantial risk in introducing invasive species. We plot cumulative invasive vulnerability for all subbasins and for the top 10% of subbasins (Appendix 3).

I. Introduction

Prevention and preparedness to identify and control incipient infestations are key to effective invasive species management. Establishing a regionally relevant watch list is foundational to this goal (e.g., Davidson et al. 2021). A watch list is an index of invasive species to be prioritized for surveillance (pre-discovery), reporting, monitoring (post-discovery), and other possible response measures to reduce the risk of impact to valued assets (Reaser et al. 2020). To date, a formal watch list has not been developed for non-native aquatic invasive species (AIS) in Arctic and Subarctic freshwaters in Alaska. A systematic and inclusive process to prioritize AIS (i.e., freshwater invertebrates, vertebrates) to target for prevention, early detection, and rapid response may increase efficiencies in prevention and preparedness to mitigate high-risk taxa.

Invasive species have impacted ecological and socio-economic systems globally (Vitousek et al. 1997; Wilcove et al. 1998; Pejchar and Mooney 2009). Not only do they harm native species, ecosystems, and human health (USFWS 2012), but they can also cost millions of dollars depending on the severity of invasion or damage inflicted (Lovell et al. 2006; USFWS 2012). Climate warming and the accelerating rates of invasions linked to human activities have elevated the need to address questions of invasion ecology for Subarctic and Arctic ecosystems (Stachowicz et al. 2002; Wonham & Carlton 2005; Ricciardi 2007). Climate warming is facilitating the establishment of invasive species by creating suitable conditions for species from lower latitudes, thereby expanding their potential ranges into northern latitudes (e.g., Sharma et al. 2007). Human activities are increasing the number of invasive species pathways and vectors, resulting in higher numbers of introductions for Subarctic and Arctic ecosystems (see Carlson and Shephard 2007). An invasive species pathway is an activity or process through which a species may be transferred to a new location where it could become invasive (e.g., shipping, air travel). Vectors are the specific means by which an invasive species moves within a particular pathway (e.g., a ship, agricultural products, boots) (ANSTF 1994; Brancatelli and Zalba 2018; Ruiz and Carlton 2003). The synergistic combination of climate warming and increased potential for introduction is substantial in freshwater ecosystems of the Subarctic and Arctic such as Alaska.

Relative to lower latitude ecosystems in North America, Alaska has relatively few AIS at present. Numerous factors are likely to have restricted the emergence of AIS across Alaska. First, there is the state's geographic isolation along with minimal freshwater connectivity to other ecosystems which prevents natural migrations of AIS established in adjacent areas (e.g., Canada). Second, Alaska's typically harsher winter climate has also likely limited the viability and establishment of introduced species. Third, human development across Alaska (and its potential to be an invasive species pathway) is more limited than other regions of North America; for example, major road systems have been identified as the primary pathway for a substantial increase in the presence of invasive plant species (Carlson and Shephard 2007) and road mile density is fifty times lower in Alaska than in the continental United States (FHWA, 2019). In addition to roads, aircraft traffic is being realized as a pathway of AIS to Alaska's freshwater systems (Carey et al. 2016; Schwoerer et al. 2020; Schwoerer et al. 2022). As climate continues to warm and human development increases in the region, so is the threat of AIS to show up in Alaska's freshwater ecosystems.

Incipient populations of AIS in Alaska, include northern pike (*Esox lucius* –a native transplant species to parts of Alaska), goldfish (*Carassius auratus*), yellow perch (*Perca flavescens*), fathead minnow (*Pimephales promelas*), muskellunge (*Esox masquinongy*),

largemouth bass (Micropterus salmoides), blackfish (Dallia pectoralis – in parts of Alaska), signal crayfish (Pacifastacus leniusculus), red swamp crayfish (Procambarus clarkia), oscar (Astronotus ocellatus), and freshwater eel (Anguilla sp.). One of the most notable aquatic invasive plants, *Elodea* spp., is also found in Alaska (Larsen et al. 2020). The introduction, establishment, spread, and impact of *Elodea* spp. in Alaska provides insight into what Subarctic and Arctic regions might experience in the future as invaders become an increasing problem for high latitude ecosystems. The success of *Elodea* spp. in Alaska suggests that long winters and short growing seasons of Subarctic and Arctic climates are not sufficient impediments to prevent the establishment and spread of an invasive species, and that remoteness is not necessarily a protective attribute of Subarctic and Arctic ecosystems (Carey et al. 2016); on the contrary, the remoteness of Subarctic and Arctic ecosystems may serve as the proverbial weakness in Alaska's armor. As observed with Elodea, human activity is substantial enough to introduce and spread the invaders in remote Alaska. However, it can be expensive and/or impractical to access remote areas for surveillance, monitoring, or eradication, efforts. The difficulty of enacting prevention, early detection, and rapid response to an invader in remote locations reinforces the usefulness of emphasizing precautionary treatment and proactive management of AIS in Subarctic and Arctic systems such as Alaska (Sethi et al. 2017).

Establishing cost effective means to prevent the introduction and spread of invasive species is a priority for invasive species managers across the United States (DOI 2021). Decision support tools such as horizon scanning (e.g., Roy et al. 2019) and predictive modeling (e.g., INHABIT, Engelstad et al. 2022) can be used to develop watch lists which can improve the knowledge base for well-informed decision making and developing policies that strengthen prevention efforts

(e.g., prohibited species listings, inspection programs). In recognition of these important tools and the usefulness of emphasizing proactive management of AIS in Alaskan freshwater ecosystems, the objectives of this project were to:

- 1. Develop a formal ranked list of potential AIS to freshwater systems of Alaska
- Assess the level of establishment risk for potential AIS by developing habitat suitability models for waterbodies across Alaska
- 3. Identify potential pathways and specific vectors for high-risk AIS to invade Alaska and develop a framework for how vector analysis will be completed to understand transport risk

Our approach follows similar methods successfully used for marine invasive species in the Bering Sea and non-native plants in Alaska (Goldstein et al. 2005; Droghini et al. 2017). This project focused on amphibians, crustaceans, invertebrates, fish, and mollusks thought to be the most likely to invade Alaskan freshwaters. Producing a list of potential invaders coupled with modeled estimates of invasion risk will provide key information for prioritizing locations and methods for surveying. Throughout the remaining document, we present our methods and results of the following three project components: a) a semi-quantitative ranking system that evaluates the ecological risk of each AIS; b) spatial habitat suitability analyses; and c) a general evaluation of potential pathways and vectors for AIS introduction to Alaska. We conclude the report with a discussion of the results and potential data acquisition that will likely improve the function of the habitat suitability analyses and ranking system.

II. Methods

Semi-quantitative species ranking system of ecological risk

Species Selection

To develop a potential list of AIS for Alaska, we included AIS taxa documented within a) Alaska; b) regions connected geographically to Alaska (i.e., adjacent territories and provinces); and c) regions that are connected to Alaska via transportation of watercraft (identified by documentation of boats entering Alaska at the Alaska-Canada Border). We chose to include records from across the United States as some ecological thresholds were represented across all states for species on our initial list.

Freshwater AIS lists were compiled using the following methods:

- Freshwater AIS lists were obtained for all U.S. states from the Nonindigenous Aquatic Species (USGS 2020). Taxa identified as "Marine" or "Marine – Freshwater" were omitted from the dataset. Taxa identified as "Freshwater" or "Freshwater – Marine" were retained. The NAS is limited to U.S. locations only.
- Freshwater AIS data for British Columbia, Canada were obtained from the British Columbia Invasives website and data portal (BC 2020). Occurrence records categorized as 'Native' were removed from the dataset.
- Freshwater AIS data for Yukon Territorywere obtained from a species list posted on the Yukon Invasives website (YISC 2021).

We created a master AIS list that totaled 302 species. The master AIS list for Alaska was then cross-referenced with the <u>Ecological Risk Screening Summary</u> (ERSS) species list categorizing

species as 'High', 'Low' and 'Uncertain' Risk (USFWS 2012). Both the ERSS and The Aquatic Species Invasiveness Screening Kit (AS-ISK) tool developed by Cefas (Centre for Environment, Fisheries and Aquaculture Science; Copp et al. 2016) was used to develop the AIS list as the ERSS only examines risk at the scale of the conterminous United States. Taxa listed by ERSS as low or uncertain risk were omitted from the master AIS list.

We removed taxa from the master list of potential aquatic AIS using the following criteria:

- 1. Determined to be taxa native to any part of Alaska;
 - a) Freshwater fish were determined to be native to Alaska if they were listed as native on the Alaska Department of Fish and Game (ADF&G) Alaska Freshwater Fish Inventory (AFFI) (ADF&G 2021).
 - b) Amphibian and reptile native states native status determined by the AK
 Herpetological Society webpage (AHS 2021).
- 2. Determined to be tropical (23.5 North, 23.5 South);
- 3. Determined to be taxonomically synonymous with another retained taxon;
- 4. Determined to be only facultatively aquatic or could be defined as riparian, and,
- 5. Determined to be a lack of taxonomic clarity to at the species level.

We then subdivided the remaining taxa from above to identify 40 potentially invasive species of interest for invasiveness ranking and subsequent habitat suitability assessment. We aimed to select species across a range of categories to include amphibians, crustaceans, fish, and mollusks. Species were excluded that were similar (e.g., sharing a common genus) or if they are native to certain parts of Alaska (e.g., Northern pike and didymo *Didymosphenia* *geminate*; Appendix 1). Some species were excluded due to time constraints, but we picked representative species that could serve as surrogates (Appendix 1). A final total of 28 species were selected to be ranked and modeled for habitat suitability (Table 1).

			-
	Taxon Name	Common Name	Category
1	Lithobates catesbeianus	American bullfrog	Amphibian
2	Mysis diluviana	Mysid crustacean	Crustacean
3	Pacifastacus leniusculus	Signal crayfish	Crustacean
4	Alosa sapidissima	American shad	Fish
5	Carassius auratus	Goldfish	Fish
6	Channa argus	Northern snakehead	Fish
7	Cyprinus carpio	Common carp	Fish
8	Esox masquinongy	Muskellunge	Fish
9	Gambusia holbrooki	Eastern mosquitofish	Fish
10	Ictalurus punctatus	Channel catfish	Fish
11	Lepomis gibbosus	Pumpkinseed	Fish
12	Micropterus dolomieu	Smallmouth bass	Fish
13	Micropterus salmoides	Largemouth bass	Fish
14	Morone americana	White perch	Fish
15	Notemigonus crysoleucas	Golden shiner	Fish
16	Perca flavescens	Yellow perch	Fish
17	Pimephales promelas	Fathead minnow	Fish
18	Pomoxis nigromaculatus	Black crappie	Fish
19	Richardsonius balteatus	Redside shiner	Fish
20	Salmo trutta	Brown trout	Fish
21	Salvelinus fontinalis	Brook trout	Fish
22	Sander vitreus	Walleye	Fish
23	Tinca tinca	Tench	Fish
24	Pectinatella magnifica	Magnificent bryozoan	Invertebrate
25	Corbicula fluminea	Asiatic clam; Asian clam	Mollusk
26	Dreissena bugensis	Quagga mussel	Mollusk
27	Dreissena polymorpha	Zebra mussel	Mollusk
28	Potamopyrgus antipodarum	New Zealand mudsnail	Mollusk

Table 1. Species included in final invasiveness ranking and habitat suitability modeling (see Appendix 2).

Invasiveness Ranking Tool

We used the Aquatic Species Invasiveness Screening Kit (AS-ISK) tool developed at (Copp et al. 2016) to rank the relative invasiveness of individual species. AS-ISK is a screening tool for any non-native aquatic plant or animal taxa, regardless of the aquatic ecosystem type (marine, brackish or fresh) or climatic zone. The AS-ISK is comprised of 55 questions that address the taxon's biogeographical and historical traits (13 questions), biological and ecological interactions (36), and the taxon's potential response to climate change (6). The first 49 questions comprise the Basic Risk Assessment (BRA) and the additional six climate-related questions comprise the Climate Change Assessment (CCA). Tallying scores for each module (BRA and CCA) allows for the comparison of taxa that may be lacking climate change data. In addition, an 'assessment outcome' is generated for each module which gives a categorical score of 'Low', 'Medium' or 'High'.

Our evaluation area was the entire state of Alaska, and we considered potential effects of the species in all the various ecoregions in Alaska answering for the state as a whole. Thus, if we had reason to believe a species would affect any part of Alaska, we would answer YES to an evaluation question for the rank calculator. We conducted a pre-assessment to calibrate responses and develop the assessment guide.

Questions for the Rank calculator are divided into 3 focal areas:

 Biogeographical/historical (13 questions) – These questions have to do with known domestication (more than 20 generations) or cultivation of the species, preferred climate conditions, existing distribution, introduction risk, and whether this species is invasive elsewhere.

- Biology/Ecology (36 questions) –These questions have to do with undesirable or persistent traits, resource exploitation, reproduction, dispersal mechanisms and tolerance attributes of potential invasive species.
- Climate change (6 questions) These questions have to do with future predicted climate change and the possibility of these changes affecting risk of entry, establishment, dispersal, magnitude of future potential impacts on biodiversity, ecosystem structure/function and socio-economic impacts.

Confidence in our answers was assigned a value based on very high, high, moderate, and low; equating to very high scored 4 points and low scored one point.

Habitat Suitability Assessment

Determining Model Criteria

We sought to characterize habitat suitability by selecting variables that were spatially consistent and comprehensive to include Alaska as well as the lower 48 States. This approach was necessary to account for the current habitats occupied by the focal AIS (see Table 1). The HydroATLAS dataset was chosen for its seamless global coverage and an array of 56 separate variables at a resolution roughly equivalent to the HUC12 sub-watershed product (Linke et al. 2019). We used the basin atlas data version 10 from this data set and converted the layers to rasters for modeling using a 2.5 minute resolution.

From the HydroATLAS variables, we selected predictors we thought would be important for the species to be modeled based on natural history knowledge of the individual species (Table 2). We decided to use a frost-free days predictor from another source (Wang et al. 2016) rather than the snow cover extent predictor within the HydroATLAS data (Linke et al. 2019) set upon review of the spatial data.

Table 2. Habitat suitability model variables included from HydroATLAS (Linke et al. 2019) and ClimateNA (Wang et al.2016).

Variable	Definition/spatial unit/ source
Natural Discharge (annual average, minimum or	Sub-basin pour point annual average, minimum and
maximum m³/sec)	maximum
Subbasin Surface Runoff (mm)	Sub basin annual average
Upstream Lake Volume (millions m ³)	Sum of total watershed upstream of sub-basin pour
	point
Sub-basin Average Terrain Slope (degrees)	Average in sub-basin

Sub-basin Wetland Extent (percent cover)	Percent cover in sub-basin of all wetland classes
	including lakes, reservoirs and river
Lithological Class (Geology)	Spatial majority of sub-basin
Average Organic Carbon Content in Top 5cm of Soil	Average in sub-basin or in total watershed upstream of
(tonnes/ hectare)	sub-basin pour point
Average Silt Fraction in Top 5cm of Soil (percent)	Average in sub-basin or in total watershed upstream of
	sub-basin pour point
Sub-basin Average Annual Snow Cover Extent (percent	Sub-basin annual average
cover)	
Human Footprint in Sub-basin (2009)	Index value in sub-basin for 2009
Climate – Frost free days (ClimateNA data set; Wang et	From ClimateNA v6.30 for 1981 to 2010 at 1km ²
al. 2016)	

Species Occurrence Records

Occurrence records for the focal AIS were queried from two primary datasets, both of which are maintained by the U.S. Geologic Survey (USGS): Biodiversity Information Serving Our Nation (BISON; this site is now called Global Biodiversity Information Facility North America Region (GBIF, 2022) and the Nonindigenous Aquatic Species (NAS) databases (USGS 2020) (Figures 1 and 2). Additional occurrence records were gathered from the British Columbian provincial government (BC 2020). Unfortunately, we were unable to locate a source for Yukon Territory AIS records that was digital and spatial. Only eight of the twenty-eight modeled species had species occurrence records for Alaska, all with 25 or fewer records statewide (Figure 3).

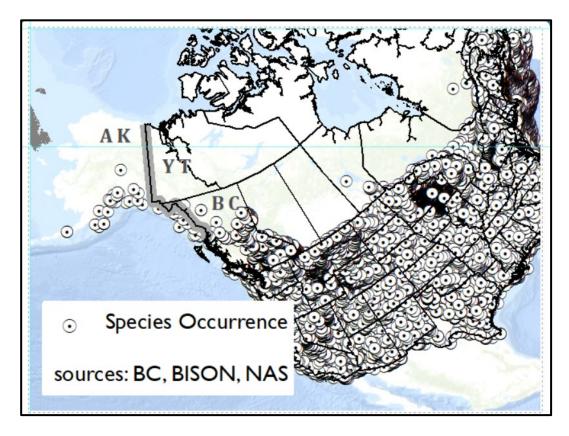


Figure 1. Map of species occurrence data across North America (2021). (BC 2020, GBIF 2021, and USGS 2021.)

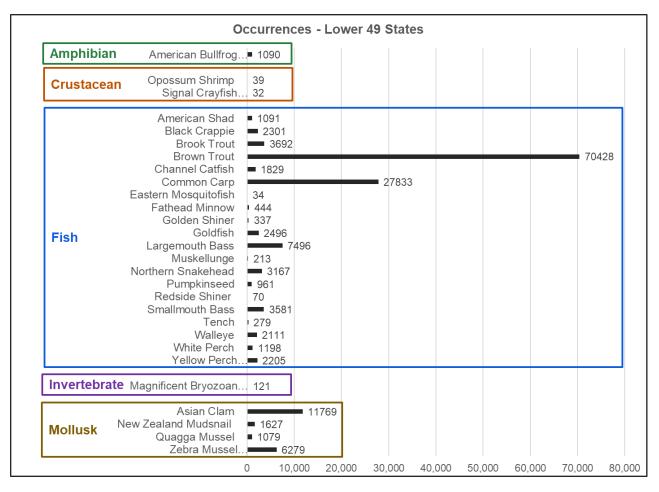


Figure 2. Aquatic invasive species occurrences for lower 48 and Hawaii from 1980 to January 2020 (USGS 2020; GBIF

2022).

Additionally, more than one third of the modeled species had less than 1000 occurrence records in the lower 48 states informing the habitat suitability models (10 of 28 species) (Figure

2).

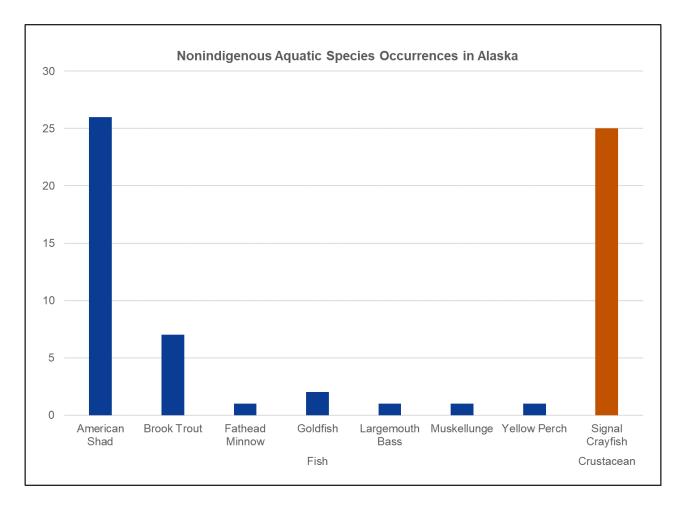


Figure 3. Aquatic invasive species occurrence records for Alaska as of April 2022. (GBIF 2022; USGS 2022). The blue

bars are species of fish and the orange bars are Crustacean.

Habitat Suitability Modeling Details

We reduced the occurrence data to a single point per pixel (4176 m²) to reduce pseudoreplication in modeling. Because we had only aggregated occurrence data, we used the target background approach (Phillips et al. 2009) to generate background points, using locations in the data set for all other species as background locations.

We used the Mollweide equal area projection with a 4176 m² resolution. For each species, we chose a subset of the predictors with pairwise correlations <0.7 following Dormann et al. (2013). Additionally, for species with less than 100 pixels with an occurrence we further reduced the predictors based on natural history knowledge to meet the 10s rule (rounding up; Hosmer and Lemeshow 2000) where we maintained a ratio of at least 10 occurrences per predictor.

We used the VisTrails Software for Assisted Habitat Modeling (SAHM) software (version 2.2.1; Morisette et al. 2013) to fit models including random forest, maxent, multivariate adaptive regression splines, generalized linear models, and boosted regression tree algorithms with 10-fold cross validation (Figures 4 and 5). We used default settings initially, but for any model algorithm with a large difference in training and average cross-validation area under the curve (AUC) values (i.e., >0.05) or highly erratic response curves we varied the algorithm parameters to simplify the model. In some cases, particularly for species with low record counts, we dropped an algorithm if we were unable to reduce these signs of overfitting. For all algorithms retained, we used four different threshold rules to discretize the continuous relative suitability maps including minimum predicted occurrence (all training occurrences correctly predicted as suitable), one percentile threshold (99% of training occurrences correctly predicted as suitable), ten percentile threshold (90% of training occurrences correctly predicted as suitable), and maximum of the sum of

sensitivity plus specificity, listed in decreasing order of inclusivity (precautionary to more targeted).

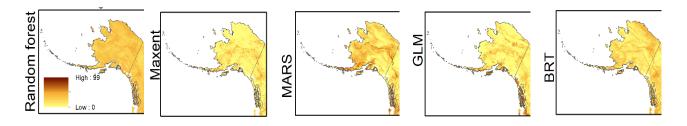


Figure 4. Illustrations of the five different habitat suitability model algorithms for species X using the same predictor variables.

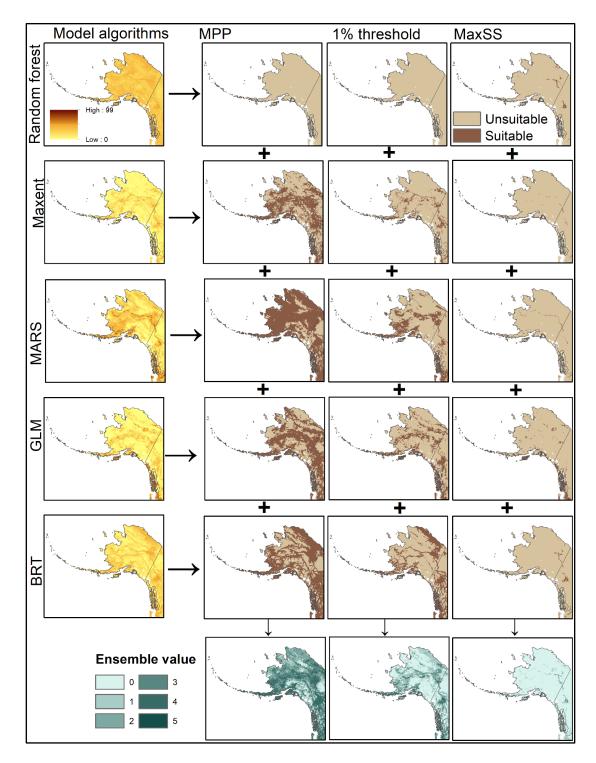
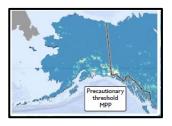
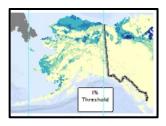


Figure 5. Illustrations of the habitat suitability model output. The model output is arranged from precautionary to targeted spectrum for all fit models including random forest, maxent, multivariate adaptive regression splines, generalized linear models, and boosted regression tree algorithms from the VisTrails Software for Assisted Habitat Modeling (SAHM) software (version 2.2.1; Morisette et al. 2013).

We then created an ensemble of the models by summing the five threshold algorithm outputs. In our results we chose to display three of the four thresholds to increase readability of the maps, and we highlighted the mid-range one percent threshold along with mapping the minimum and maximum setting as boundaries of habitat suitability (Figure 5 and 6).



Precautionary **MPP** - Minimum Predicted Presence (translates to maximum amount of suitability)





1% (99% of training occurrences correctly predicted as suitable)

Targeted MSS – Maximum Sensitivity and Specificity (typically translates to minimum number of

suitable models)

Figure 6. Illustrations of the three habitat suitability model thresholds.

Pathways and Vectors

A literature review was conducted to determine potential vectors for the introduction of AIS. Many pathways and vectors we initially considered (Davidson et al. 2017; Harrower et al. 2018; Bailey et al. 2020) did not meet the criteria for Alaska or freshwater systems. These include pathways such as aquaculture escapement, biological control, live food market, and marine debris. Table 3 lists the pathways considered relevant for the state and are described further below.

Relevant Pathways	Examples from Alaska
	Northern pike introduced into southcentral Alaska (native to North
Transfer in-state	and western Alaska)
	Atlantic salmon (Salmo salar) migrating into AK waters from escaped
Natural migration	pens in British Columbia and Washington state.
Stowaways and	
contaminants	Zebra mussels found within aquarium moss balls from local pet stores
Importation and	
release	Signal crayfish introduced into Buskin watershed on Kodiak Island
	Elodea detections in Chena Slough and goldfish introduced to Cuddy
Aquarium release	Pond, Anchorage and lakes on the Kenai Peninsula

Table 3. List of pathways considered relevant for the species analyzed in this assessment (ADF&G 2021).

III. Results

Species Ranking

Using the Aquatic Species Invasiveness Screening Kit (AS-ISK tool), we generated invasiveness scores for all 28 species. These values were tabulated following completion of all questions for an overall 'Confidence Factor', most of which were less than 0.7 meaning confidence was overall rated between moderate and high. The invasiveness rankings ranged from a high of 28 (*Micropterus dolomieu*, smallmouth bass) to a low of -6 (*Richardsonius balteatus*, redside shiner) using the AS-ISK tool (Copp et al. 2016). The ranked species were divided into three equivalently numbered groups of very high, high, and moderate invasiveness for this simple analysis. Future analyses could consider statistical techniques to assess invasiveness. Species categories were distributed among the three groups with fish and mollusks occurring in all three invasive risk groups (Figures 7 and 8).

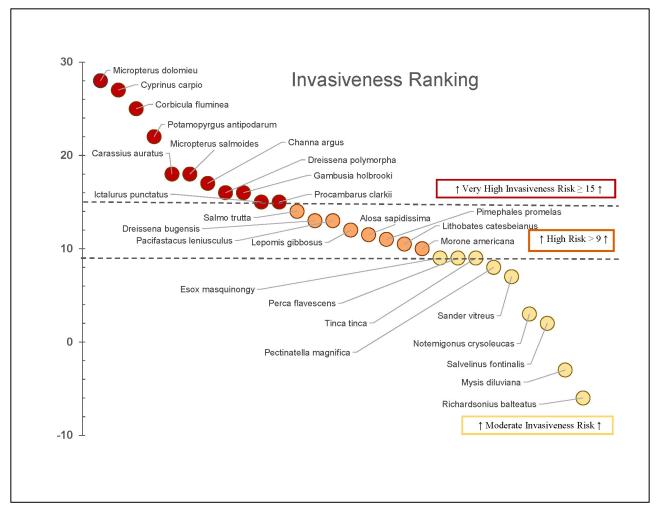


Figure 7. Invasiveness ranking of focal aquatic invasive species by taxonomic name.

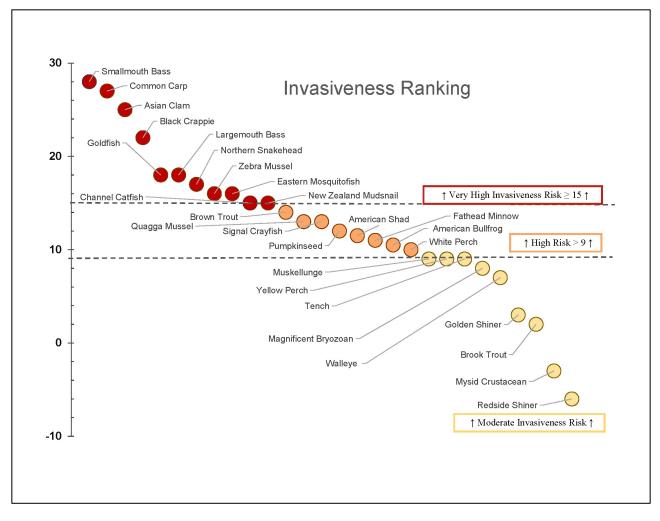


Figure 8. Invasiveness ranking of focal aquatic invasive species by common name.

Habitat Suitability Modeling

The habitat suitability model outputs were mapped with major river systems, larger communities, and the boundaries of the 164 HUC8 subbasins across Alaska to provide regional context (Linke et al. 2019; Appendix 2). Each species' habitat suitability model results are presented on maps at the three model thresholds explained earlier to provide a range of possible suitability. A fourth map is scaled to show Alaska along with much of the lower 48 states and western Canada to display the relative proximity or distances between Alaska and current documented species occurrence locations (Figure 9 and Appendix 2).

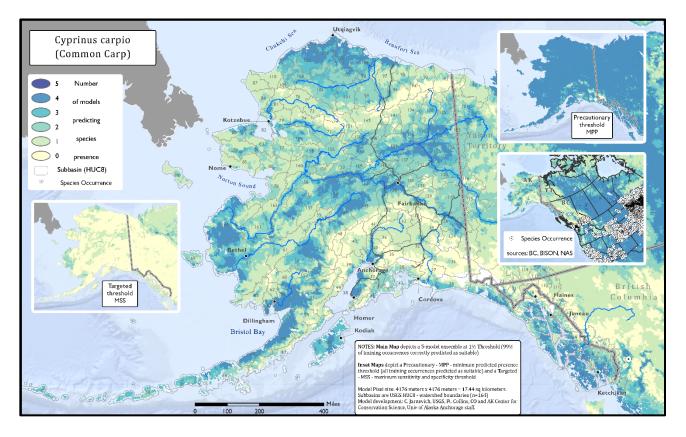


Figure 9. Example habitat suitability maps for common carp (Cyprinus carpio) with three thresholds and species occurrence map. The shading represents the number of models that predicted suitable habitat across HUC8 subbasins. Habitat suitability maps for the other AIS are available in Appendix 2.

The main map shows the one percent threshold (99% of training occurrences correctly predicted as suitable), the lowest predicted suitability is mapped in the lower left by the MSS (Maximum Sensitivity and Specificity), the highest predicted suitability is mapped in the upper right by the MPP (Minimum Predicted Presence), and the map in lower right shows the documented species occurrence records across most of the study area and all of Alaska. Habitat suitability model results were also summarized by species and by HUC8 subbasin in histograms grouped by HUC4 including the following regions: Southeast, Southcentral, Southwest, Northwest, Arctic, Upper Yukon, Middle Yukon, and Lower Yukon River (Figure 10 and Appendix 2).

Across the three thresholds of habitat suitability maps, we found a range of suitable habitat within a species and high variability across species. For example, the Common carp (Figure 9) prediction for the targeted threshold predicts almost no suitable habitat in a HUC8 subbasin, while the precautionary threshold predicts suitability across Alaska. Looking across species, we found similar variability such as the low amount of habitat suitability found for the Eastern mosquitofish (*Gambusia holbrooki*) compared to the high predicted suitability across model thresholds of Brook trout (*Salvelinus fontinalis*) (Appendix 2).

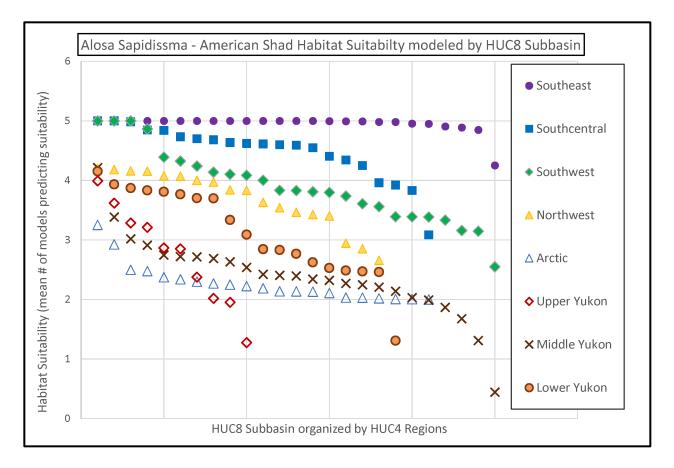
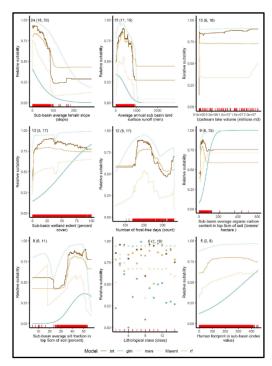


Figure 10. Habitat suitability model results were also summarized by species at different spatial resolutions. Example of habitat suitability by HUC8 subbasins in Alaska for American shad (Appendix 2). The habitat suitability is the mean of all fit models including random forest, maxent, multivariate adaptive regression splines, generalized linear models, and boosted regression tree algorithms from the VisTrails Software for Assisted Habitat Modeling (SAHM) software (version 2.2.1; Morisette et al. 2013).

Habitat Suitability Model Response Curves and Variable Importance

The response curve graph matrix represents the relative importance of each of the predictors included in the species' model from the set in Table 2 (Figure 11; Appendix 2). The relative importance means that all importance values were divided by the maximum so that the values indicate the percent each contributed out of a total of 100. These graphs plot the relative habitat



suitability (y-axis) across the range of values for each predictor (x-axis). The red lines along the xaxis represent values for the occurrence points used in modeling. Each line in the graph represents one model algorithm for a total of five lines possible; missing lines indicate the predictor was dropped from that model algorithm during the model fitting process. The numbers in the top left of each graph represent the average relative importance of the predictor with the range across model algorithm shown in parentheses. The graphs are arranged by average relative importance for the species' models, with the top left predictor contributing most to models on average.

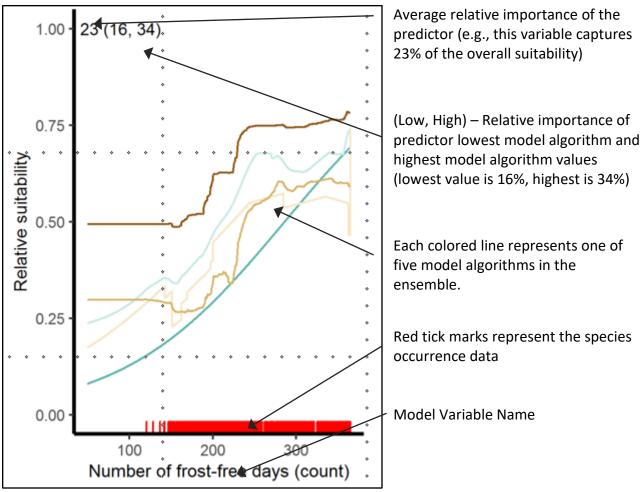


Figure 11. Example illustration of a response curve with explanation on components (Appendix 2).

The variable importance graphic shows the range of variable importance, measured as permutation importance, across species and algorithms, indicating what predictors were most important on average across the entire study (Figure 12). Thus, the individual response curve graphics may have a different order that is species specific. Figure 12 illustrates the variable importance for each of the potential predictors (Table 2) along the y-axis with the permutation importance, measured by mixing up predictor values between occurrence and background locations for a single predictor and seeing how that changes model performance, along the x-axis. The box and whiskers plots were created using the values from all species/algorithm combinations that included the predictor (number shown on the right side of the plot). Some species' models did not include a predictor, and some algorithms for a species may have dropped a predictor as being unimportant through a variable selection process.

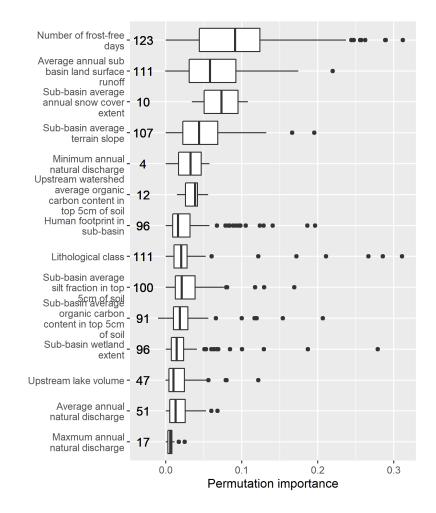


Figure 12. Illustration of the variable importance for each of the potential predictors used in the habitat suitability models, where the number of models including the variable is shown along the y axis next to the predictor name.

Overall, 'Frost-free days' was identified as the most important predictor in the habitat suitability models (Figure 12) and is the most important variable in models for most species (15 out of 28 species; Appendix 2). The second most important predictor is 'subbasin land surface runoff', a variable that indicates that amount of discharge and runoff, while the third most important predictor is 'snow cover' an indication of winter conditions and precipitation (Figure 12). Variables that were not important overall, were maximum and minimum discharge, along with upstream volume (Figure 12). All species-specific habitat models are presented in four map formats along with explanatory response curve summaries and model results as HUC8 subbasin histograms in the Habitat Suitability Atlas (Appendix 2).

The cumulative vulnerability across species was calculated for each subbasin (Appendix 3). This was done by calculating the mean habitat suitability score for each species for the mapping unit values within a subbasin and then dividing by the number of map units. Then we added the mean species scores to create a cumulative vulnerability for the subbasin. We plot cumulative invasive vulnerability for all subbasins and for the top 10% of subbasins (Appendix 3).

Pathways and Vectors

Several pathways including transfer in-state, natural migration, stowaways, and release were identified as relevant to Alaska waters (Appendix 2).

Transfer in State

The first pathway considered is transferring organisms that are already within Alaska to other bodies of water. Anglers worldwide have been documented to introduce AIS (e.g., fish) into new and unauthorized locations either for increased sport fishing opportunities or as prey for other fish (Fernandez et al. 2019). Although it is illegal for the transportation and/or release of live fish or fish eggs (5 AAC 41.005), this has not stopped people from doing so. There are several species that are native to Alaska but are only found within specific geographical regions and not throughout the entire state. For example, northern pike are native to Alaska and found north and west of the Alaska Range (Massengill et al. 2020). However, this fish was illegally introduced to southcentral Alaska and has been damaging native species who have not evolved with this predator (Massengill et al. 2020). Alaska blackfish (*Dallia pectoralis*) is another example of an Alaskan native fish that has been illegally transported and introduced outside its native ranges such as into Southcentral Alaska (Eidam et al. 2016).

Natural Migration

Natural migration through connected streams and water bodies is important to consider given Alaska shares a border with Canada. British Columbia and the Yukon Territory are the only two potential locations in which natural migration of freshwater species may come from. The land between British Columbia and Alaska connects in the southeast region of the state and is mostly covered by mountains. Although this does not easily facilitate freshwater migration, there are a few rivers (e.g., Taku, Stikine, Whiting, and Tatshenshini Rivers) which connect the countries. Further north, the Yukon Territory has more potential for non-native migration as major rivers cross between the border such as the Yukon and Porcupine Rivers, as well as many creeks, streams, and drainages. The likelihood of invertebrates migrating such distances is low and much slower, therefore we focus on freshwater and anadromous fish for this pathway.

Range expansion is a naturally occurring process. Depending on many factors such as time scale, habitat suitability, and obstacles, fish may expand their ranges by hundreds to thousands of miles. Pink salmon, *Oncorhynchus gorbuscha*, intentionally stocked into the Great Lakes, expanded their range over 1,000 miles from Lake Superior to Lake Ontario from 1956 to 1979, a

matter of 23 years (Lee 1981). With climate change, researchers also expect marine fish to migrate to waters farther north in the coming decades. They predict large shifts for the Gulf of Alaska and west coast of the United States and Canada, with some shifts over 1,000 km (Morley et al. 2018). While this research specifically points to marine species, freshwater species have also been predicted to encounter range shifts and expansion (Chu et al. 2005; Van Zuidan et al. 2016).

When comparing native fish in Alaska to those found in British Columbia and the Yukon Territory, many occur throughout all three. The Yukon Territory does have several invasive fish that can be found near the Yukon River (YISC 2021), but these species are already native to Alaska (ADFG 2021a). Though these species may already occur in Alaska, they do carry the potential to bring in non-native parasites and diseases. One species found in British Columbia, the mountain whitefish, Prosopium williamsoni, has a range close to the Alaskan border (BC 2021) and therefore has potential to expand into the state. Atlantic salmon, Salmo salar, may also extend its range into Alaska's fresh waters. Although Atlantic salmon are native to the northeastern United States and adjacent regions of Canada (Fuller et al. 2021), this species was introduced to Canadian waters for aquaculture and subsequently escaped some of their facilities. Atlantic salmon were found in marine waters of southeastern Alaska (Wing et al. 1992; Ray 2005) and the NAS database (USGS 2023) indicates it has been found throughout many locations in coastal southeast Alaska as well the Gulf of Alaska and the lower Kenai Peninsula. It has spawned in freshwater rivers in British Columbia (Fay 2002; Ray 2005) and has the potential to spread to freshwater systems of Alaska as well (Fay 2002). Additionally, there is also potential for species not considered in this review or newly introduced species to expand their range into Alaska from Canada (e.g., Schwoerer et al. 2022).

Stowaways and Contaminants

Stowaways and contaminants are similar and common pathways in the spread of invasive species (Davidson et al. 2017). An organism is considered a stowaway when it is attached to or contained within a piece of equipment or vessel such as fishing gear, buoys, boats, floatplanes, or other vectors (Harrower et al. 2018). Organisms that are small (e.g., zebra mussels) or readily attach to surfaces are easily undetected and transported to various water bodies, allowing the spread of AIS (ERSS 2021). Contaminants are transported in similar ways but are attached to objects they interact with naturally (Harrower et al. 2018). For example, golden shiner juveniles, Notemigonus crysoleucas, have "cement" like heads that attach to plants (Stone et al. 2016). This introduces the potential for these fish to go undetected and be inadvertently transferred. Aiding in their survival during transportation is the ability for some organisms such as crayfish and mussels to survive out of water for extended periods of time (Piersanti et al. 2018; ERSS 2021). Many U.S. states and Canadian provinces are contaminated with invasive species such as zebra mussels (Leung and Finster 2016; ISP 2018) and pose a legitimate risk of spreading species across their borders through aquatic equipment and vehicles (Leung and von Finster 2016). Boats and similar equipment may be subjected to an inspection and quarantine if thought to be contaminated, but inspection of watercraft entering Canada and Alaska overland is not mandatory (CBSA 2019).

Online purchase, importation, and release

There are several similar sub-categories of vectors that can be combined into one

category, and that is importation through online purchase. Although the shipping costs are high, it is possible to order a large variety of live freshwater species online and have them shipped globally (Padilla and Williams 2004; Walters et al. 2006; Mazza et al. 2015; Olden et al. 2020), including to Alaska. While most U.S. states could simply transport species by vehicle, Canada has rigorous policies about travelling with aquatic species across their border with several being prohibited, and others requiring permits (Government of Canada 2020). Therefore, many individuals may turn to online ordering. This includes aquarium species such as fish, snails, and shrimp, live bait fish such as crayfish, and pond stocking fingerlings and fry such as trout. There is also demand for ornamental fish for aquarium and hobbyists that can lead to introductions. In Alaska, aquatic species defined as amphibians, fish, aquatic invertebrates, and plants (5AAC 41.005 -41.600; ADFG 2021), are authorized for importation, but are subject to regulations and permitting processes depending on intended use. One may import ornamental fish (which includes aquarium fish, plants, invertebrates, and amphibians) without a permit, but the species in question must fit a specific description listed in 5 AAC 41.899 (ADFG 2021). Some characteristics of ornamental fish include species that are maintained for the pet industry or personal use, are not used for human consumption or fishing, and are not capable of surviving in the wild in Alaska. Importation for scientific research, educational purposes, or aquaculture are also authorized but require permits (ADFG 2021). Within the state, transportation, handling, and disposal are also regulated, but ultimately these organisms, their eggs, and their waste are prohibited from being released into water or on land.

Live food, including bait and illegal stocking

Besides importing species for aquarium or hobby interests, which is covered in another section, there are various reasons consumers may import species into the state, and subsequently illegally release them into the wild. One reason is for the consumption of live food. Not only has the release of live food being linked to invasive species been documented in the United States and other countries (Kerr et al. 2005; Nico et al. 2019), it is potentially the source of introduction (though speculative) for *Pacifastacus leniusculus*, signal crayfish, within the Buskin Lake and River on Kodiak Island, AK (Barrett 2015). This species has been documented since 2002 (Dunker 2018) and recent surveys (STK 2018) indicate this population is established in this location (Dunker 2018). Additionally, importation may occur for scientific or educational purposes, pond or lake stocking (illegal in Alaska), live bait (illegal in Alaska under most scenarios; ADFG 2021), or religious prayer release (Nico et al. 2019). These activities have been documented as pathways for AIS introductions in the U.S. and other countries, leading to the impairment and interruption of ecosystems, declining native species, and economic impacts (Padilla and Williams 2004; Kerr et al. 2005; Nico et al. 2019). Although these activities are regulated in Alaska, the increased shipping capacity in society today, combined with the vast amount of land and small number of wildlife enforcement in the state, leaves the door open to introduction of invasive species through the online importation pathway.

While the law in Alaska is clear, much of importation through online ordering is unregulated and sometimes bypasses the permitting process. There are various reasons individuals import aquatic species into the state and this can be accomplished through multiple avenues such as large online retailers, bidding sites, or small or family-owned businesses. While ultimately it is the responsibility of the individual ordering online to make sure their purchases are legal and follow state regulations, online sources do not always know or follow the destination's laws. Permitting processes or inspections may be bypassed, which increases the risk for the importation of invasive species.

Aquarium release

Another major pathway is the release of aquarium organisms into natural water bodies (Padilla and Williams 2004; Strecker et al. 2011). This event occurs globally and is categorized as one of the top five methods for invasive aquatic species (Ruiz et al. 1997). Reasons for aquarium release include the pets aren't wanted anymore, they have grown too big for the tanks, or individuals believe it is more humane to release into the wild rather than euthanize (Padilla and Williams 2004). In Alaska, there are several stores that sell freshwater species including big store chains and locally owned businesses. These businesses can be found in several regions across the state including central, south-central, and southeast Alaska in towns like Fairbanks, Anchorage, Soldotna, Cordova, and Juneau. The pet store itself may be required to have a permit depending on the species, but it is not needed for an individual to purchase from these locations.

Although it is illegal to release any aquatic organism, its waste, or wastewater into waters of the state (5AAC 41.005 -41.600; ADFG 2021), this event still occurs. *Carassius auratus*, goldfish, have been found in several locations in the state including Anchorage (Cordova 2019) and water bodies on the Kenai Peninsula (Massengill et al. 2020). Additionally, aquarium hobbyists have been known to dump entire contents of aquariums into the water rather than just fish. This is equally as dangerous, as some organisms hide within plants or aquarium accessories. Recently, moss balls obtained in local stores in Alaska have been discovered to contain the highly invasive and destructive *Dreissena polymorpha*, zebra mussel (Davis 2021). This is the first known introduction of this catastrophic organism into the state. The highly invasive aquatic plant *Elodea*, which is a staple in aquariums, has been introduced and spread throughout many water bodies in the state (Larsen et al. 2020). It is believed to have been introduced from individuals dumping aquarium water or contents into natural water bodies. Although aquatic plants are not covered in this review, the introduction of *Elodea* highlights the high risk of aquarium release as an invasive pathway.

IV. Discussion

Species Ranking

The introduction and establishment of AIS poses one of the greatest risks to native biodiversity and can have negative impacts to freshwater ecosystems (Panlasigui et al. 2018). The first line of defense is prevention, which is becoming more difficult due to increased economic connectivity and more moderate winter conditions (Carey et al. 2016). Thus, our goal was to identify potential invasive, freshwater species to Alaska's freshwater ecosystems. We identify those species most likely to become introduced and established in Alaska based on species life cycles, biological thresholds, and introduction pathways and vectors. Importantly, this research also identifies statewide dataset gaps that, if available in the future, would better inform the modeling of habitat suitability in Alaska.

In the 28 ranked species that we categorized as very high, high, and moderate invasiveness, all three risk groups included fish and mollusks. Note that our divisions between

very high, high, and moderate rank did not follow the existing threshold breaks in the rank calculator, instead we equally divided the ranked species into three groups (see Methods above). Our justification for this division was to recognize that freshwater invasive species establishment in Alaska could have a significant effect on an ecosystem even for those with relatively lower ranks. One commonality of the very high-invasiveness-ranked species was the availability of ERSS documents, except for the goldfish and the New Zealand mudsnail. These summaries allowed the evaluator to easily know more about the species life history and ecological effects. It is not coincidental that these species were chosen by USFWS to evaluate in more detail (ERSS reports) for invasive risk, as these species were causing the most ecological damage or threat at the time of evaluation. The species that have ERSS reports that did not rank as the most invasive from the rank calculator include yellow perch and tench, which have primarily expanded beyond their native range in the northeast U.S. due to transport of live specimens to other water bodies for sport fishing (ERSS). This pathway is an active area of prevention for Alaska as dumping of aquarium species is illegal. Alaska now has a list of aquatic invasives that are illegal to possess import, propagate, transport, release, purchase, and/or sell without a permit. Many of the species on this list are on our evaluation list, thereby reducing the pathway of "accidental" aquarium dumping introduction (ADF&G 2024).

Of the species we specified as very high or high risk, many of them were fish species. Many of the top fish are sportfish species along with aquarium fish and species with management plans (e.g., stocking) for fishing, suggesting the importance of these pathways for establishment. Four of the invasive fish species' (e.g., smallmouth bass, Carey et al. 2011) impacts could stem from being top predators, whereas other species such as common carp (Carey and Wahl 2010) would likely impact Alaskan waterbodies by altering ecosystem processes. This pattern is partly due to more information being available on warm-water fish that are then favored in the rank calculator due to being able to answer questions with more confidence and references (see below: Approach limitations and Data deficiencies). The lack of information on cool-water species is another data gap that future assessments could address. The high-risk category included more invertebrates and fish species that are invertivores. Confidence statistics from the rank calculator never exceeded 0.75 and most species had 0.60 or lower, indicating that there were data gaps when answering the rank calculator questions and the calculator was only moderately effective at discriminating between very high, high, and moderately high species invasiveness, keeping in mind we had no pre-existing ranking of these species form freshwater ecosystems to compare our results. Below we compare what factors are important to a species establishment by evaluating habitat suitability of each species.

<u>Results comparison to other assessments</u>

Our ranking identifies species that fit with expectations of invasive effects in high-latitude ecosystems and prioritizes species for Early Detection and Rapid Response. Our ranking, however, does differ from similar efforts in Arctic and Subarctic regions. Recent evaluation of freshwater species in the Yukon Territory, Canada produced a ranking of relative risk of establishment of aquatic invasive species. There are four species that were evaluated by Yukon Environment with an established risk assessment tool (IASWG 2009) that were common to the list of species evaluated herein. These species are zebra mussels (Figure 2: rank 14), New Zealand mudsnail (Figure 2: rank 22), common goldfish (Figure 2: rank 19) and the northern snakehead (Figure 2: rank 18). While the metrics used to quantify the risk of invasion were different than the AS-ISK rank calculator, their results had a slight difference in the relative order of risk rank. Zebra mussels were identified as having the highest risk, followed by the New Zealand mudsnail, goldfish, and northern snakehead. The higher risk of zebra mussels in the Yukon Territory versus Alaska is due to more inbound pathways from neighboring regions for the zebra mussel along with more established populations adjacent to the Yukon Territory.

Species ranking approach limitations and data deficiencies

AS-ISK is an easy-to-use tool for screening any aquatic non-native plant or animal taxa, regardless of the aquatic ecosystem (marine, brackish, or fresh) or climatic zone. The rank calculator allowed the relative comparison of species invasiveness across taxa. Two factors affected the ranking outcomes. First, some species that were ranked had Ecological Risk Screening Summary (ERSS) documents prepared by USFWS and some did not have these summaries. These ecological summaries were extremely helpful in understanding the risk of invasiveness and likely influenced the outcome of our ranking. In most cases, if the species had an ERSS report it was because it was already considered invasive in other states or countries, and researchers compiled all they knew about the species' ecology. Therefore, the availability of an ERSS report created a bias towards ranking species higher (unintentional but likely an outcome of all the information in these reports). More summaries of background information for species without ERSS reports could benefit future efforts.

Second, species that have species occurrence records in Alaska offered an insight into where they have been established and possible life-cycle requirements including climate

limitations. For those species that have not yet established in Alaska, the results were based on what we could gather from the species introduced range in the lower 48 United States and Canada. In general, the more data we found about a species, whether it was established in the lower 48 and/or Alaska led to a more accurate and possibly unintentionally higher ranking with our ranked AIS calculator (e.g., goldfish). The use of invasive species occurrence record locations to predict future suitable habitat does appear to offer predictive power, however, for some of the modeled species we had less than two hundred total records (see figures 4 and 5) as there were few documented records across the lower 48 states. Further complications were determining locations to consider as a place of origination and determining what databases represent those areas best.

Habitat Suitability

The habitat suitability models generate outputs which provide geographic specificity that differentiate the results from the statewide invasiveness rankings. This approach would appear to be a favorable method in Alaska, a vast state with incredible habitat variation, while also recognizing the difficulty of answering questions for the invasiveness ranking method that describe the entire state. Conversely, our modeling methods compare environmental characteristics of species occurrences in the lower 48 United States and some Canadian provinces to Alaskan settings. This technique necessitated aquatic environmental datasets that were continental in scale. While the HydroAtlas data met this requirement, the data informing the HydroAtlas values were often interpolated from very coarse resolution source data layers, particularly in Alaska (Linke et al. 2016). Better spatial data representing aquatic environments would likely improve this approach. Another challenge is only 8 of the 27 species modeled had occurrence records in Alaska. While this is encouraging for prevention of invasive species, it creates complications for habitat suitability modeling. Furthermore, for 10 of the 28 species, there were less than 1000 occurrence records for habitat suitability from outside Alaska (primarily the lower 48) to inform habitat suitability models.

Despite these challenges, the habitat suitability modeling is an informative first step in mapping potential locations of establishment by our representative species. The habitat suitability modeling criteria represent landscape characteristics that might represent suitable habitat. To further help provide a range of habitat suitability due to these data limitations, the presented maps have three model thresholds to provide a range of possible suitability. The maps first show the high habitat variation across Alaska. Considering the different thresholds, the targeted threshold shows limited suitability for warmwater species. This pattern is visible when comparing the warmwater largemouth bass versus the cool-water smallmouth bass. For the precautionary threshold, only two species (northern snakehead and mosquito fish) predicted limited habitat suitability that we suspect is driven by temperature thresholds for these species. Some species, such as Brook trout, have high habitat suitability no matter what threshold approach is taken. The widespread suitable habitat for brook trout is not surprising as dolly varden (S. malma), native to Alaska and in the same genus as brook trout, has a very wide distribution through the state. Other noticeable patterns across species for the main map include limited habitat for goldfish, snakeheads, Asain clam, mosquito fish, channel catfish, pumpkinseed, and black crappie, while signal crayfish have a very patchy distribution.

Examining the variables that drive the habitat suitability modeling confirms the

importance of temperature in determining the suitability of Arctic and Subarctic ecosystems for establishment. Comparing the importance of potential predictor variables across species and algorithms identified 'Frost-free days' as the most important predictor (Figure 12). Moreover, the variable 'Frost free days' is the most important variable in models for most species (15 out of 28 species) as expected due to harsh winter conditions in Arctic and Subarctic regions. Temperature is a driving factor, especially for ectotherms such as fish, that could overwhelm the influence of other factors. The second most important predictor is 'subbasin land surface runoff', a variable that indicates that amount of discharge and runoff suggesting whether species is adapted to low or high- volume reaches of rivers or amongst different stream orders, while the third most important predictor is 'snow cover' and indication of winter conditions and precipitation. Variability in importance of the other predictors variables fits with the range of physiological thresholds of the species considered (Figure 12). The large range in physiological thresholds across species is also the driver of variation in permutation importance within an individual predictor.

Overall, the ability to understand the effect of future climate scenarios on the establishment of AIS was challenging. Alaska is a large state with a multitude of eco-regions defined by different climate regimes. A detailed dataset of freshwater temperatures would complement the existing climate classification and greatly improve the ability to predict invasiveness of freshwater species to Alaska's ecosystems on a regional basis. Now that it is available, creating a model that used reaches as well as lakes and ponds from the National Hydrographic Dataset (NHD) (USGS 2021) as a network might more closely address the issues of hydrologic connectivity and represent potential species spread from points of introduction.

'Frost-free days' has the largest confidence interval of permutations, while 'Maximum annual natural discharge' has the lowest range of confidence intervals and lowest level of importance. Discharge has been considered an important variable and an analysis with reaches from NHD may help explore the influence of discharge on invaders to Alaska. Low importance of 'maximum annual natural discharge' suggests the potential invasive species can inhabit a large range of river conditions.

Pathways and Vectors

Based on the findings of the literature review and the evidence assessed from freshwater invasions in Alaska, we conclude that the two most prevalent pathway risks for invasive freshwater organisms are in-state transfer and stowaways/contaminants. Although there are examples of introductions from other pathways, the risk is somewhat mitigated by Alaska's climate and regulations. For example, aquarium released goldfish into Cuddy Pond and lakes on the Kenai Peninsula did not, as far as researchers and officials know, sustain reproducing populations and were able to be chemically eradicated (Massengill et al. 2020). Statutes in place make it clear which species are authorized for importation and the stipulations for their intended use. Furthermore, the Alaska Department of Fish and Game has passed new regulations that would include a list of banned species in the state including yellow perch, and Asian carp, *Hypophthalmichthys spp* (ADF&G 2024). In-state transfer of northern pike have already had major impacts to sport fishing and native species decline. Alaska is so large with many ecoregions that species can be transported to novel ecosystems. Additionally, the loose protocols for inspection and cleaning of fishing gear, watercraft, and other similar items while crossing into Alaska as well as transferring from waterbody to waterbody within the state creates a substantial risk in introducing invasive species.

Future studies may benefit from a more focused geographic scope examining a group of HUC8 subbasins or regional basins rather than the entire state. These drainages could be selected based upon the mostly likely locations of introductory pathways and vectors. Additional projects could emphasize one species or a grouping of similar species by family.

V. Acknowledgements

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