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Ports, Prosperity, and Pests: Assessing the Threat of Aquatic Invasive Species Introduced by Maritime Shipping Activity in Cuba

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PORTS, PROSPERITY, & PESTS:
ASSESSING THE THREAT OF AQUATIC INVASIVE SPECIES INTRODUCED BY MARITIME SHIPPING ACTIVITY IN CUBA

By

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Abstract

Aquatic invasive species (AIS) are biological pollutants that cause detrimental ecological, economic, and sociological effects on non-native communities. With increasing globalization through maritime trade, coastal ports are vulnerable to AIS introductions transported by commercial vessels. As Cuba's Port Mariel becomes a competitive transshipment hub within the Caribbean, it is essential to identify the potential threat that AIS may pose with a likely increase in shipping activity. It is equally important to understand the status of established AIS in Cuba and control measures presently being implemented by the country. This information can provide guidance for establishing or improving Cuban AIS preventative and remedial actions. For this study, publically accessible information was used to conduct threat assessments of present and potential AIS in Cuba and to identify feasible international donors of AIS due to trade with Port Mariel. Fifteen species were identified as established Cuban AIS, eight of which were associated with harmful impacts to the environment, economy, and human health. Only one established AIS, *Perna viridis* (the Asian green mussel), was recorded as having repeated, negative influences in Cuba. Regional trade partners of Port Mariel were identified as the most likely donors of AIS due to ecological similarity and minimal voyage duration between countries. These trade partners also represented the busiest ports and transshipment hubs in the wider Caribbean region and, therefore, could expose Port Mariel to 'stepping-stone' invasions. Five species associated with international trade partners were identified as potentially detrimental to Cuba if introduced into Port Mariel. There were no significant differences between the salinity and temperature tolerances of the AIS already established in Cuba and the possible AIS of concern, suggesting that these potential invaders could survive the environmental conditions of Port Mariel and subsequently become established throughout Cuba. The results presented herein are a preliminary assessment of AIS threats in Cuba and emphasize the importance of prioritizing AIS prevention and management. This study also establishes a baseline inventory of potential AIS in Cuba and a methodology that can be followed for future analyses outside of the study region.

Keywords: Port Mariel, Cuba, shipping, pathways, maritime trade, threat assessment, aquatic invasive species

1.0 Introduction

Cuba is in pursuit of economic growth. Since opening a billion-dollar container terminal at Port Mariel, Cuba in 2014, the island country has received international attention from government officials and private businesses alike seeking investment opportunities (Gallagher 2016). Industrial interests in Port Mariel have been attributed to a number of port features, including the creation of a special economic development zone, operational assistance from some of the world's leading maritime entities (i.e., port operator PSA International and shipping company CMA CGM), and the availability of adjacent land for future development (Jessop 2014; González 2015; Burnson 2016). In addition, the anticipated completion of dredging in Mariel Bay by 2017 will allow entrance for "post-Panamax" vessels, the largest commercial ships capable of traversing the recently expanded Panama Canal (Drewry 2015). Accommodation of these vessels will likely result in greater volumes of goods, encourage business, and enhance Port Mariel's potential of becoming a regional transshipment hub for the Caribbean (Jessop 2014; Drewry 2015; Burnson 2016). Improving diplomatic relations with the United States has also led to discussions of individual feeder services with ports in the Gulf of Mexico (e.g., New Orleans, LA; Mobile, AL; and Houston, TX) once the U.S. trade embargo is lifted (Jessop 2014; Gallagher 2016; Miller 2016a).

In the two years since Port Mariel's expanded operation, container throughput in the port has risen from 160,000 twenty-foot equivalent units (TEU) in 2014 to 330,000 TEU in 2015 (Miller 2016b). Increased container traffic, media coverage, and prospective investors are encouragement to the Cuban people and the international commerce community that Port Mariel will fulfill its promise of prosperity. While important to draw attention to the economic gain associated with improvements to the port, it is also necessary to consider relevant ramifications – specifically, the potential biological consequences of aquatic invasive species (AIS) known to be associated with increased international trade (Molnar et al. 2008; Kaluza et al. 2010; Keller et al. 2011).

1.1 Mariel Bay

Situated 46 km west of the capital of Havana on the northern coast of Cuba, Mariel Bay is an economically and ecologically valuable entity comprised of mangrove forests, two western coves, and the mouth of the Bongo River at the southern end (Morrison et al. 2008; Ruiz et al. 2008; EY & CONAS 2015). The 7.8 km^2 estuarine bay provides a habitat for marine species; however, anthropogenic influences have negatively altered the bay's conditions and threatened the health of the aquatic ecosystem (Joyce 1996; Ruiz et al. 2008). Input from the adjacent thermoelectric plant and cement factory as well as urban and agricultural runoff have polluted the water (Ruiz et al. 2008; Gonzalez-Diaz 2010). Additionally, the construction of Port Mariel and industrial activity within the Special Economic Development Zone (Figure 1) has further disturbed natural conditions within the bay (Núñez et al. 2005). Compared to other Cuban bays, Mariel Bay is considered 'contaminated', with mean petroleum hydrocarbons in the sediment measuring 316 mg/kg (Ruiz et al. 2008). This concentration exceeds the petroleum hydrocarbon tolerance limit of 20 mg/kg (dry matter) proposed by the Marine Pollution Research and Monitoring Program in the Caribbean (Ruiz et al. 2008).

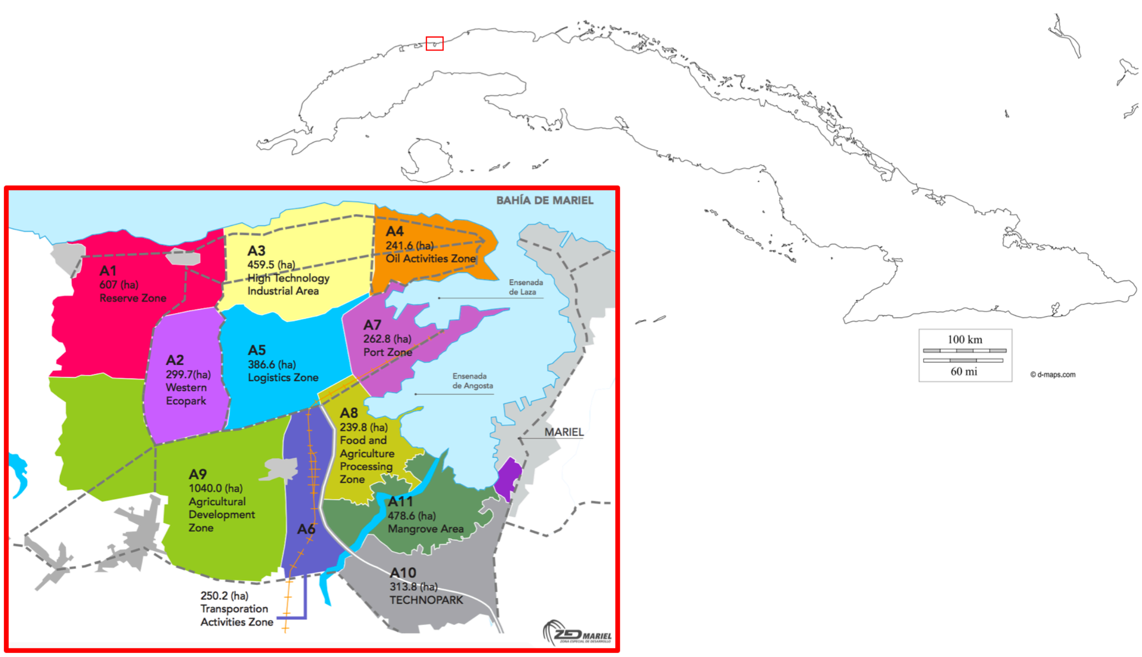


Figure 1. Sectors of the Special Economic Development Zone in Mariel Bay, Cuba published by Zona Especial de Desarrollo Mariel (ZED Mariel) (2014). The map of Cuba was acquired from d-maps.com.

Alterations and pollution of Mariel Bay have diminished its environmental quality, impacting the health of native species and providing opportunities for AIS establishment (Cohen & Carlton 1998; Morrison et al. 2008). The local human population is also at risk because of the bay's deteriorating health. Mariel Bay is a popular site for sport fishers in the community, who have been known to also sell their catches in local markets (Morrison et al. 2008). In the past, the aquatic resources of Mariel Bay have been a cause for concern due to outbreaks of ciguatera poisoning (Morrison et al. 2008). The introduction of toxigenic AIS in Mariel Bay could negatively affect individuals who consume the species. The combined threat of increasing industrial activity resulting in greater anthropogenic input along with increased maritime trade and, consequently, exposure to AIS in Port Mariel, may seriously impact the environment, economy and health of the local population who are reliant on Mariel Bay resources.

1.2 Maritime Activity as a Pathway for AIS

Increasing globalization and the dominant use of maritime transport for international trade has resulted in shipping being the primary unintentional vector of AIS, responsible for over two-thirds of known introductions (Molnar et al. 2008; Hulme 2009; Kaluza et al. 2010; United Nations Conference on Trade and Development 2015). The main mechanisms of AIS dispersal via ocean-going ships are ballast water exchanges and biofouling. Ballast water is ambient water that is taken on or released from a vessel's on-board storage tanks in order to provide stability during transit, balance weight changes (i.e., due to fuel consumption and adjusted cargo volume), and aid in maneuverability (International Maritime Organization (IMO) 2016a). Ballast water uptake in a donor region and subsequent discharge in a recipient site introduces species (e.g., phytoplankton, algae, zooplankton) into ecosystems outside of their native range (Carlton & Geller 1993; Hallegraeff 1993; National Research Council Marine Board 1996). An estimated 7,000 AIS are transported in ballast tanks every hour and three to five billion tons of ballast water are transported around the world each year (Tamelander et al. 2010). The exterior wetted surface area of vessels, including propellers, sea chests (intake recesses for ballast water), and hulls, offers additional space for fouling and encrusting species (e.g., crustaceans and bivalves) (Gollasch 2002). In recipient locations, biofouling AIS can be introduced through the release of gametes while remaining attached to vessels or by detachment (Minchin & Gollasch 2003; Coutts & Taylor 2004).

As sites that receive maritime trade goods, ports are at high risk of AIS introduction (Carlton & Geller 1993). The degree of international trade that a port receives is indicative of the frequency of exposure that the port and surrounding region has to AIS (Bax et al. 2003; Westphal et al. 2007). AIS that do become established may reflect connectivity of global and regional shipping networks (Drake & Lodge 2007; Keller et al. 2011; Seebens et al. 2013). Port susceptibility to AIS is also in part due to disturbances caused by human activity in the port harbors such as channel dredging and run-off from coastal industrial sites (Cohen & Carlton 1998). Consistent with the intermediate disturbance hypothesis, anthropogenic input can create aquatic environments that may not be hospitable for native species and, thus, become available niches for AIS (Hobbs & Huenneke 1992; Hulme 2009).

In order to become an AIS, a species must survive a series of stages spanning transportation, release, and establishment. With introduction through unintentional shipping vectors, the species must first become attached to the exterior of a ship or be taken in with ballast water (Carton 1985; Gollasch 2002). Second, during the voyage, biofouling species must remain attached to the exterior surface area of vessels despite exposure to fluctuating coastal and oceanic conditions and swift water flowing over the vessel hull (Wonham et al. 2001). For organisms transported within ballast water, the species must endure water quality changes in the ballast tank such as light conditions and oxygen concentrations that decrease over time (Carlton 1985; Galil & Hülsmann 1997; Lavoie et al. 1999). Although survival times during transit vary, the greatest loss of species abundance in ballast tanks occurs within the first five days (Gollasch et al. 2000; Cordell et al. 2009). With faster vessels and decreasing transit times, the likelihood of species survival between port stops is increasing (Minchin & Gollasch 2003; Dunstan & Bax 2008). Upon release, the introduced species need to tolerate the conditions of the non-native ecosystem - in particular, the ambient salinity and temperature (Kinne 1963; Barry et al. 2008). After surviving transportation and introduction, the non-native organisms must produce a self-sustaining, reproductive population which may be capable of dispersing to neighboring habitats (Lodge et al. 2006).

1.3 Impacts of AIS

Invasive populations distinguish themselves further by negatively influencing ecosystems into which they are introduced (Executive Order No.13112 1999). While only a small number of introduced species become classified as invasive, AIS still impose ecological, economic, and health-related damages that can be severe and intensify over time (Molnar et al. 2008). In the absence of natural predators and competitors, AIS can overtake available resources, disrupt the local food web balance, alter habitat structures (i.e., as ecosystem engineers), and act as secondary vectors of parasites and disease (Pysek & Richardson 2010; Tamelander et al. 2010). The breadth of AIS effects, however, is not limited to biological impacts on native marine species; the impacts can also result in monetary loss. Fouling of artificial structures, including ships, piers, and industrial intake pipes, can be safety hazards that require maintenance costs, dry-docking (i.e., time and money lost while a vessel is out of service for repairs), and even replacement of damaged structures (Molnar et al. 2008). Competition or extensive population growth can initiate mass mortality of economically important species or damage fisheries and aquaculture gear, potentially causing closures and unemployment (Washington Invasive Species Council (WISC) 2009). Direct influences on human health range from causing cuts and stings to life threatening aliments, such as paralytic shellfish poisoning (PSP) and cholera (WISC 2009; Cohen et al. 2012).

An invasive population is nearly impossible to eradicate and costly to control (Molnar et al. 2008). In the United States, an estimated $120 billion dollars are spent annually to manage terrestrial and aquatic invaders (Pimentel et al. 2005). The most effective strategies for reducing these unwelcomed species are prevention and early, rapid response (Molnar et al. 2008). Efforts to reduce or eliminate the transfer of AIS via shipping include ballast water treatment and anti-fouling strategies, which continue to be tested and improved (United Nations Conference on Trade and Development 2015). International conventions, regional workshops, and national management plans offer resources and opportunities for collaboration (Tamelander et al. 2010). Limited funds and time, however, require policy makers to prioritize prevention initiatives and control regimes.

1.4 AIS Threat Assessment

A tested method to measure the potential future establishment and impacts of AIS is a threat assessment. Threat assessments can be conducted through a variety of methods and are dependent upon the availability and quality of data to parameterize such analyses (Mazzotti & Briggs-Gonzalez 2015; McGeoch et al. 2016). While not all factors that drive invasions are well understood, recorded invasion histories and abiotic tolerances of AIS, environmental similarities of trade-connected ports, ship frequency, and voyage time have been examined historically to rank AIS by levels of concern, as well as identify potential future AIS donor regions and AIS threats (Gollasch & Leppakoski 2007; Molnar et al. 2008; Kaluza et al. 2010; Keller et al. 2011; Seebens et al. 2013; Wonham et al. 2013; Xu et al. 2014). Threat assessment scoring systems provide a standardized format for examining AIS with different taxonomies, impacts, and distributions (Molnar et al. 2008; Pysek & Richardson 2010). In such an analysis, qualitative information can be converted into numerical scores that can be used to prioritize control efforts and species of concern and identify gaps in knowledge where further research is warranted (Molnar et al. 2008). Scoring formats produce standardized results that can be applied to national and global-scale analyses and be used by other researchers for comparison. The flexibility of such a system also allows the scoring criteria to be improved and applied for future analyses if additional or higher-resolution quantitative data become available (Leung & Dudgeon 2008; WAISC 2009). Results of a threat assessment are relatively easy to comprehend, allowing government officials, scientists, and citizens alike to understand AIS risk and the feasibility of removing present AIS as well as preventing future AIS introductions (McGeoch & Squires 2015).

1.5 Motivation and Purpose of the Study

Cuba continues to expand international trade relations and as Port Mariel evolves into a regional transshipment hub, exposure to AIS will escalate. The motivation of this study was to emphasize the importance of AIS prevention and mitigation within the country and identify those species that may pose the greatest risk, particularly in anticipation of increased maritime trade. The research was organized into several major components:

- A scored threat assessment of established, ship-related AIS in Cuba (hereafter referred to as 'Established Cuban AIS' – i.e., ECAIS) was conducted in order to facilitate future prioritization of management resources of those species already established.
- Existing and projected container shipping routes to Port Mariel were examined to determine international ports (hereafter referred to as 'Potential AIS Donors' – i.e., PAISD) that could possibly introduce harmful AIS into Cuba.
- A select number of species recorded within the PAISD and surrounding regions (hereafter referred to as 'Potential AIS of Concern' – i.e., PAISC) were assessed by invasion risk and the severity of impact the species may impose on Cuba.
- Recorded salinity and temperature values of the PAISC and the ECAIS were compared to evaluate inter-species similarities in distribution capabilities due to environmental tolerances.
- Cuba's AIS control initiatives and collaborations on a national, regional, and global scale in relation to AIS shipping pathways were reviewed.

In order to take efficient and effective actions against AIS, it is necessary to understand the current ecological, economic, and sociological impacts of these species as well as the risks of the AIS introductory pathways. While the threat assessments for this study were specific to Cuba, the methodology used here is applicable to other countries in need of similar AIS anlayses.

2.0 Methodology

2.1 Scoring System of AIS Threat Assessment

The AIS scoring system utilized in this study was based on the impact assessment frameworks proposed by Molnar et al. (2008) and the Washington Invasive Species Council (2009). The threat assessment was divided into six categories: ecological impact, economic impact, human health impact, geographic extent, invasive potential, and management difficulty (Table 1). 'Ecological impact' was used to classify the magnitude in which AIS disturbed the native biotic and abiotic environments (Molnar et al. 2008). 'Economic impact' characterized the severity of an AIS impact on natural and artificial maritime systems and activities that could result in monetary loss (WISC 2009). 'Human health impact' was the direct effect an AIS posed on the human body (WISC 2009). 'Geographic extent' represented the global distribution of an AIS and was scored based on the number of non-native ecoregions in which the species was present (Spalding et al. 2007; Molnar et al. 2008). As defined by Spalding et al. (2007), ecoregions are the smallest unit of the coastal and shelf-area bioregionalization scale that are defined by distinct, "homogenous species composition" and are regulated by biogeographic forces such as nutrient concentrations, currents, freshwater inflow, and temperature fluctuations (Spalding et al. 2007). 'Invasive potential' was based on the rate in which an AIS is presently establishing in new, non-native regions and the life history traits of the species such as dormancy in ballast water tanks during vessel transit, reproduction frequency, etc. (Molnar et al. 2008). Finally, 'management difficulty' described the amount of time and resources required to eradicate or control an AIS population once established (Molnar et al. 2008).

Scoring of AIS in this study was based on information derived from publically accessible databases, published reports, and primary literature (Appendices 1- 4). Combined, the assessment categories described the current or potential threat AIS may or do inflict upon introduction. Every category, with the exception of human health impact, had a scoring system of zero to four in increasing order of threat. Human health impacts were valued from zero to two following WISC (2009) and did not influence the analysis, as the final ranking of AIS by overall threat was based on the cumulative score of the six categories. The highest threat score attainable was 22. AIS that had ecological or economic impact scores of three or four, or a human health impact score of one or two, were considered ‘harmful’ following Molnar et al. (2008).

Ecological Impact*: influence on native biodiversity	
4	Impact both biotic and abiotic features of the entire ecosystem, which include large scale changes to community structure, clogging of waterways, altering of natural habitats, and causing localized or widespread extinctions
3	Influence species of wider ecosystem importance (e.g., keystone or threatened species) or multiple species without creating localized extinction
2	Minor disruption to a single species with no wider ecosystem effects
1	Little or no disruption; Co-exists with native species
0	Unknown or not enough information to determine score
Economic Impact:** influence on maritime activities or systems with direct monetary value	
4	Temporary or permanent closure of industry for control of AIS (e.g., regulatory cleaning of clogged intake pipe); Cause unstable physical infrastructure that could be expensive to replace; Elimination of recreational activities (e.g., fishing, swimming)
3	Significant influence on aquaculture (e.g., major reduction in yield), physical infrastructure (damage to water intake systems, piers, vessels, aquaculture equipment), or recreational activities
2	Minor impact on aquaculture (e.g., reduced resources for cultured species, reduced production yield without mass mortality), physical infrastructure (e.g., impediments to aquaculture or fisheries equipment, piers, ships), or recreational activities
1	Little or no disruption
0	Unknown or not enough information to assign score
Human Health Impact:** influence on the health of individuals or human population	
2	Is a vector or organism of disease that causes serious individual or widespread illness; May result in death
1	Causes physical injury (e.g., stings or cuts); Provides habitat for a disease vector or organism
0	Unknown or not enough information to assign score

Geographic Extent*: invasive range of AIS	
4	Multiple ecoregions (three or more); Transoceanic; Cross-continental
3	One or two adjoining ecoregions
2	More than one occurrence in a local ecosystem or sub-ecoregion
1	One site
0	Unknown or not enough information to assign score
Invasive Potential*: rate of distribution and potential for future invasion; takes into account life histories and known spread	
4	Recent or active rapid spreading (doubling in < 10 years); Potential for future spreading quickly after new invasions
3	Recent or active spreading at a slower rate; Potential for future spread
2	Present but not actively spreading; Has life history traits that indicate high potential for future spread
1	Present but not actively spreading; Has life history traits that indicate little or no future spread
0	Unknown or not enough information to assign score
Management Difficulty*: effort required to control or reverse the threat of AIS once established	
4	Cannot be contained or controlled; No known successful eradications or management efforts of local AIS populations
3	Removal or control with difficulty that require significant use of resources and time; Regular monitoring and management efforts may be necessary
2	Removal or control with some difficulty but do not require significant use of resources and time; Seasonal monitoring and management efforts may be necessary
1	Easy removal or control that do not require significant use of resources and time
0	Unknown or not enough information to assign score

Table 1. Scoring system for aquatic invasive species (AIS) threat assessment. *Threat category adapted from Molnar et al. (2008); **Threat category adapted from Washington Invasive Species Council (2009).

2.1.1 Established Cuban AIS

ECAIS were defined as non-native, aquatic species already recorded in Cuba at the start of the study and that were most likely introduced through shipping vectors (i.e., ballast water or biofouling). Due to the historical introductions of AIS via shipping vessels, and limited information on native distributions, species with unknown origins were also included in the threat assessment if they were recorded as inflicting harmful ecological, economic, or human health effects in Cuba or other Caribbean locations following Bickford et al. (2006). Species chosen for analysis were derived from the resources listed in Appendix 1.

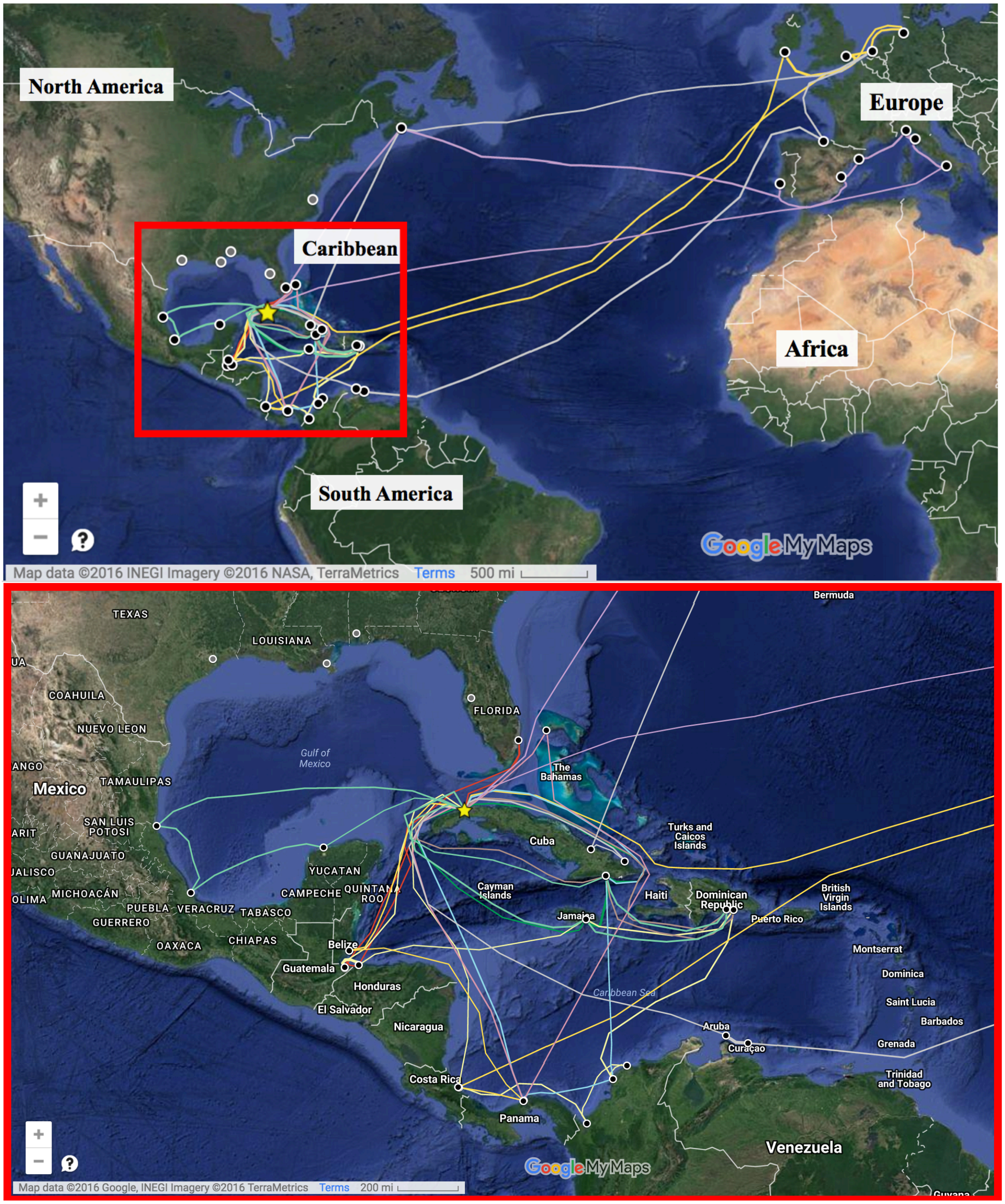
2.1.2 Data Analysis

The scoring system was used to convert semi-qualitative data into continuous variables (Appendix 2), which produced a ranked outcome for discussion. In order to examine the strength and direction (i.e., positivity or negativity) of covariation between the ECAIS threat assessment categories, inter-category correlations were run using the R statistical software package (Ricciardi & Cohen 2007; Molnar et al. 2008; Gotthardt & Walton 2011; R Core Team 2015). Due to an uneven distribution of data within the categories, the parametric assumption of data normality for Pearson Correlation could not be met. Therefore, the Spearman Rank Correlation was chosen as a robust, non-parametric alternative (Logan 2010).

2.2 International Trade Partners as Potential AIS Donors

To identify PAISD of Port Mariel, known container vessel routes were surveyed to determine international trade partners of the port. Routes were derived from "Maritime Routes Maps of the Greater Caribbean" (http://www.cocatram.org.ni/rutas/), an on-going project by the Central American Commission on Maritime Transport (COCATRAM), the Association of Caribbean States, the Cuban Ministry of Transportation, and the Maritime Authority of Panama (Figures 2 and 3) (Reyes et al. 2016). While container ships do not account for all of the vessel arrivals to Port Mariel, the port handles 80% of the island's container traffic and as container ships follow a regular, repeated path, the routes provide a robust indicator of consistent exposure from PAISD (Gollasch & Leppakoski 2007; Kaluza et al. 2010; González 2015).

Information on port stops and traverse times between ports were collected from the associated shipping companies' websites and published route schedules after preliminary identification of routes from the COCATRAM website. Only international ports that were prior stops to Port Mariel on the active shipping routes were evaluated following Seebens et al. (2013). International ports that may trade with Port Mariel in the future were also examined and selected from news articles that discussed recent trade negotiations and port tours (Appendix 3).



Figures 2 and 3. Active container routes to Port Mariel at a global and regional scale, respectively. Information was derived from the Central American Commission on Maritime Transport (COCATRAM) and displayed in Google Maps. The yellow star represents Port Mariel, Cuba; the black points symbolize ports along the container routes connected to Port Mariel; the gray points are ports that have expressed an interest or have been reported as potential future trade partners of Port Mariel.

2.2.1 Scoring System for Potential AIS Donors

PAISD were categorized by voyage duration and ecological similarity to Port Mariel. These variables were indicators of possible AIS survival during voyage and subsequent introduction into the Cuban port (Carlton 1985; Gollasch et al. 2000; Spalding et al. 2007; Cordell et al. 2009). The scoring systems for PAISD were derived from Gollasch & Leppakoski (2007) and Verna et al. (2016). To integrate the negative correlation between AIS survival and population density spanning the transit time, voyage duration to Port Mariel of less than six days was classified as high risk (3), 6 to 10 days as medium risk (2), and greater than 10 days as low risk (1) (Gollasch & Leppakoski 2007; Cordell et al. 2009; Verna et al. 2016). For future trade partners, voyage duration was calculated as:

$$\text{Voyage duration (days)} = \frac{\text{Distance (natutical miles)}}{\text{Vessel speed (knots)}} \times \frac{1\ \text{knot}}{1\ \text{nautical mph}} \div 24\ \text{(hours)}\ \text{(Miller 2013)}$$

The distances between future ports and Port Mariel were calculated from inter-port distances published on the website SeaRates.com (SeaRates LP 2016). Vessel speed was set as the average speed (i.e., 13.36 knots) of container ships on active routes to Port Mariel, calculated from vessel data collected by marinetraffic.com and containership-info.com (Svendsen & Tiedemann 2014; MarineTraffic 2016). Ecological similarity of international ports that trade with Port Mariel reflected the relative "physical proximity" between ports, whereas facilities in the same ecoregion, as defined by Spalding et al. (2007), were considered high risk (score of 3), adjacent ecoregions were medium (2), and non-adjacent ecoregions were low (1) (Verna et al. 2016). Ports on multiple container routes were ranked by their highest score. The highest possible score was six, which represented donor ports with the greatest probability of AIS transference and survival into Port Mariel (Appendix 3).

2.2.2 Data Analysis

The identification of present and future trade partners and their ranking as PAISD offered preliminary insight into the global connectivity and AIS exposure of Port Mariel. In addition, PAISD were used to select PAISC for further analysis.

2.3 Potential AIS of Concern

PAISC were selected with the following criteria:

- Species recorded in or near PAISD ports as established AIS, harmful species of unknown origins, or native species that are invaders in other regions
- Documented ecological, economic, or human health related damages in non-native regions
- Known to be transported on ocean-going vessels
- Capable of withstanding the physical conditions of Mariel Bay (Ruiz et al. 2008):
    - Salinity (range: 35.73 – 36.18 PSU; mean: 36.11 PSU)
    - Temperature (range: 27.2 – 29.9 °C; mean: 28.3°C)

The level of threat that a PAISC may impose upon Cuba if introduced to Port Mariel was assessed through the use of publically accessible information (Appendix 1) and the threat scoring system (Table 1). Salinity and temperature tolerances (i.e., minimum, maximum, and range) of the PAISC and the ECAIS (Appendix 5) were compared to determine if PAISC could withstand similar environmental conditions, indicating that the potential AIS are capable of becoming established in Cuba.

2.3.1 Data Analysis

PAISC were ranked by cumulative threat score (Appendix 4). An independent, two-sample t-test was initially chosen to compare salinity and temperature tolerances. However, due to relatively large difference in sample sizes of ECAIS (n= 15) versus PAISC (n= 5), the t-test's parametric assumption of homogenous variance could not be met. Therefore, an alternative two-sided Mann-Whitney-Wilcoxon test was used following Logan (2010). The statistical analyses were conducted in R.

3.0 Results

3.1 Threat Assessment of Established Cuban AIS

Fifteen species were identified as ECAIS. *Perna viridis* and *Gymnodinium catenatum* had both the highest threat assessment scores of 21 out of a possible 22. *P. viridis*, *G. catenatum*, *Teredo bartschi, Teredo furcifera, Teredo navalis, Lyrodus pedicellatus,* and *Charybdis hellerii* had both ecological and economic impact scores greater than or equal to three, classifying them as 'harmful' in these categories. *Sphaeroma terebrans* was also considered ecologically harmful but did not receive high impact scores for economic or human health impact. *P. viridis* and *G. catenatum* had invasive histories associated with negative effects to human health. Every species analyzed had known non-native distributions in multiple marine ecoregions and, therefore, they all received the highest impact score (four) for geographic extent. The eight highest-ranking species scored three or greater for invasive potential. Management difficulty scores were greatest for *P. viridis, G. catenatum, Tubastraea coccinea,* and *Amphibalanus reticulatus*.

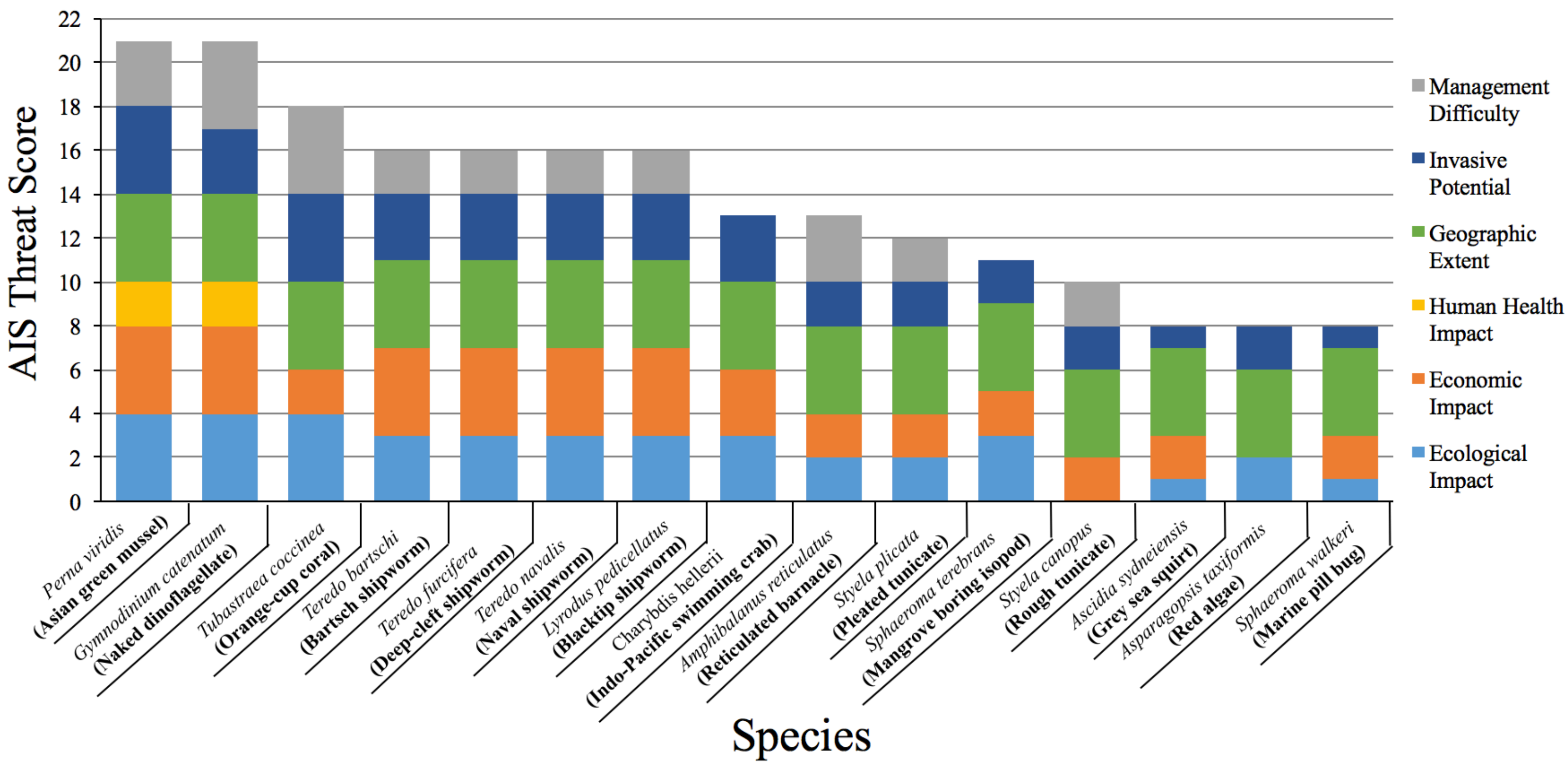


Figure 4. Threat scores of Established Cuban AIS (ECAIS). Species with ecological or economic impact scores of 3 or 4 or human health impact scores of 2 or 1 were considered harmful.

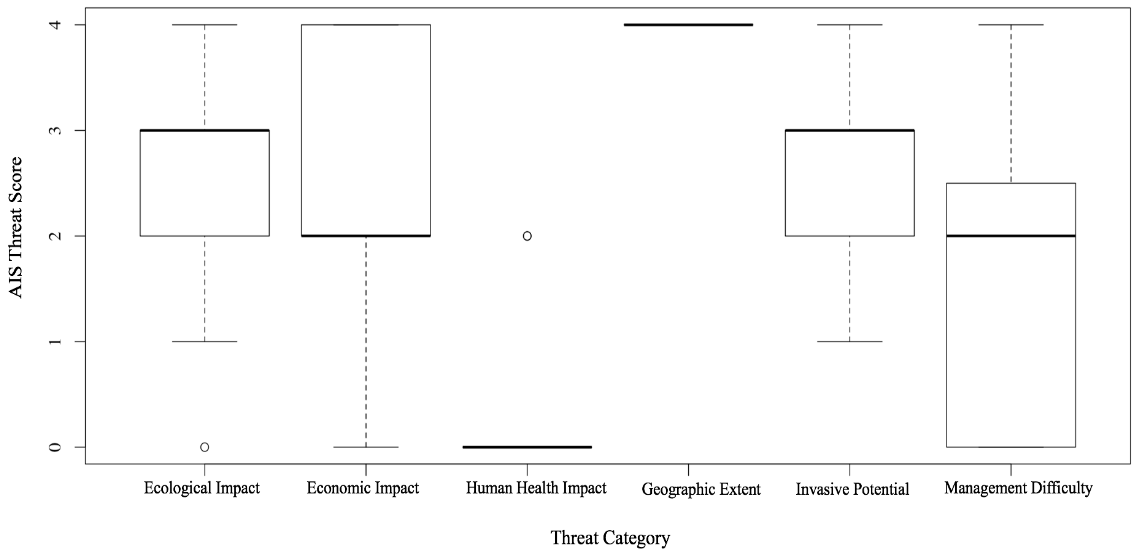


Figure 5. Distribution of Established Cuban AIS (ECAIS) (n= 15) scores by threat assessment category. The lines above and below the boxes represent the largest and smallest non-outliers of the data sets, respectively. The darkened lines within the boxes are the median values of the data sets.

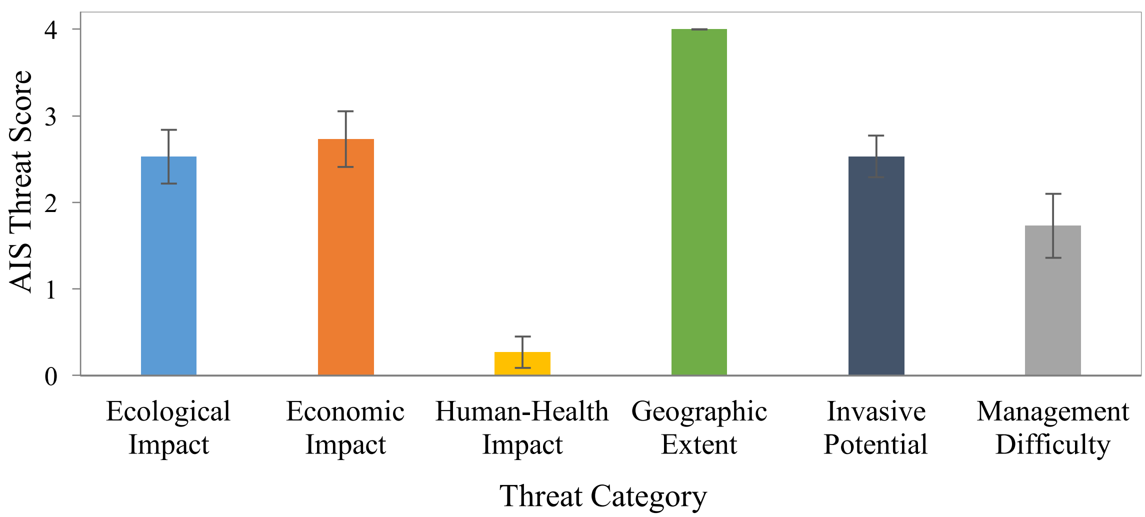


Figure 6. Mean Established Cuban AIS (ECAIS) (n= 15) scores by threat assessment category. The lines that extend above and within the bars represent one positive and negative standard error measurement (± SEM).

The average impact scores (± SEM) for ECAIS were 2.53 ± 0.31 for ecological, 2.73 ± 0.32 for economic, and 0.27 ± 0.18 for human health. All ECAIS received an impact score of 4 for geographic extent. The mean impact score for invasive potential was 2.53 ± 0.24 and 1.73 ± 0.37 for management difficulty. Human health impact and geographic extent varied the least with scores of 0.495 and 0, respectively, while management difficulty had the greatest disparity, measuring 2.067.

3.1.1 Correlation of Established Cuban AIS Threat Categories

	Ecological Impact	Economic Impact	Human Health Impact	Invasive Potential
Ecological Impact				
Economic Impact	0.6220283			
Human Health Impact	0.5674536	0.446406		
Invasive Potential	0.8901608*	0.6773693*	0.4310548	
Management Difficulty	0.5672689	0.4014803	0.5268447	0.6312127

Table 2. Spearman rank correlation coefficients of Established Cuban AIS (ECAIS) (n= 15) threat assessment categories; (*) represents significant correlation at a two-tailed, 0.01 p-value level. Geographic extent category was excluded from the analysis due to no comparable variation.

All inter-category comparisons of ECAIS threat assessment categories (i.e., ecological impact, economic impact, human health impact, invasive potential, and management difficulty) had positive but varying relationship strengths. One comparison - ecological impact and invasive potential (rho= 0.89; p= 8.75e-06) - showed a very strong, significant correlation (i.e., rho= 0.80-1.00; p<0.01). Correlation between economic impact and invasive potential (rho= 0.677; p= 0.006) was significantly strong (rho= 0.60-0.79) based on Weir (2014). Ecological impact and economic impact (rho= 0.622; p= 0.013), and management difficulty and invasive potential (rho= 0.631; p= 0.012), were also found to be strongly correlated but not significantly so. Correlations between ecological impact and human health impact (rho= 0.567; p= 0.446), ecological impact and management difficulty (rho= 0.567; p= 0.027), and human health impact and management difficulty (rho= 0.527; p= 0.044) were classified as moderate relationships (rho= 0.40-0.59) (Weir 2014). The three weakest correlations were between economic impact and management difficulty (rho= 0.401; p= 0.138), human health impact and invasive potential (rho= 0.431; p= 0.109), and economic impact and human health impact (rho= 0.446; p= 0.095). Geographic extent was excluded from the correlation analyses because the ECAIS scores were invariant for this category.

3.2 Potential AIS Donors of Port Mariel

The port of Kingston, Jamaica received the highest PAISD score of six out of a possible six. All ports that received scores of five or six were located within or surrounding the Caribbean Sea. Ports with the lowest ranking (i.e., two) were located in Europe and the Mediterranean. One potential future trade partner (Tampa, USA) received a PAISD score of five, while the remaining future trade partners - Houston, Mobile, New Orleans, and Norfolk, USA - were assigned scores of four.

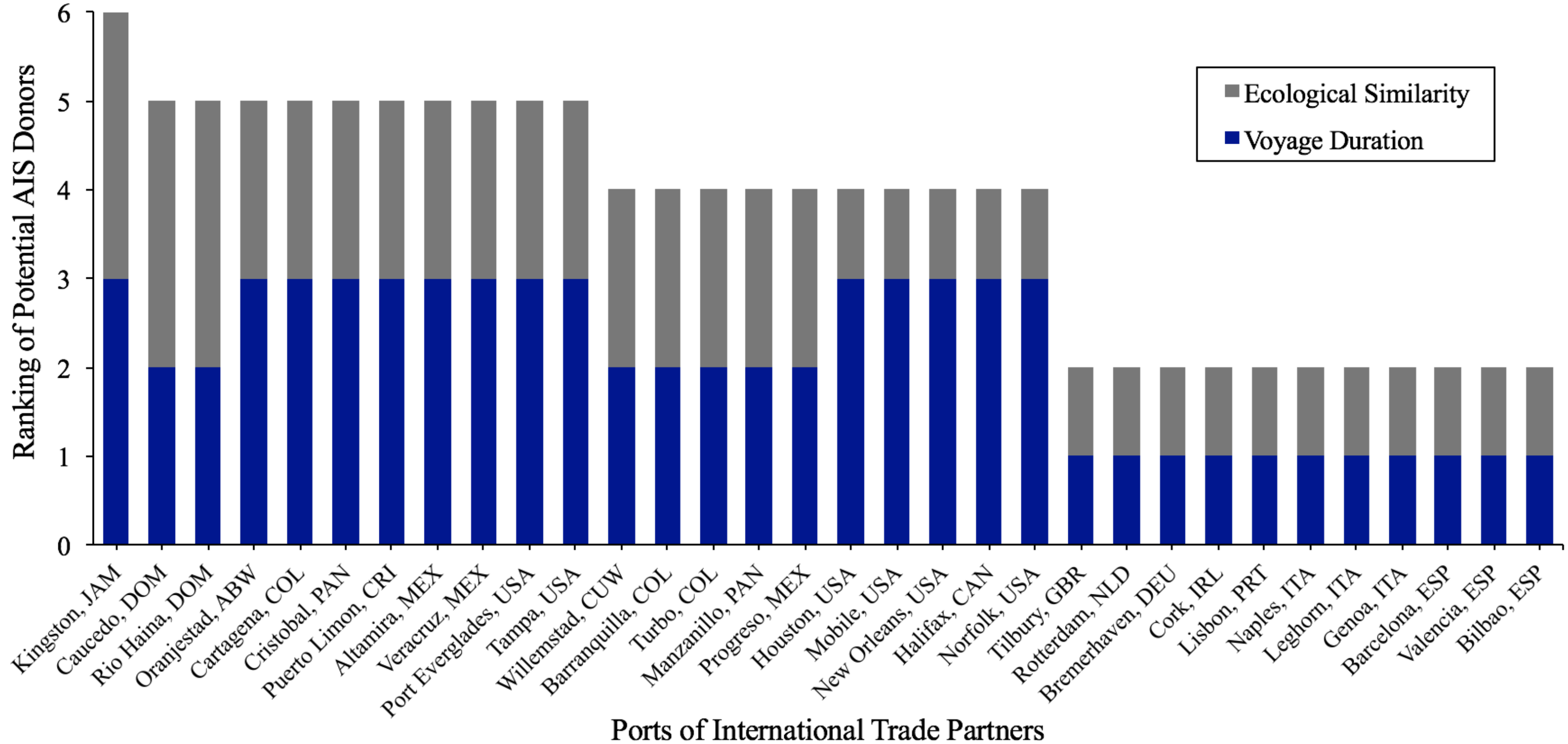


Figure 7. Ranking of Potential AIS Donors (PAISD) from greatest to least concern due to ecological similarity (proximity to Cuba's ecoregion) and voyage duration to Port Mariel.

PAISD Rank	Port, Country	PAISC
6	Kingston, Jamaica (JAM)**	***Alexandrium minutum, Phyllorhiza punctata*** (Ranston et al. 2007, Bayha & Graham 2009)
5	Caucedo, Dominican Republic (DOM)	
	Rio Haina, DOM **	
	Oranjestad, Aruba (ABW)	
	Cartagena, Colombia (COL) **	***Vibrio cholerae*** (Lopez et al. 2010)
	Cristobal, Panama (PAN)	
	Puerto Limon, Costa Rica (CRI)	
	Altamira, Mexico (MEX)	***Hydroides elegans, Perna perna*** (Hicks & Tunnell 1995; Bastida-Zavala & Ten Hove 2002)
	Veracruz, MEX	***P. perna, P. punctata, H. elegans*** (Hicks & Tunnell 1995; Bastida-Zavala & Ten Hove 2002; Ocaña-Luna et al. 2010)
	Port Everglades, United States (USA)	
	Tampa, USA *	***H. elegans*** (Zibrowius 1971)
4	Willemstad, Curacao (CUW)	***H. elegans*** (Ten Hove 1974)
	Barranquilla, COL	***A. minutum*** (Ramos 2005)
	Turbo, COL	
	Manzanillo, PAN	
	Progreso, MEX	
	Houston, USA *	***P. perna*** (Hicks & Tunnell 1995)
	Mobile, USA *	***P. punctata, V. cholerae*** (DePaola et al. 1992; Graham et al. 2003)
	New Orleans, USA*	***P. punctata*** (Bolton & Graham 2004)
	Halifax, Canada (CAN)	
	Norfolk, USA *	***V. cholerae*** (Louis et al. 2003)

2	Tilbury, Great Britain (GBR)	
	Rotterdam, the Netherlands (NLD) **	***H. elegans*** (Ten Hove 1974)
	Bremerhaven, Germany (DEU)	***V. cholerae*** (Böer et al. 2013)
	Cork, Ireland (IRL)	***A. minutum*** (Lilly et al. 2005)
	Lisbon, Portugal (PRT)	***A. minutum, H. elegans*** (Zardoya et al. 1995; Ramos 2010)
	Naples, Italy (ITA)	***H. elegans*** (Guerrieo et al. 2007)
	Leghorn, ITA	
	Genoa, ITA	
	Barcelona, Spain (ESP)	***A. minutum, P. perna, H. elegans*** (Buccheri & Palisano 1976; Zingone et al. 2005; Ramos 2010)
	Valencia, ESP	
	Bilbao, ESP	

Table 3. Ranking of international trade partners by Potential AIS Donor (PAISD) score with corresponding Potential AIS of Concern (PAISC). (*) are potential future trade partners of Port Mariel; (**) represents ports that were on two active container routes to Port Mariel, which were ranked by their highest PAISD score.

3.3 Threat Assessment of Potential AIS of Concern

Five species were identified as PAISC. *Alexandrium minutum* had the greatest overall threat assessment score of 22 out of a possible 22. *A. minutum*, *Phyllorhiza punctata,* and *Hydroides elegans* had harmful ecological and economic impact scores as well as the highest management difficulty scores. *A. minutum*, *P. punctata, Perna perna*, and *Vibrio cholerae* were associated with negative human health impacts. All of the species had non-native distributions in multiple ecoregions and therefore scored four for geographic extent. *A. minutum* and *V. cholerae* scored the highest values for invasive potential.

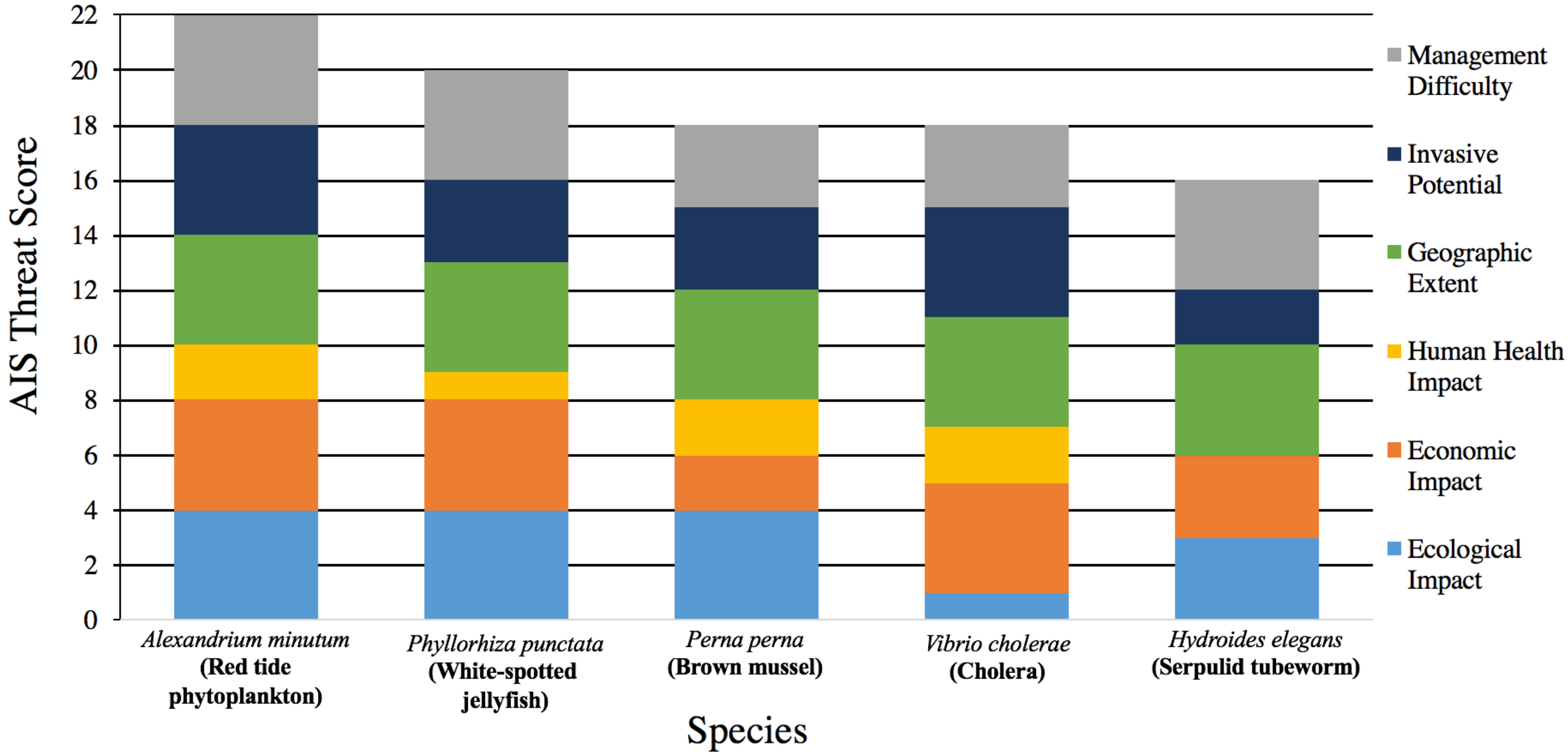


Figure 8. Threat scores of Potential AIS of Concern (PAISC). Species with ecological or economic impact scores of 3 or 4 or human health impact scores of 2 or 1 were considered harmful.

3.3.1 Temperature Tolerances of AIS

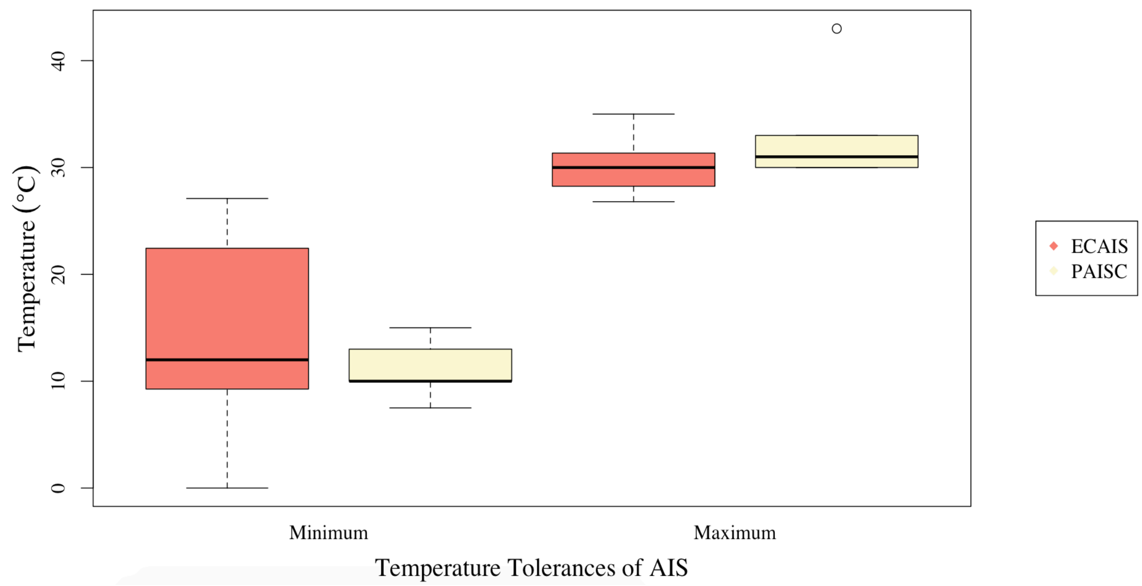


Figure 9. Minimum and maximum temperature (°C) tolerances of Established Cuban AIS (ECAIS) (n= 15) and Potential AIS of Concern (PAISC) (n=5). The lines above and below the boxes represent the largest and smallest non-outliers of the data sets, respectively. The darkened lines within the boxes are the median values of the data sets.

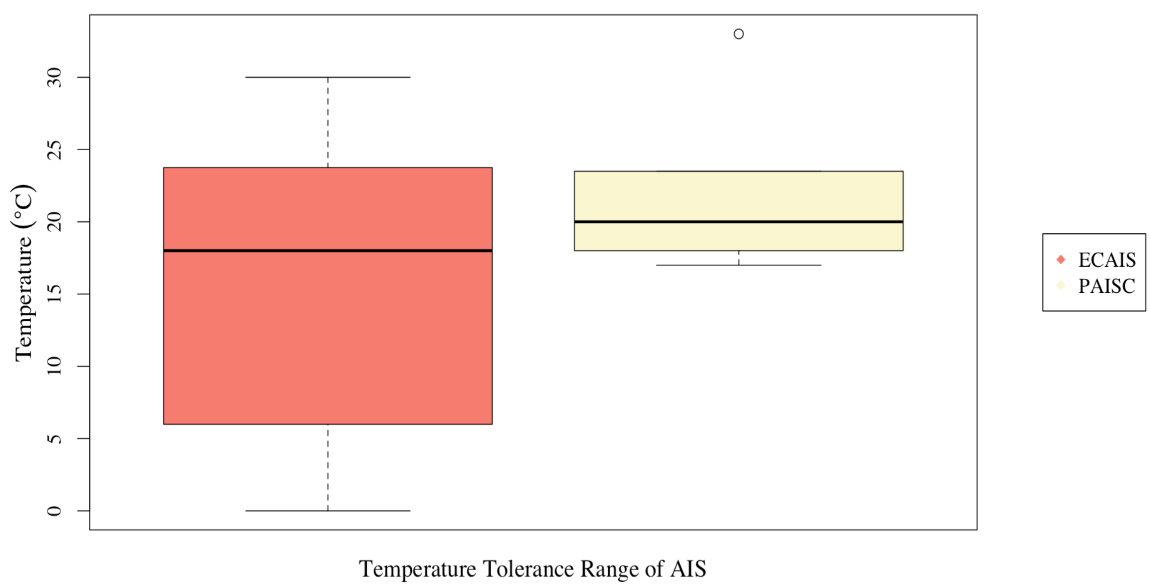


Figure 10. Range of temperature (°C) tolerances of Established Cuban AIS (ECAIS) (n= 15) and Potential AIS of Concern (PAISC) (n= 5). The lines above and below the boxes represent the largest and smallest non-outliers of the data sets, respectively. The darkened lines within the boxes are the median values of the data sets.

		Mean Tolerance (± SEM)		Mann-Whitney Wilcoxon Test (p-value)
		ECAIS (n=15)	**PAISC (n=5)**	
Temperature (°C)	**Minimum**	13.97 ± 2.23	11.1 ± 1.3	0.599
	Maximum	30.01 ± 0.70	33.4 ± 2.5	0.124
	Range	16.04 ± 2.66	22.3 ± 2.9	0.359

Table 4. Mean minimum, maximum, and range (± one standard error measurement (SEM)) of Established Cuban AIS (ECAIS) (n= 15) and Potential AIS of Concern (PAISC) (n= 5) temperature tolerances (°C). Two-tailed Mann-Whitney-Wilcoxon tests were conducted in order to compare the temperature tolerances of the AIS data sets (significance at p<0.01)

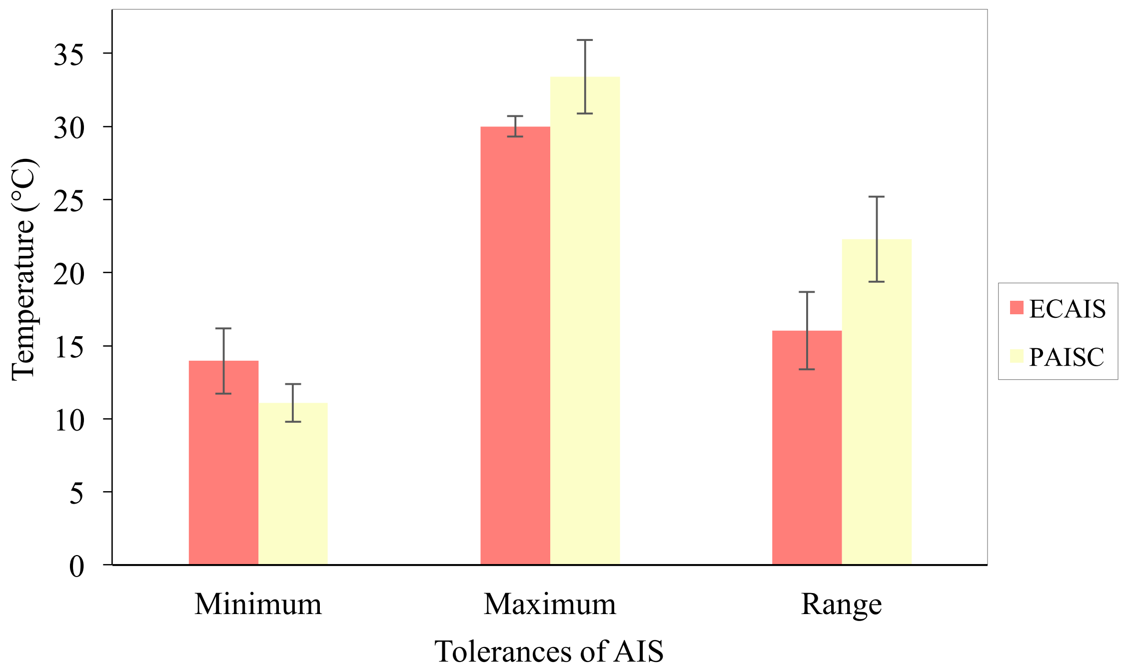


Figure 11. Mean temperature (°C) tolerances of Established Cuban AIS (ECAIS) (n= 15) and Potential AIS of Concern (PAISC) (n= 5). The lines that extend above and within the bars represent one positive and negative standard error measurement (± SEM).

The average minimum temperature tolerances of the ECAIS (13.97 ± 2.23 °C) and the PAISC (11.1 ± 1.3 °C) did not differ significantly (p= 0.599). The average maximum temperature tolerances of the ECAIS (30.01 ± 0.70 °C) and the PAISC (33.4 ± 2.5 °C) were not significantly different (p= 0.124). The average range between the minimum and maximum thermal tolerances for the ECAIS (16.04 ± 2.66 °C) and the PAISC (22.3 ± 2.9 °C) were also not significantly different (p=0.359).

3.3.2 Salinity Tolerances of AIS

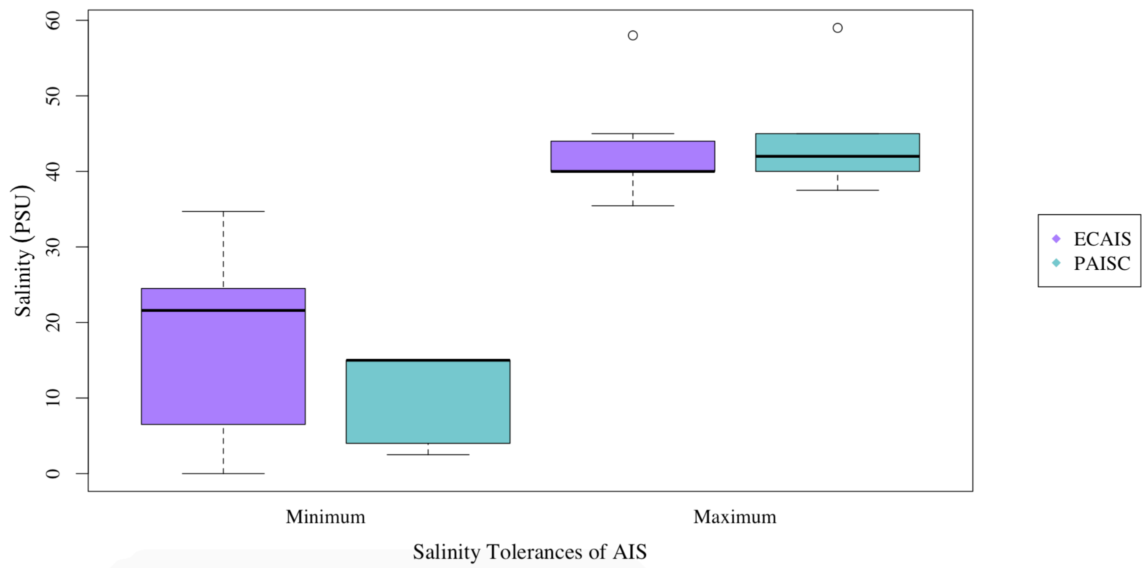


Figure 12. Minimum and maximum salinity (PSU) tolerances of Established Cuban AIS (ECAIS) (n= 15) and Potential AIS of Concern (PAISC) (n= 5). The lines above and below the boxes represent the largest and smallest non-outliers of the data sets, respectively. The darkened lines within the boxes are the median values of the data sets.

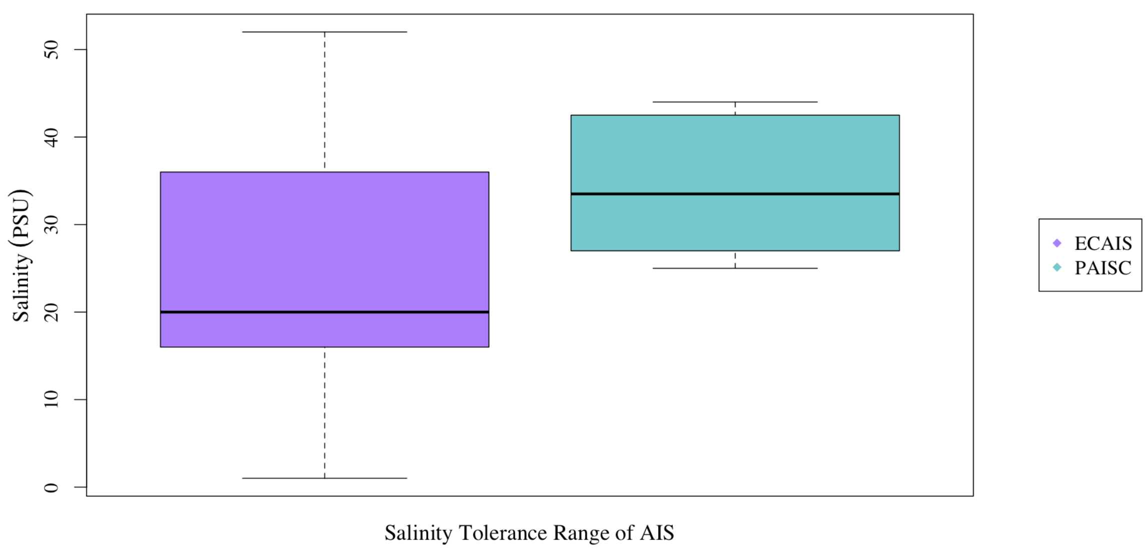


Figure 13. Range of salinity (PSU) tolerances of Established Cuban AIS (ECAIS) (n= 15) and Potential AIS of Concern (PAISC) (n=5). The lines above and below the boxes represent the largest and smallest non-outliers of the data sets, respectively. The darkened lines within the boxes are the median values of the data sets.

		Mean Tolerance (± SEM)		Mann-Whitney Wilcoxon Test (p-value)
		ECAIS (n= 15)	PAISC (n= 5)	
Salinity (PSU)	Minimum	17.62 ± 2.88	10.3 ± 2.9	0.137
	Maximum	41.71 ± 1.39	44.7 ± 3.8	0.469
	Range	24.09 ± 3.72	34.4 ± 3.9	0.1157

Table 5. Mean minimum, maximum, and range (± one standard error measurement (SEM)) of Established Cuban AIS (ECAIS) (n= 15) and Potential AIS of Concern (PAISC) (n= 5) salinity tolerances (PSU). Two-tailed Mann-Whitney-Wilcoxon tests were conducted in order to compare the salinity tolerances of the AIS data sets (significance at $p<0.01$).

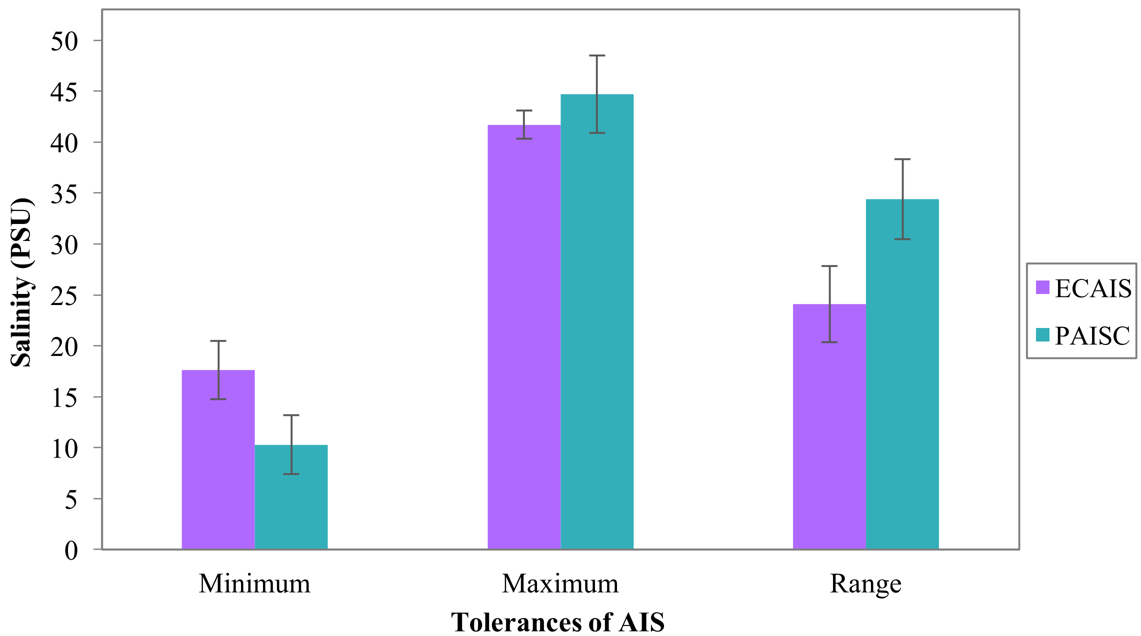


Figure 14. Mean salinity (PSU) tolerances of Established Cuban AIS (ECAIS) (n= 15) and Potential AIS of Concern (PAISC) (n= 5). The lines that extend above and within the bars represent one positive and negative standard error measurement (± SEM).

The average minimum salinity tolerances of ECAIS (17.62 ± 2.875 PSU) and PAISC (10.3 ± 2.9 PSU) did not differ significantly (p= 0.137). Mean maximum salinity tolerances of ECAIS (41.71 ± 1.39 PSU) and PASIC (44.7 ± 3.8 PSU) were not significantly different (p = 0.469). The average range between minimum and maximum salinities for ECAIS (24.09 ± 3.72 PSU) and PAISC (34.4 ± 3.9 PSU) was also not significantly different (p = 0.137).

4.0 Discussion

As Port Mariel, Cuba transforms into a regional transshipment hub for the Caribbean, the port will not only receive greater volumes of international trade but also more frequent exposure to AIS transferred via ocean-going commercial vessels (Carlton & Geller 1993; Bax et al. 2003; Drake & Lodge 2004; Westphal et al. 2007). The purpose of this study was to assess the threat levels that ship-related AIS do (or could) impose on Cuba in order to prioritize prevention and management actions in the country. The research exposed the limited availability of information on AIS abundance and distribution in Cuba. In order to gain better insight into the damage established and potential AIS may cause in Cuba, regional and global AIS records were utilized as supplemental data for the threat assessments. The study results indicated that management efforts for ECAIS should be primarily directed toward monitoring the spread and minimizing the impact of harmful species, such as removing Asian green mussels (*Perna viridis*) from industrial cooling systems, limiting anthropogenic input in harbors that may contribute to toxic blooms of the naked dinoflagellate (*Gymnodinium catenatum*), and potentially eradicating isolated occurrences of orange-cup coral (*Tubastraea coccinea*). Recent attention to the national invasive species action plans have indicated that the Cuban government is focused on improving the control of AIS in the country.

This study suggests that active and future international trade partners of Port Mariel, such as the ports of Jamaica, Mexico, United States (Florida), and Colombia, may be AIS donors due to their 'ecological' and spatial proximity to the port. In addition, these PAISD are busy maritime hubs in the Caribbean and, thus, potential stepping stones for AIS dispersal to Cuba. The identified PAISC – i.e., red tide phytoplankton (*Alexandrium minutum*), white-spotted jellyfish (*Phyllorhiza punctata*), brown mussel (*Perna perna*), cholera (*Vibrio cholera*), and serpulid tubeworm (*Hydroides elegans*) - have similar physical environmental tolerances of those AIS presently established in Cuba and, if introduced, these non-native species could cause serious ecological, economic, and health-related damage to Cuba. The threat of these species underscore the importance of strong preventative measures and international collaborative efforts in order to minimize the potential risk of future AIS introductions in Port Mariel and dispersal at a national and regional scale. Going forward, this information can be utilized by: (1) the Cuban government to allocate resources for combating AIS, (2) scientists for further research on propagule pressure from increased maritime activity and potential expansion of AIS populations within Cuba, and (3) Cuban citizens for educational purposes and to encourage community participation to help control AIS population densities and dispersal. The threat assessment herein can also be used as a template for future AIS analyses.

4.1 Established Cuban AIS

The ECAIS identified in this analysis represent species of different taxonomies, origins, and reporting of establishment in Cuba. Two commonalities among the ECAIS were their multi-ecoregional, invasive distributions and the method (i.e., shipping pathways) by which these species were likely introduced to Cuba. Individual threat assessments revealed a range in the abundance, impact, and management potential of the ECAIS. Seven of the fifteen ECAIS were not scored as 'harmful' for two or more of the impact categories. These species did not pose a risk to human health and caused relatively minor ecological and economic impacts, such as competition with native species that did not result in local mass mortality (marine pill bug, *Sphaeroma walkeri*) and attachment to artificial structures without significant fouling-damage (rough sea squirt, *Styela canopus;* and reticulated barnacle, *Amphibalanus reticulatus*) (Fofonoff et al. 2016). Of the eight harmful species, the Asian green mussel received one of the two highest ECAIS threat scores and was the only invader that was recorded as having multiple negative impacts in Cuba. Due to limited available data on AIS in Cuba, other harmful ECAIS (including the naked dinoflagellate and orange-cup coral) were assigned high threat scores based on their known impact in other countries and documented concern as established AIS within Cuba. Under favorable conditions (e.g., nutrient inputs, disturbances, habitat changes), these species have the potential to expand their populations beyond their initial sites of establishment and cause serious damage in Cuba if left unmanaged (Lodge 1993; Kolar & Lodge 2001; Sakai et al. 2001).

Comparing the categorical variation of threat scores showed positive correlations between the assessment categories. These results indicated that for all of the ECAIS, increasing threat scores in one category corresponded with increasing threat scores in another. Three of the four strongest correlations were associated with the "invasive potential" category. Invasive potential had a very strong, statistically significant relationship with ecological impact. In addition, invasive potential had strong correlations with economic impact (also significant) and management difficulty. Variation in ecological impact and economic impact were found to also have a strong positive relationship. These associations are representative of classic invasive species traits (Moyle 1986). In the threat assessment, invasive potential was a measure of the species' ability to spread beyond the initial site of introduction. AIS with robust life history traits (e.g., prolific reproduction of small orange-cup coral colonies, dormancy of naked dinoflagellate cysts, and high fecundity of the deep-cleft shipworm (*Teredo furcifera*) may take advantage of niches in non-native locations that lack the predation and competition present in indigenous regions (Richards et al. 1984; Fofonoff et al. 2016; Global Invasive Species Database (GISD) 2016). The AIS, therefore, may be capable of outcompeting native species and dominating local habitats and resources, which can cause negative ecological and economic impacts, such as displacement of native coral species by orange-cup coral, fishery closures due to naked dinoflagellate blooms, and destabilized wooden structures from boring deep-cleft shipworms (Hewitt et al. 2002; Molnar et al. 2008; Silva et al. 2011). Strong, positive covariations between invasive potential, ecological and economic impacts, and management difficulty categories illustrate the scoring system's accuracy and can, therefore, aid in prioritizing ECAIS management in Cuba.

4.1.1 Harmful Established Cuban AIS

***Perna viridis* (threat score: 21/22)**

The Asian green mussel (*P. viridis*) received one of the two highest scores in the ECAIS threat assessment. This species is also the AIS that had the most information on their invasiveness specific to Cuba. The green mussel has caused harmful economic impacts in Cuba and has the potential to negatively affect the environment and human health in the country in the future. The species was first detected in Cuba in 2005 as blockage in cooling channels at Carlos Manuel de Céspedes thermoelectric plant in Cienfuegos Bay, located on the southern central coast of the island (Fernández-Garcés & Rolán 2005). As a major industrial port for Cuba, Cienfuegos Bay possesses artificial structures and retains high nutrient loads from anthropogenic pollution that fuels plankton blooms upon which the mussels feed (Burke 1986; Rodriguez et al. 1993; Diaz-Asencio et al. 2005; Moreira et al. 2010; Garces et al. 2012). Available habitat and ample resources in the bay facilitated establishment of this ECAIS and densities after detection were measured at upwards of 18,000 individuals per m^2 (Fernández-Garcés & Rolán 2005). Since establishment of the green mussel, the thermoelectric plant must stop biannually in order to remove obstructions caused by the invasive population (Fernandez 2015). The species has also been found in natural habitats, colonizing roots of mangroves within the bay. Fouling of ecologically important mangroves may displace native species from nursery sites and alter the structure of habitats as mangrove roots prevent erosion (Lopeztegui–Castillo et al. 2014). The appearance and subsequent impacts of the green mussel has resulted in research into the species' abundance, distribution, and potential use for scientific and financial purposes (Alonso-Hernández et al. 2012; Lopeztegui–Castillo et al. 2014). However, at the end of the year 2012, recorded green mussel populations in Cienfuegos Bay were low, with the exception of the cooling channels of the thermoelectric plant. Explanations for the decreased abundance of green mussels included fluctuations in abiotic factors (e.g., dissolved oxygen concentrations, water temperatures, and air exposure) as well as harvesting activities (e.g., bio-monitoring of heavy metals, consumption, and creation of handicrafts) (Lopeztegui–Castillo et al. 2014). Reduced population sizes in 2012 suggested that the green mussel was contained within Cienfuegos Bay; however, the species was subsequently discovered in Mariel Bay in 2013 and Santiago Bay in 2015, confirming that the population was not controlled (Lopeztegui–Castillo et al. 2013; Lopeztegui–Castillo et al. 2014; Fernandez 2015) (Figure 15).

Figure 15. The distribution of the Asian green mussel (*Perna viridis*) in Cuba with the year of population establishment.

Population densities of the green mussel within Mariel Bay were not considered high in 2012 (5 individuals per 10 m^2) but all specimens that were collected were adults, suggesting that the species had been established for at least six months (Lopeztegui–Castillo et al. 2013). Similar to Cienfuegos Bay, green mussels in Mariel Bay were found in the channels of the Central Thermoelectric Maximo Gomez plant (Lopeztegui–Castillo et al. 2013). The recent expansion of the green mussel is a cause of concern as further economic damage as well as possible harmful ecological and health impacts may be in store for Cuba from this species. The invasive history of the green mussel outside of Cuba includes high oyster mortality in Tampa Bay, United States as a result of competition for space as well as PSP outbreaks in Trinidad and China (causing two human fatalities and four illnesses) (Zhou et al. 1999; Ammons et al. 2001). Consumption of PSP-contaminated green mussels can result in numbness, nausea, diarrhea, vomiting and, in more serious cases, paralysis, respiratory failure, and death (Ansdell 2015). Multiple impacts in several regions, and also the geographic extent of invasion of the species, has led to speculation that the green mussel could become the marine equivalent of the Asian zebra mussel (*Dreissena polymorpha*), an infamous freshwater invader of the Great Lakes in the United States (Power et al. 2004). Containment of the well-established green mussel population within Cienfuegos Bay and removal of the mussels from the bay's thermoelectric plant are the most feasible options for this region (United Nations Development Programme (UNDP) 2011; Fernandez 2015; Alexander Lopeztegui-Castillo, personal communication, August 4, 2016). Due to early detection in Mariel Bay and Santiago Bay, rapid response with the purpose of eradication has been implemented by Cuban officials (Lopeztegui–Castillo et al. 2013; Lopeztegui–Castillo et al. 2014; Fernandez 2015).

***Gymnodinium catenatum* (threat score: 21/22)**

The naked dinoflagellate (*G. catenatum*) is a cryptic, coastal species ranging from temperate waters to the tropics with recorded introductions via shipping pathways and aquaculture (GISD 2016). The AIS threat score of the naked dinoflagellate was based on the species' well-documented capability to produce toxins that cause PSP (Morey-Gaines 1982; Mee et al. 1986). The species has been linked to three separate outbreaks of PSP-contaminated shellfish in Mexico that caused 460 illnesses and 32 human fatalities (Band-Schmidt et al. 2004). Due to safety concerns for human health, detections of PSP-contaminated, economically valuable organisms can result in fishery closures (Hewitt et al. 2002). Additionally, toxins produced by rapid population growth or "blooms" of naked dinoflagellates can cause mass mortality of native species (Alonso-Rodriguez & Paez-Osuna 2003). The naked dinoflagellate was discovered in two regions of Cuba: Sabana-Gamaguey in 1999 and Cienfuegos Bay in 2009. The species has not been linked to any PSP-outbreaks in Cuba, but rapid blooms could be detrimental to the region if the dinoflagellate reproduces uncontrolled (Leal et al. 2003; Moreira-González 2014). Local harvesting of the invasive Asian green mussel in Cienfuegos Bay is prevalent and may be a vector of PSP as the mussels can become contaminated through the consumption of the dinoflagellate (Moreira-González et al. 2014). Moreira-González et al. (2014) suggested management of the naked dinoflagellate and other toxigenic dinoflagellates in Cienfuegos Bay should include reducing anthropogenic discharge in the bay to prevent eutrophication and subsequent blooms as well as monitoring bivalves in the bay for PSP presence.

Tubastraea coccinea **(threat score: 18/22)**

The non-indigenous orange-cup coral *T. coccinea* (native to the Indo-Pacific) was first recorded in the western Atlantic in the early 1940s and has since spread throughout this region, including the first reported sighting on Cuba's eastern coast in 1982 (Vaughan & Wells 1943; Zlatarski & Estallela 1982; Cairns 2000). Specific information on the impact and distribution of the species in Cuba is limited; therefore, the ECAIS threat was based on data from the surrounding regions. Although primarily found on artificial structures such as oil platforms, artificial reefs, and shipwrecks, the orange-cup coral's spread to natural ecosystems may displace native sponges and corals and significantly disturb reef and hard-bottom ecosystems (Fenner 2001; Fenner & Banks 2004). Research conducted in Brazil demonstrated that the orange-cup coral inhibits reef productivity by preventing coral reef fishes from feeding and also reduces algal recruitment (Lages et al. 2010; Moreira & Creed 2012). In addition, mortality of the native coral *Mussismilia hispida* has been associated with physical contact from the orange-cup coral and the non-native congener, *T. tagusensis* (Silva et al. 2011). Mantelatto and Creed (2015) speculated that the expansion of this coral in *Perna perna* (a commercially important native mussel) beds in Ilha Grande Bay, Brazil could result in economic impacts on the local mussel fishery. Efforts to reduce orange-cup coral in its invasive range include physical removal in the western region of the Flower Garden Banks National Marine Sanctuary (FGBNMS) in the Gulf of Mexico and plastic or raffia to smother introductions in Brazil (FGBNMS 2015; Mantelatto et al. 2015). Beyond the initial record of the orange-cup coral, abundance and distribution of the ECIAS in Cuba could not be determined at the time of this analysis. Control of the species within Cuba may be possible if it is found in isolated locations, given previous successful removals in other regions. However, widespread distribution in the region and reproductive proficiency suggests that the orange-cup coral may be difficult and costly to manage (Fenner 2001; Figueira de Paula et al. 2014).

Shipworms *(Teredo bartschi, Teredo furcifera, Teredo navalis, Lyrodus pedicellatus)* (threat scores: 16/20)

For centuries, ocean-going vessels have transferred shipworms globally across ocean basins, which has made identifying their native ranges a challenge (Fofonoff et al. 2016). Typically, multiple shipworm species, whose phylogeny is often in question, co-occur. Determining their ecological and economic influences as individual AIS, therefore, can be difficult (Fofonoff et al. 2016). Shipworms are highly-modified, wood-boring mollusks. At an ecological level, these species are ecosystem engineers that can alter habitat structure, creating available niches for other AIS, and diminish available resources (Fofonoff et al. 2016). From an economic perspective, high abundances of shipworms undermine wooden structures (e.g., ships, piers) that then become unsafe due to diminished stability and are also costly to repair (Turner 1973; Hoppe 2002; Foderaro 2011). Shipworms, however, have no known effect on human health. Four species of shipworms have been recorded in Cuba: *T. bartschi* (Bartsch shipworm), first documented in 1960, *T. furcifera* (deep-cleft shipworm) in 1953, *T. navalis* (naval shipworm) in 2010, and *L. pedicellatus* (blacktip shipworm) in 1960 (Wallour 1960; Miloslavich et al. 2010; Fofonoff et al. 2016). Limited information was available for analysis on the specific impacts shipworms have on the Cuban environment and economy. Regional and global data were therefore utilized for the threat assessments. Controlling the spread and damage of shipworms has been effective by chemically treating wood with creosote or by using alternative materials, such as concrete or metal in place of wood, in water-exposed structures (Ray 2005).

***Charybdis hellerii* (threat score: 13/20)**

C. hellerii, the Indo-Pacific swimming crab, was most likely introduced to the western Atlantic in the late 1980s via ocean-going ships from previously-invaded Mediterranean ports (Campos & Türkay 1989; Galil & Zenetos 2002). The species was recorded in Cuba in 1987 in Cienfuegos Bay and Bahia de Gibara (Gómez & Martinez-Iglesias 1990). Impacts of the swimming crab in Cuba are not documented; therefore, regional and global information was used for scoring the threat in this analysis. Despite establishment and spread in non-native regions, the crab does not seem to cause significant ecological and economic impacts. However, in Belize, the species may be a competitor of several native crustaceans and has been associated with a decreased abundance of these populations in shallow-water habitats (Felder et al. 2010). If the non-native swimming crab population was to displace economically important crustaceans, fisheries could be negatively affected, as noted as a concern in the *Callinectes sapidus* (blue crab) fishery in Florida, US (Dineen 2001). In addition, this ECAIS is a potential host of the White Spot Syndrome Virus, a disease that causes mass species mortality in aquaculture facilities (Joint Subcommittee on Aquaculture 1997; Tavares & Amouroux 2003). Information on control efforts for established, non-native populations were not provided in the resources used in this analysis. However, preventative measures in the form of ballast water management are proposed here to help suppress the expansion of the Indo-Pacific swimming crab in Cuba and the introduction to the surrounding region (IMO 2009).

4.2 Potential AIS Donors

Active and future container routes to Port Mariel provide insight into regional and global maritime connectivity of Cuba to other nations. The routes represent repeated and frequent visits to the Port (e.g., 2016 schedules were every 7 to 13 days) from specific international locations that may act as donors of ship-introduced AIS to Cuba. European and Mediterranean ports (i.e., Great Britain, Ireland, the Netherlands, Germany, Portugal, Spain, and Italy) received the lowest scores (two) as PAISD due to ecoregional separation from Cuba by the Atlantic Ocean and relatively long voyages of over ten days to Port Mariel. With the exception of Tampa, United States (a score of five), the USA sites that may be adding cargo services to Port Mariel in the future – i.e., Norfolk, Mobile, Houston, and New Orleans – were not prioritized (four) as PAISD. International trade partners that ranked the highest as donor ports – i.e., Kingston, Jamaica (a score of six), Cristobal, within the port city of Colon, Panama (five), Cartagena, Colombia (five), and Rio Haina, Dominican Republic (five) - not only were found at regional risk of AIS expansion but also demonstrate Port Mariel's direct connection to major transshipment ports of the Caribbean. Kingston, Colon, and Cartagena, as well as Limon, Costa Rica (five), Veracruz, Mexico (five), and Caucedo, Dominican Republic (five) were ranked in the top 20 Latin ports by volume of container throughput in 2015 (Economic Commission for Latin America and the Caribbean 2016). Connectivity with these ports demonstrates that Port Mariel is susceptible to invasion via "stepping stones", wherein AIS are transported from busy, contaminated hubs to other ports where they are not native (Floerl et al. 2009; Keller et al. 2011). Due to relatively similar ecosystems, short transit durations, and high maritime activity, transshipment ports of the Caribbean such as those highlighted above could feasibly expose Port Mariel to PAISC. Monitoring future invasions in these locations, therefore, may forewarn Cuban officials of the potential spread of AIS to Cuba from these transshipment hubs and should be management priorities.

4.3 Potential AIS of Concern

Compared to the ECAIS, the PAISC did not have significantly different salinity and temperature tolerances. These results indicate that, overall, the PAISC are capable of surviving in similar environments as the invasive species already established in Cuba. The capability of these species to tolerate broad environmental conditions is also supported by their present, multi-ecoregional ranges. The threat assessments in this study showed that the PAISC could not only invade new regions such as Cuba but also inflict a multitude of serious impacts to the ecosystem (e.g., mortality of native species), economy (e.g., closure of fisheries), and health (e.g., PSP) of the local community. Without implementing stringent preventative and early response measures, Port Mariel could be vulnerable to future AIS invasions and also promote AIS dispersal throughout Cuba.

4.3.1 Harmful Potential AIS of Concern

***Alexandrium minutum* (threat score: 22/22)**

The red tide phytoplankton, *A. minutum,* was ranked as the highest PAISC threat for Cuba. This planktonic dinoflagellate is found in enriched coastal zones from temperate to tropical regions worldwide (Faust & Gulledge 2002). The invasive potential of the red tide phytoplankton is associated with the species' widespread distribution (i.e., broad environmental tolerances) and sexual reproduction of resting cysts, which can remain dormant in sediment for years until favorable conditions exist (Hewitt 2002; Probert et al. 2002). These traits allow the red tide phytoplankton to survive extended transit times in ballast water from one contaminated port into another. Blooms of this PAISC (also known as 'red tides') produce large amounts of toxins that can cause serious impacts to the ecosystem in the form of mass mortality of native species, to the economy by requiring temporary closures of local shellfish harvests, and to human health from PSP outbreaks (Hallegraeff 1993; Faust & Gulledge 2002; GISD 2016). Once the red tide phytoplankton is established and undergoes rapid population growth, the species is difficult to eradicate (Centre for Agriculture and Biosciences International 2016). Moreover, detecting its presence in a harbor requires time-consuming, taxonomically challenging research (GISD 2016). Moreira-González et al. (2014) reported the occurrence of the red tide phytoplankton in Cienfuegos Bay, Cuba in 2009, without indication of native or invasive status. Apart from that study, however, the species has not been noted in Cuba by any of the databases utilized for this study (Appendix 1). To reduce the likelihood of the red tide phytoplankton establishing in Mariel Bay, preventative measures (i.e., ballast water management) for both international and national maritime traffic should be implemented (Hallegraeff & Bolch 1992).

***Phyllorhiza punctata* (threat score: 20/22)**

For over half a century, the white-spotted jellyfish (*P. punctata*) has been sporadically recorded in the western Atlantic basin in areas such as eastern United States, Puerto Rico, Jamaica, and Mexico (Garcia & Durbin 1993; Silveira & Cornelius 2000; Masterson 2014; Ocaña-Luna et al. 2010; Bayha & Graham 2011; U.S. National Museum of Natural History 2016). This Indo-Pacific species has also been introduced to Brazil, western United States (southern California and Hawaii) and the Mediterranean (Fofonoff et al. 2016). The white-spotted jellyfish alternates between asexual and sexual reproduction and may be transported by the two main AIS shipping vectors: ballast water (i.e., the life history stages planktonic planula, ephyra, or medusa) and biofouling (i.e., the benthic scyphistoma or strobili stage) (DeFelice et al. 2002). Under favorable conditions, a non-native population can increase to ecologically and economically destructive proportions.

During the summer of 2000, for example, a white-spotted jellyfish bloom in the Gulf of Mexico caused as much as $10 million in economic losses. The damages were attributed to reduced catches of commercially important species in the form of competition for resources and direct predation of larvae and also damages to fishing equipment from clogged shrimp nets and boat intakes (Graham et al. 2003; Fuller 2005). The bloom in the Gulf of Mexico decreased by September 2000 and reemergence of the species in the Gulf has not been as severe since (Fofonoff 2016). The 2000 event represents the most extreme scenario that Cuba may witness if the white-spotted jellyfish is introduced and establishes a reproductive population in the country. In the majority of non-native regions, this species has caused minimal negative economic impacts, primarily due to low abundance (Fofonoff 2016). However, blooms in the Gulf of Mexico as well as Puerto Rico and Brazil demonstrate that the white-spotted jellyfish is capable of disturbing native species and, potentially, the local economy (Garcia & Durbin 1993; Haddad & Nogueira Júnior 2006). At the time of this study, information was lacking on successful control measures for the invasive jellyfish (GISD 2016). Due to the species' ability to remain within the small polyp stage for years, however, it is probable that it would be difficult to remove the white-spotted jellyfish from areas of establishment (Molnar et al. 2008).

***Perna perna* (threat score: 18/22)**

Although not considered as harmful as the related Asian green mussel (*P. viridis*), the brown mussel (*P. perna*) is capable of displacing native species, changing habitat structures as an ecosystem engineer, and fouling artificial substrates such as oil platforms and buoys (Hicks & Tunnell 1995; Souza et al. 2003; Carranza & Borthagaray 2009). Impacts attributed to the brown mussel have been reported in Texas, USA and Mexico, where the non-native population is forecasted to expand "farther east to the Yucatan peninsula and farther north along the Gulf of Mexico coast" (Molnar et al. 2008). In Brazil, the brown mussel has long been cultivated as an important food source (Souza et al. 2003). However, similar to other filter-feeding bivalves, the species is a secondary vector of PSP and has been linked to a 1991 PSP outbreak in Venezuela (La Barbera-Sanchez et al. 2004). The invasive potential of the brown mussel is of particular concern to tropical regions where the species is capable of spawning year round (Hicks & McMahon 2002). Additionally, brown mussel larvae can settle on a wide variety of surfaces, including vegetation, rocks, and wood (Gulf States Marine Fisheries Commission 2003). The brown mussel is more sensitive to abiotic conditions than the green mussel and it has been suggested that established populations in cooling systems can be controlled with chlorine treatments (Rajagopal et al. 1996).

***Vibrio cholerae* (threat score: 18/22)**

The introduction of toxigenic strains of the bacterium *V. cholerae* (i.e., serogroups O1 and O139) can cause life-threatening outbreaks of the disease cholera (Cohen et al. 2012). After consuming cholera-contaminated food (e.g., shellfish) and water (i.e., poor sanitation), individuals experience acute diarrhea and, if left untreated, can die within hours (Seas & Gotuzzo 2009; Todar 2012). Additionally, cholera in non-endemic areas, where the majority of the population is not immune, can infect all age groups and cause infection rates as high as 10% (Levy 2004). Transmission of cholera is possible through a number of vectors, including insufficient sewage treatment, contaminated drinking water after a natural disaster, and ballast water discharge (Ruiz et al. 2000; Cohen et al. 2012; Centers for Disease Control and Prevention (CDC) 2014). The ability of the cholera bacterium to tolerate both marine and freshwater habitats and to remain dormant in algae and zooplankton enhances the species' aquatic invasive potential (Levy 2004; Todar 2012). The cholera epidemics in Latin America in 1991 and Haiti in 2010 have sparked concern over the spread of the bacterium from contaminated ports to recipient, uninfected harbors (CDC 1993; Cohen et al. 2012). As the main container port for Cuba, Port Mariel could be vulnerable to exposure of this PASIC following international cholera outbreaks and, if introduced, the species could be devastating for the Cuban population (Cohen et al. 2012; Miller 2016a). Thankfully, recent international ballast water treatment protocols and governances have been important efforts to minimize the risk of cholera transfer to Cuba and internationally (Cohen et al. 2012; IMO 2016a).

***Hydroides elegans* (threat score: 16/22)**

H. elegans is a widely distributed serpulid tubeworm found from warm-temperate to tropical marine ecosystems, including the southern Caribbean Sea (Bastida-Zavala & Ten Hove 2002; Hewitt et al. 2002; Fofonoff et al. 2016). The species is a common fouling organism on ship hulls, capable of withstanding vessel speeds due to minimal drag from its tube structure (Woods Hole Oceanographic Institution 1952; Nelson-Smith 1971).

Additionally, the tubeworm is relatively tolerant of AIS treatments applied to vessels such as wood preservatives and tributyltin antifouling compounds (Udhayakumar & Karande 1996; Tarakanadha et al. 2004). In Japan and Florida, US, the species has inhabited oyster beds, creating competition for resources and space (Wang & Huang 1993; Boudreaux et al. 2006; Masterson 2014; Fofonoff et al. 2016). Oysters are ecologically important as natural water filters and ecosystem engineers and economically valuable as food. It follows that displacement of oysters by the invasive tubeworm can negatively affect local environmental conditions and impact harvesting activities (Masterson 2014). Introduced populations of this PAISC are dominantly found in polluted, artificial sites such as harbors and have created fouling problems in industrial intake systems (Zibrowius 1971; Kocak & Kucuksezgin 2000). At Port Mariel, the water intake systems in the economic development zone and the thermoelectric plant could be negatively affected by the tubeworm if introduced (Lopeztegui–Castillo et al. 2013; Zona Especial de Desarrollo Mariel 2014).

4.4. AIS Impacts Beyond Port Mariel

The exposure of Port Mariel to AIS and subsequent threat is not limited to Mariel Bay - the port may act as a vector of AIS dispersal throughout Cuba and to other regions that are connected by water flow (Figure 16). While specific information on water flow within Mariel Bay was unavailable for this study, oceanic conditions along the northern coast of Cuba have been examined in previous studies. Cuba flanks major oceanic currents that flow through the Caribbean Sea and Gulf of Mexico (Peterson et al. 2012). For example, the warm waters of the Central Caribbean current pass northward between the Yucatan Peninsula and western edge of Cuba, entering the Gulf of Mexico, becoming the Loop Current. After exiting the Gulf of Mexico, the Loop Current becomes the Florida Current and flows eastward in between southern Florida and northern Cuba. Subsequently, the Florida Current transforms into the Gulf Stream and travels along the southeastern coast of the United States (Peterson et al. 2012). While the eastern flow of the Florida Current is a strong dispersal barrier to the north along northwestern Cuba, other factors influencing water flow and dispersal in the region are coastal cyclonic and anticyclonic eddies (Alvarez et al. 2009).

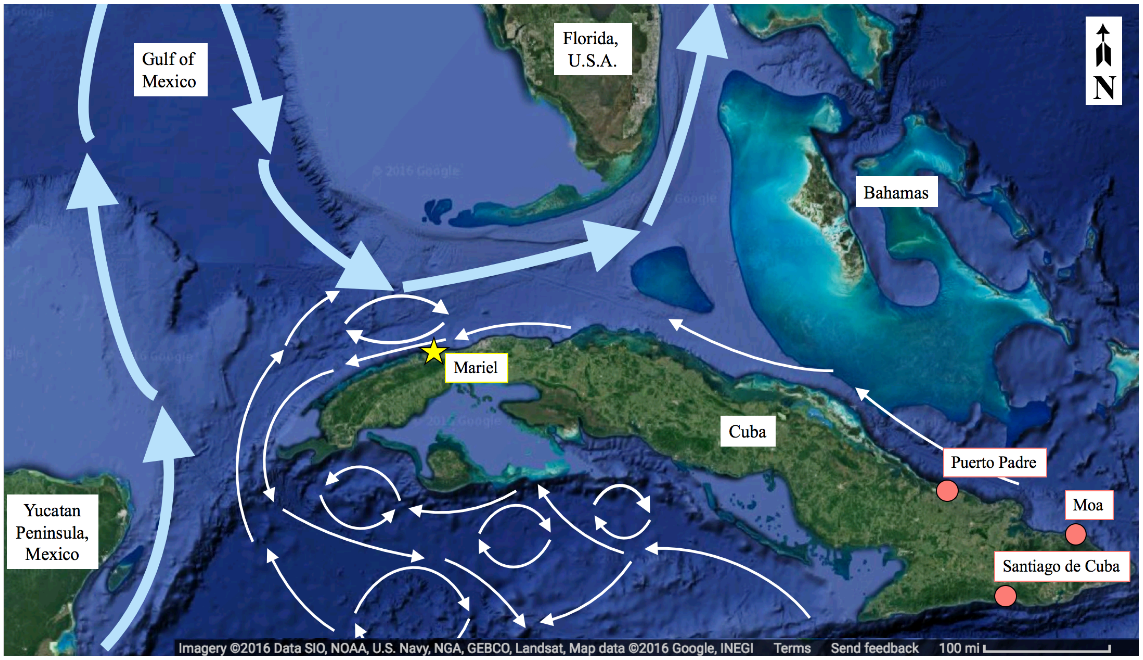


Figure 16. Potential natural and anthropogenic AIS dispersal from Port Mariel, Cuba. The yellow star represents the location of Port Mariel. Light blue and white lines represent the direction of major oceanic and coastal currents, respectively, that influence water flow along the northern coast of Cuba (Alvarez et al. 2009; Peterson et al. 2012). Pink dots illustrate Cuban ports that are stops after Port Mariel on active containership routes (CMA-CGM 2015; Melfi Marine Corporation S.A. 2016; Nirint Shipping 2016).

Modelling of native snapper (*Lutjanidae analis, L. cyanopterus, L. griseus, L. jocu,* and *L. synagris*) and non-native lionfish (*Pterois volitans/miles*) spawning in the northwest region of Cuba indicates larval dispersal to north-central (i.e., east of Port Mariel) and southwestern Cuba with little larval retention in the northwest (Paris et al. 2005; Johnston & Purkis 2015; Kough et al. 2016). If AIS introduced to Port Mariel are capable of expanding their range by natural dispersal in similar directions as those modeled for the mentioned species, a number of coastal ecosystems in Cuba could be at risk of AIS establishment, potentially threatening ecological and economic entities that are valuable to the country. The rich biodiversity within marine protected areas of Guanahacabibes National Park (a UNESCO Biosphere Reserve on the southern tip of the island) and the Archipelago Sabana-Camagüey (north-central Cuba) could be impacted by the introduction of AIS through competition of resources and habitat displacement, threatening the overall balance of the native ecosystems (UNDP 2008; National Oceanic and Atmospheric Administration (NOAA) 2015). Reductions in native species abundance due to AIS within these regions as well as the Gulf of Batabanó (southwest Cuba) could also damage the fishing industry, the country's fourth largest economic sector, which produced a $36.4 million (USD) crustacean export in 2014 (Claro et al. 2001; UNDP 2008; Muñoz-Nuñez 2009; Simoes et al. 2016).

Additional spread of AIS within Cuba may be enhanced via intra-national port connectivity. Although the commercial infrastructure (i.e., roads and rails) supporting Port Mariel is improving, shipping reportedly remains the most efficient method for transporting goods within Cuba (Chao 2016). For example, the Port of Santiago de Cuba, located on the southern end of the country, is the stop immediately after Port Mariel for both the CMA-CGM (2015) and Melfi Marine Corporation S.A. (2016) routes. Along the Nirint route, the ports of Moa and Puerto Padre, both on the eastern coast of Cuba, follow Port Mariel (Nirint Shipping 2016) (Figure 16). Similar to PAISD from regional Caribbean transshipment hubs, Port Mariel may facilitate "stepping stone" invasions within Cuba (Floerl et al. 2009; Keller et al. 2011). With a dependency on national maritime traffic and more frequent AIS exposure in Mariel Bay due to increased port activity, other regions in Cuba are at risk of AIS introductions due to anthropogenic dispersal.

4.5 Present AIS Prevention and Management Initiatives in Cuba

The purpose of the ECAIS and PAISC assessments was to emphasize the importance of implementing and maintaining proper protocols to help control established AIS populations in Cuba and to forewarn of future AIS introductions through international trade in Port Mariel. Since 1992, the Cuban government has prioritized biological conservation and as a result, the country is considered the "ecological crown jewel of the Caribbean." (Whittle & Santos 2006; Peterson et al. 2012). However, invasive species were identified in the 2007-2010 Cuban National Environmental Strategy as a serious threat (Ministry of Science, Technology, and Environment (CITMA) 2007; UNDP 2011). In collaboration with the Global Environmental Facility (GEF) and the United Nations Development Programme (UNDP), the Cuban Ministry of Science, Technology, and Environment (CITMA) led a five-year project (approved: 2009; implemented: 2011- June 2016) focused on enhancing invasive species (IS) control and prevention efforts within Cuba (UNDP 2011). The principal components of the project were to strengthen IS policy frameworks and improve stakeholder knowledge, IS data collection, institutional capacities, public awareness, and IS management effectiveness in the field. The $15 million

(USD) project was comprised of developing and implementing IS strategies (UNDP 2011). All Cuban agency branches were involved in the project in order to promote the coordination and delineation of IS duties. In addition, IS in select terrestrial and aquatic (i.e., wetland, coastal and marine) sites were examined to determine species of significant ecological and economic threat. The ECAIS Asian green mussel (*P. viridis*) identified here was considered an invasive priority. The invasion success of the green mussel in Cuba was attributed to the lack of policies regarding ballast water management and anti-biofouling measures, the non-compliance with global IS preventative standards, and the absence of environmental authorities charged with the responsibility of border inspections (UNDP 2011).

The Directorate of the Maritime Security and Inspection at the Cuban Ministry of Transport (MITRANS) was assigned the role of establishing new AIS legislation and quarantine systems, maintaining regulations regarding shipping pathways, and training 50 ballast water and biofouling control specialists (UNDP 2011). In response, MITRANS created the resolution, "Instrucción DSIM No. 05-2011", which outlined provisions for managing ballast water (e.g., discharge and treatments), monitoring trade partners as AIS donors (e.g., risk from cholera-contaminated regions), and establishing partnerships with other agencies (e.g., CITMA and entities of Public Health) in the event of an AIS introduction (Martinez Moreno 2011; Alexander Lopeztegui-Castillo, personal communication, September 27, 2016).

The timing of the CITMA project and the identification of necessary preventative measures intersect with the September 2016 ratification of the IMO's "International Convention for the Control and Management of Ships' Ballast Water and Sediments" (the "Convention"; IMO 2016b). Under this reform, all international, ocean-going ships are required to implement ship-specific ballast water management plans, maintain valid IMO certifications and record books, and, eventually, install IMO approved on-board ballast water treatment systems, with mid-ocean ballast exchange as an intermediate solution. The Convention was originally adopted in 2004 and with the 2016 ratification, vessels and port states have one year to prepare for the convention to come into effect (September 8, 2017) (IMO 2016b). This is the second IMO convention related to AIS shipping vectors; the "International Convention on the Control of Harmful Anti-fouling System on Ships", which bans the use of harmful organotins in anti-fouling paints, was entered into force in 2008 (IMO 2016c). To promote IMO compliance and regional cooperation with AIS prevention, IMO created the GloBallast Program, which provides training sessions, technical support, and assistance in contingency plan development (GEF 2016). Cuba has participated in this program with the Wider Caribbean Regional Coordination Organization, attending workshops on ballast water management and anti-fouling systems in the past decade (RAC-REMPEITIC Caribe 2014; GEF 2016). Communication and collaboration with regional states and organizations not only forms a support system for improving control measures but also has the potential to offer additional resources for combating AIS.

To further combat the Asian green mussel and other IS in Cuba, the country has improved monitoring programs by developing a national invasive database, an early warning and rapid response system (SATRR), and a strengthened format for invasive impact assessments (UNDP 2011). Implementation of SATRR has been credited with controlling green mussel populations in Mariel Bay and Santiago Bay (Alexander Lopeztegui-Castillo, personal communication, August 4, 2016). Identification and correction of the gaps in IS management has created the opportunity for Cuba to retain its title as the crown jewel. It is essential that the IS control programs implemented by the national project and international conventions are enforced and periodically reviewed in order to remain up-to-date on effective and efficient IS management.

4.6 Recommendations and Future Research

Information from this study provides insight into the established threat of AIS in Cuba, the national and international maritime connectivity of Port Mariel, and the potential impact of future invasions in the country due to increased ocean-going trade. As important, AIS risk in Cuba was identified as a topic that merits further research.

The method chosen for the study was a semi-qualitative analysis of AIS and trade partners, which were scored based on publically accessible information. For the PAISD, the data was limited to active, consistent containership routes and, therefore, exposure frequency of Port Mariel to AIS from specific regions (Kaluza et al. 2010). However, in order to better understand propagule pressure at Port Mariel from AIS introduced via ocean-going vessels, multiple additional shipping and environmental parameters should be examined. A model integrating the following variables can offer quantitative forecasts of AIS risk and aid Cuban officials in improving the regulation and monitoring of in-coming, AIS-contaminated vessels.

- All vessel arrivals (e.g., containerships, bulkers, tankers) into Port Mariel would provide a comprehensive understanding of Cuba's international trader partners as PAISD. Data could be collected by government officials in charge of the port (i.e., MITRANS) or from commercial databases, such as Lloyd's List Intelligence (www.lloydslistintelligence.com) (Kaluza et al. 2010; Keller et al. 2011).
- Networks of shipping routes would offer better insight into the global dynamics of AIS transfers and potential introductions of AIS in Cuba due to Port Mariel's international connectivity (Kaluza et al. 2010; Seebens et al. 2013; Xu et al. 2014)
- Ballast water discharge records in Port Mariel would help identify the origin and volume of AIS exposure in Port Mariel. For example, the US Coast Guard and the Smithsonian Environmental Research Center have published this information for the United States on the National Ballast Information Clearinghouse website (http://invasions.si.edu/nbic/) (Keller et al. 2011).
- Information on vessel transit speed and duration to Port Mariel would help quantify the survival and introduction potential of AIS (Davidson et al. 2016).
- Effectiveness of preventative efforts (e.g., ballast water exchange and treatment, anti-fouling paints) would help determine the survival of AIS transported to new regions by ocean-going vessels (Dickman & Zhang 1999; Floerl 2005; Tsolaki & Diamadopoulos 2010).
- Abiotic conditions (e.g., salinity, mean temperatures of warmest and coldest months) of ports would aid in defining AIS risk from specific donor ports. In previous research, these variables have been used to calculate the Euclidian distance between ports, determining environmental similarity. Data on oceanic salinities and temperatures can be attained from Lloyd's List Intelligence and the World Ocean Database (http://www.nodc.noaa.gov/ OC5/WOD/pr_wod.html) (Keller et al. 2011).
- Abiotic tolerances of AIS could be used to project the distribution of specific AIS that may be of greatest threat to new, non-native regions (Seebens et al. 2016).

- The influence of climate change on oceanic temperatures may factor into determining the future distribution of AIS in relation to the species' thermal tolerances (Seebens et al. 2016).

Biophysical modelling could also be useful to forecast natural and anthropogenic AIS dispersal from Port Mariel. AIS biological data, such as larval duration and spawning frequency, and physical data in the region, such as coastal and oceanic influences around Mariel Bay and maritime connectivity of the port, could be utilized to forecast AIS expansion not only in Cuba but also the wider Caribbean region (Floerl et al. 2008; Johnston & Purkis 2015; Seebens et al. 2016). Previous research of larval dispersal from northwestern Cuba demonstrated self-recruitment within Cuba as well connectivity to the Bahamas and some to Florida, USA (Paris et al. 2005; Johnston & Purkis 2015; Kough et al. 2016). Identifying possible national and regional AIS expansion from Port Mariel could assist in prioritizing AIS monitoring in Cuba and prompt collaborative efforts among countries that may be recipients of AIS that first establish in Cuba.

With an improving diplomatic relationship, Cuba and the United States have increased economic and ecological opportunities, including an agreement on the protection of shared marine resources between southern Florida and northern Cuba (NOAA 2015; Miller 2016a). This collaboration signifies the progress the countries have made in joint stewardship and communication. The success of the cooperative endeavor will be reliant on the countries' ability to share tools and knowledge (NOAA 2015). By understanding the risk of AIS exposure in the protected area that is shared by these two nations, the countries can work together to reduce the threat of AIS and preserve the region's valuable marine assets.

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Appendices

Appendix 1. Resource List for AIS Threat Assessments

Resources utilized for initial research of Established Cuban AIS and Potential AIS of concern.

Resources	URL
AlgaeBase	www.algaebase.org/
Bishop Museum and University of Hawaii- Guidebook of Introduced Marine Species of Hawaii	http://www2.bishopmuseum.org/HBS/invertguide/index.htm
Centre for Agriculture and Biosciences International (CABI)	www.cabi.org/
Census of Marine Life- Ocean Biogeographic Information System (OBIS)	www.iobis.org/
Delivering Alien Invasive Species Inventories for Europe (DAISIE)	www.europe-aliens.org/default.do
Encyclopedia of Life (EOL)	www.eol.org
European Network on Invasive Alien Species (NOBANIS)	www.nobanis.org/
Global Invasive Species Database (GISD)	www.iucngisd.org/gisd/
Kairo M, Ali B, Cheesman O, Haysom K, Murphy S. 2003. "Invasive species threats in the Caribbean Region". Report to The Nature Conservancy. 134 pp	www.issg.org/database/species/reference_files/Kairo et al, 2003.pdf Last updated April 25, 2012: www.ciasnet.org/wp-content/uploads/2012/11/IAS-in-the-Caribbean-Database-.pdf
Lopez V, Krauss U. 2006. "National and Regional Capacities and Experiences on Marine Invasive Species, Including Ballast Waters, Management Programmes in the Wider Caribbean Region - a Compilation of Current Information." Report to United Nations Environment Programme Report on Marine Invasive Species. 105 pp	www.icmyl.unam.mx/pdf/GRAMED/ Assessments_Delivery-Item1/New%20Assessments/ New_ Assessments _pdf_support%20information/ National%20and%20Regional%20Capacities %20and%20Experiences.pdf
Molnar JL, Gamboa RL, Revenga C, Spalding MD. 2008. "Assessing the global threat of invasive species to marine biodiversity" *Front Ecol Envrion* 6: 485-492.	https://www.conservationgateway.org/Conservation Practices/Marine/Pages/marineinvasives.aspx
National Exotic Marine and Estuarine Species Information System (NEMESIS) California Non-native Estuarine and Marine Organisms (Cal-NEMO)	invasions.si.edu/nemesis/ http://invasions.si.edu/nemesis/calnemo/intro.html
National Park Service (NPS)- Marine and Great Lakes Invasive Search	http://www.nature.nps.gov/water/marineinvasives/search.cfm
Nonindigenous Aquatic Species (NAS)	nas.er.usgs.gov/
O'Loughlin E, McCloud C, Sierp M, Westphalen G. 2006. "Temperature and Salinity Tolerances of Priority Marine Pests." Adelaide, Australia: South Australian Research and Development Institute (Aquatic Sciences).	www.pir.sa.gov.au/__data/assets/pdf_file/0009/231597/ TempandSalTolerances_ Final_Report.pdf
Smithsonian Natural History Museum Department of Botany-Identifying Harmful Marine Dinoflagellates (Faust & Gulledge 2002)	botany.si.edu http://botany.si.edu/references/dinoflag/
Smithsonian Marine Station at Fort Pierce- Indian River Lagoon Species Inventory	http://www.sms.si.edu/irlspec/Search.htm
World Register of Marine Species (WoRMS)	www.marinespecies.org/

Appendix 2: Threat Assessment Scores of Established Cuban AIS

Species (common name)	Ecological Impact	Economic Impact	Human-Health Impact	Geographic Extent	Invasive Potential	Management Difficulty
Perna viridis (Asian green mussel) [4] (Total Score: **21** out of 22)	**(4)** *In Cuba*: Colonization of mangrove roots, especially in semi-natural and enclosed ecosystems- habitat competition on roots with fouling native species and potential disruption of mangroves' role in erosion control [10] *Presumed*: Displacement of native species; Ecosystem engineers (i.e., creation of surface area niches for mobile and other fouling species) [4] *In other regions*: High oyster mortality in Tampa Bay, United States oyster reefs (most likely caused by excess *P. viridis* fouling) [2, 3, 4]	**(4)** Frequent fouler of artificial structures (i.e., buoys, piers & ship hulls) [4] *In Cuba:* Blockage of intake pipes and channels of thermoelectric plants at Cienfuegos Bay (densities as high as thousands per square meters) and Mariel Bay. [6] *In other regions* [4]: Fouling of industrial zones and aquaculture sites in Tampa, USA [3] and Japan [8] *Potential:* Outbreaks of Paralytic Shellfish Poisoning (PSP) could restrict harvesting activities [4, 13]	**(2)** Secondary vector of PSP via accumulation of red-tide toxins; Bio-accumulation of heavy metals in the shellfish could result in food poisoning for humans [13] *In other regions:* Two human fatalities and four illness linked to consumption of species collected from Daya Bay, China (1991) [14]; PSP detected in *P. viridis* in Trinidad (1994) but no human intoxications were reported [1]	**(4)** *Multiple ecoregions*: Caribbean, Gulf of Mexico, Eastern United States, Asia, S. Pacific [4] *In Cuba*: first at Cienfuegos Bay (2005) [6] then spread to Mariel Bay (2013) [9] and Santiago Bay (2015) [5]	**(4)** Recent (within the last 10 years) and rapid spreading in the Atlantic basin and within Cuba [4, 5, 9, 10] Sexual reproduction with larval duration ranging from 13 to 41 days [4, 11] Due to impact and spread in the United States, species may become marine equivalent of *Dreissena polymorpha* (Asian zebra mussel), a highly invasive freshwater species in Great Lakes [12] Associated shipping vectors: biofouling and ballast water [9, 10]	**(3)** *In Cuba:* Frequent maintenance at Carlos Manuel de Cespedes thermoelectric plant in Cienfuegos Bay to remove species from intake pipes [6, 7]; Reductions in the population at Cienfuegos Bay has been attributed to collection for scientific research and illegal harvesting (consumption or handicrafts) [10] Rapid response at Mariel and Santiago Bays has resulted in successful management of populations [5, 9] Should not be eaten if harvested from polluted bays or ports [13]

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Species (common name)	Ecological Impact	Economic Impact	Human-Health Impact	Geographic Extent	Invasive Potential	Management Difficulty
Gymnodinium catenatum (chain-forming dinoflagellate) [6] (Total Score: **21** out of 22)	**(4)** *In other regions:* Mass mortality at shrimp farms due to bloom of species in Mexico [1, 6]	**(4)** *General:* Impact on wild and aquaculture shellfish industries- toxic blooms could result in temporary shutdown of farm [6, 8]	**(2)** Production of saxitoxins & gonyautoxins that cause PSP [6, 5]; Linked to 3 PSP out-breaks in Mexico (32 deaths & 460 illnesses) due to contaminated shellfish [2, 6, 11]	**(4)** Coastal regions world-wide from tropical to temperate waters [3, 4, 6] *In Cuba*: Sabana – Gamaguey (1999) [9]; Cienfuegos Bay (2009) [10]	**(3)** Asexual & sexual reproduction; sexual cysts can remain dormant until ideal conditions [6, 11] Associated shipping vectors: ballast water [6]	**(4)** Eradication is not feasible for established populations, even at a local management level [6, 7]

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Species (common name)	Ecological Impact	Economic Impact	Human-Health Impact	Geographic Extent	Invasive Potential	Management Difficulty
Tubastraea coccinea (Orange-cup coral) [6] (Total Score: **18** out of 22)	**(4)** *Potential:* outcompete native sponges & corals, which could compromise ecosystem functions (i.e., habitat structure and food resources) [2, 6, 11, 12] *In other regions:* Death of native *Mussismilia hispida* coral in Brazil [2, 6]	**(2)** *General*: Mainly confined to artificial substrates (i.e., oil platforms, shipwrecks, and artificial reefs) [6] *In other regions:* Spreading to native *Perna perna* beds - could threaten mussel fisheries in Ilha Grande Bay, Brazil [6, 9]	**(0)** No known effect [6]	**(4)** Multiple ecoregions: South America, Gulf of Mexico, Caribbean, [6] *In Cuba*: First recorded on eastern coast in 1982 [6,13]	**(4)** Hermaphrodites or asexual (budding) [1, 6, 8]; Highly prolific, reproduces at small colony size; long-distance dispersal (larval duration: 18 days) [3, 4, 6] Rapidly spreading in tropical W. Atlantic [6] Associated shipping vectors: biofouling [7]	**(4)** Covering corals in plastic or raffia has been effective at controlling isolated introductions in Brazil [7, 9]; Physical removal of individual colonies in Gulf of Mexico [5]; Unknown management of widespread, established populations [10]

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The following four shipworms: ** Specific impacts are difficult to determine because species often co-occurs with other shipworms (Fofonoff et al. 2016)**

Species (common name)	Ecological Impact	Economic Impact	Human-Health Impact	Geographic Extent	Invasive Potential	Management Difficulty
Teredo bartschi (Bartsch shipworm) [4] (Total Score: **16** out of 22)	(3) *General*: Ecosystem engineers (i.e., riddling of wood for mobile species) [2]; Destruction of resources [1, 4]	(4) *General:* Rapid riddling of wood in tropical & sub-tropical waters [3, 4] *Other regions:* High abundance & damage to marinas in New Jersey, USA [4, 5, 8]	(0) No known effect [4, 6]	(4) Wide-spread distribution in tropical and sub-tropical regions [4] Cannot determine native range [3, 4], Cryptic to Cuba (since 1960) [4]	(3) Protandrous hermaphrodites but unable to self-fertilize [1, 4]; 3-day larval duration [9, 4] Associated shipping vectors: biofouling [4]	(2) Effective control via chemical treatment (e.g., creosote) of wood or use of an alternative material (e.g. metal or concrete) for partially submerged man-made structures [6, 7]

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Species (common name)	Ecological Impact	Economic Impact	Human-Health Impact	Geographic Extent	Invasive Potential	Management Difficulty
Teredo furcifera (Deep-cleft shipworm) [3] (Total Score: **16** out of 22)	(3) *General*: Ecosystem engineers (i.e., riddling of wood for small mobile species) [2, 3]; Destruction of resources [1, 3]	(4) *General*: Rapid riddling of wood in sub-tropical & tropical waters [3] *In other regions*: Damage to marinas in New Jersey, USA [3, 4, 9]; Damage to aquaculture wooden rafts in Gulf of Mannar [3, 6]	(0) No known effect [3, 5]	(4) Wide-spread global distribution Cannot determine native range [3] Cryptic to Cuba (first recorded in 1953) [3, 10]	(3) Protandrous hermaphrodites but unable to self-fertilize [1, 3] Females can retain 7,000 larvae in a brood; the planktonic larval duration is 3 days [3, 8] Associated shipping vectors: biofouling [3]	(2) Effective control via chemical treatment (e.g., creosote) of wood or use of an alternative material (e.g. metal or concrete) for partially submerged man-made structures [3, 7]

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Species (common name)	Ecological Impact	Economic Impact	Human-Health Impact	Geographic Extent	Invasive Potential	Management Difficulty
Teredo navalis (Naval shipworm) [10] (Total Score: **16** out of 22)	(3) *General*: Competition with natural species for resources; Ecosystem engineers [5, 10]	(4) *In the Caribbean*: Severe damage to ships & piers [13, 16] *In other regions:* Damage to wooden structures in the Netherlands (destruction of seawall) [8, 13], New York ($200 million for repairs) [9], Nova Scotia [14], North Carolina to central Florida [3, 22], San Francisco Bay [1, 4, 5], Japan (timber logs) [21]	(0) No known effect [10, 16]	(4) Most widespread marine wood-borer, with an extensive global distribution [10] Cannot determine native range [10] Cryptic to Cuba (first recorded in 2010) [17]	(3) Protandrous hermaphrodites but unable to self-fertilize [2, 10]; Each season, females produce 1-5 million eggs [10, 11]; 11-35 day larval duration [7, 10, 12, 20] Sudden invasions and population fluctuations in new regions [1, 5, 6, 10, 13, 15, 18]] Associated shipping vectors: biofouling [10]	(2) Effective control via chemical treatment (e.g., creosote) of wood or use of an alternative material (e.g. metal or concrete) for partially submerged man-made structures [16, 19]

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Species (common name)	Ecological Impact	Economic Impact	Human-Health Impact	Geographic Extent	Invasive Potential	Management Difficulty
Lyrodus pedicellatus (Blacktip shipworm) [4] (Total Score: **16** out of 22)	(3) *General*: Competition; Ecosystem engineers (riddling of wood for other sp.) [3, 4]; Destruction of resources [1, 4]	(4) *In other regions:* Destruction of timber logs stored in harbor in Japan [4, 10]; Damage to wooden rafts used in aquacultures in Gulf of Mannar [4, 6] & Bombay [4, 7]	(0) No known effect [4, 5]	(4) Cosmopolitan (dominant in warm – temperate, sub-tropical, & tropical waters) [4] Cryptic to Cuba (1st record: 1960) [4]	(3) Protandrous hermaph-rodites but unable to self-fertilize [1, 4]; 2-24 hour larval duration [9, 4] Associated shipping vectors: biofouling [4]	(2) Effective control via chemical treatment (e.g., creosote) of wood or use of an alternative material (e.g. metal or concrete) for partially submerged man-made structures [5, 8]

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Species (common name)	Ecological Impact	Economic Impact	Human-Health Impact	Geographic Extent	Invasive Potential	Management Difficulty
Charybdis hellerii (Indo-Pacific swimming crab) [5] (Total Score: **13** out of 22)	**(3)** *Presumed*: Competition (opportunistic feeders); Changes to natural benthic communities [2] *In other regions:* Displacement of native crustacean in Belize [4] *General:* Host of White Spot Syndrome Virus (WSSV) [7, 9]	**(3)** *Potential*: Outbreaks of WSSV in aquaculture facilities can cause lethal decimation of penaeid shrimp [7]	**(0)** No known effect [5, 8]	**(4)** *Multiple* ecoregions: Caribbean, Hawaii, E. United States, E. South America, Mediterranean [5] *In Cuba:* First recorded in both northwestern and southwestern Cuban coasts in 1987 [5]	**(3)** Sexual reproduction; Long larval duration of 44 days allows oceanic transit [2, 3]; Rapid growth and short generation (easy invasive establishment) [6] Recent colonization in the Western Atlantic [5] Associated shipping vectors: ballast water and biofouling (sea chests) [2, 6]	**(0)** Unknown management efforts [8]

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Species (common name)	Ecological Impact	Economic Impact	Human-Health Impact	Geographic Extent	Invasive Potential	Management Difficulty
Amphibalanus reticulatus (Reticulated barnacle) [2] (Total Score: **12** out of 22)	**(1)** *Presumed*: Competition [2] *In other regions:* Replacement of *A. amphitrite* in Hawaii [8] and Louisiana, USA [3]	**(2)** *General:* Frequently fouls artificial structures (i.e. ships), especially in warm subtropical- tropical locations [4, 7]	**(0)** No known effect [2, 6]	**(4)** *Multiple ecoregions:* Caribbean, Central America, E. United States, E. South America, California, Hawaii, NW Africa, Mediterranean [2] *In Cuba:* Recent introduction (2012) [2]	**(2)** Hermaphroditic but capable of cross-fertilization; fertilized eggs can brood for several months; larval duration is ~ 4-5 days [1] Associated shipping vectors: ballast water and biofouling [6]	**(3)** Can be controlled (but not eradicated) in desalination plants with chlorination and high temperatures [6]

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Species (common name)	Ecological Impact	Economic Impact	Human-Health Impact	Geographic Extent	Invasive Potential	Management Difficulty
Styela plicata (Pleated tunicate) [3] (Total Score: **12** out of 22)	**(2)** *Presumed*: Competition and displacement of native solitary tunicates [3] *In Australia*: Vector for invasive *Bugula neritina* [3, 8]	**(2)** *General*: Fouler of artificial structures (i.e., ships, piers, power plants) [3] *In other regions*: Interferes with cultured bivalves in Brazil, Spain, Japan, and Hong Kong [2, 3]	**(0)** No known effect [3, 6]	**(4)** *Multiple ecoregions*: Caribbean, Gulf of Mexico, Central & S. America, California, Africa, Mediterranean, Australia, New Zealand [3] *In Cuba*: First record in 2003 [3, 7]	**(2)** Hermaphroditic, self-or external fertilization [1, 3]; Planktonic larval dispersal at short distances; Fouling for long-distance [5] Associated shipping vectors: ballast water and biofouling [4, 5]	**(2)** Not considered a dominant management priority therefore presumed to not be difficult to remove [6]

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Species (common name)	Ecological Impact	Economic Impact	Human-Health Impact	Geographic Extent	Invasive Potential	Management Difficulty
Sphaeroma terebrans (Mangrove boring isopod) [2] (Total Score: **11** out of 22)	**(3)** Debate of effect on red mangrove prop roots: Weakens & destroys the plant [2, 7] *or* Promotes branching of roots, strengthening ability to withstand wave action [2, 6]	**(2)** *General*: Fouls wooden artificial structures (i.e., piers, ships, & pilings) [2, 4]	**(0)** No known effect [3]	**(4)** *Multiple ecoregions*: E. United States, Gulf of Mexico, Central America, Australia, Africa [2] *In Cuba*: 1994 but unknown active spreading [1, 2]	**(2)** Sexually reproduces; Requires assisted transportation for further dispersal [2, 3, 8] Associated shipping vectors: biofouling [2, 5]	**(0)** Unknown management efforts [3]

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Species (common name)	Ecological Impact	Economic Impact	Human-Health Impact	Geographic Extent	Invasive Potential	Management Difficulty
Styela canopus (Rough tunicate) [3] (Total Score: **10** out of 22)	**(0)** No known effect [3]	**(2)** *General*: Common fouling organism of artificial structures (i.e., ships & piers) [2, 3, 4, 6]	**(0)** No known effect [3]	**(4)** Widely distributed in temperate and tropical coastal waters [3] *In Cuba*: since 1914 but unknown active expansion [3, 6]	**(2)** Hermaphroditic, self- or external fertilization [1, 3] Requires assistance for range expansion [4] Associated shipping vectors: biofouling [3]	**(2)** Not considered a dominant management priority therefore presumed to not be difficult to remove [4]

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Species (common name)	Ecological Impact	Economic Impact	Human-Health Impact	Geographic Extent	Invasive Potential	Management Difficulty
Ascidia sydneiensis (Grey sea squirt) [5] (Total Score: **8** out of 22)	**(1)** *Presumed*: Competition (i.e., resources & space) however impact has not been studied [3, 4]	**(2)** *General*: Common fouler of artificial structures (e.g., ships, piers) [4] *In Brazil*: attached to oyster lantern nets but no serious economic issue [2, 4]	**(0)** No known effect [4, 5]	**(4)** *Multiple ecoregions:* Caribbean, Central & S. America, Mexico, E. Africa, Hawaii, Guam, Mediterranean [4] *In Cuba*: since 1912 but unknown spread [4, 6]	**(1)** Hermaphroditic, self-or external fertilization [1, 4]; Assistance needed for non-native expansion [5] Associated shipping vectors: biofouling [7]	**(0)** Unknown management efforts [4, 5]

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Species (common name)	Ecological Impact	Economic Impact	Human-Health Impact	Geographic Extent	Invasive Potential	Management Difficulty
Asparagopsis taxiformis (Red algae) [1] (Total Score: **8** out of 22)	**(2)** *Presumed*: Competition with native species [1] *In other regions*: Low abundance and no signs of abundance in Saronikos Gulf, Mediterranean [2]	**(0)** No known effect [4]	**(0)** No known effect [4]	**(4)** *Multiple ecoregions:* Caribbean, Mexico, Mediterranean, Hawaii, Florida, Asia, N. South America [1] *In Cuba:* Recent (2005) [3] but unknown spread	**(2)** *Movement*: stolon fragments or human-assisted routes [3] Associated shipping vectors: ballast water and biofouling [2]	**(0)** Unknown management efforts [4]

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Species (common name)	Ecological Impact	Economic Impact	Human-Health Impact	Geographic Extent	Invasive Potential	Management Difficulty
Sphaeroma walkeri (Marine pill bug) [4] (Total Score: **8** out of 22)	**(1)** Pre*sumed:* Competition but specific impact is unknown [2, 4]	**(2)** *General:* Fouling of man-made structures (i.e., ship hulls, mariculture cages) [2] *In other regions*: High densities in Florida, US [2, 5], southern California, US [2, 1], and Hong Kong (i.e., densities of 12,521 m^2) [2, 3]	**(0)** No known effect [2, 4]	**(4)** One of the most wide-spread isopod invaders: N.W. Pacific, Africa, Gulf of Mexico, Central & S. America, S. California, Mediterranean, Hawaii, Caribbean, Australia [2] *In Cuba:* 1994 [2, 6] but unknown spread	**(1)** Sexually reproduces and requires assisted transportation to spread further outside non-native distribution [2, 4] Associated shipping vectors: ballast water and biofouling [4]	**(0)** Unknown management efforts [4]

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Appendix 3. International Trade Partners as Potential AIS Donors of Port Mariel

Data and corresponding scores of present and projected international maritime trade partners of Port Mariel, Cuba utilized to rank partner ports by AIS donor potential. Ports are in route order prior to Port Mariel. Cuba is located in the Greater Antilles ecoregion (numbered as 65) [5]

ACTIVE CONTAINER ROUTES TO PORT MARIEL, CUBA [1 *]					
Port, Country	**Transit Time (days)**	**Voyage Duration Score [2, 3, 4]**	**Ecoregion[5]**	**Ecological Similarity Score [4]**	**Total Donor Score**
CMA CGM - Cuba Feeder [6]					
Kingston, Jamaica (JAM)	3	**3**	65	**3**	**6**
Crowley - Central America Northern Zone Loop 3 Saturday [7]					
Port Everglades, United States (USA)	1	**3**	70	**2**	**5**
Hamburg-Süd - Caribbean Feeder [8]					
Barranquilla, Colombia (COL)	10	**2**	67	**2**	**4**
Turbo, COL	7	**2**	67	**2**	**4**
Cartagena, COL	6	**2**	67	**2**	**4**
Limon, Costa Rica (CRI)	3	**3**	67	**2**	**5**
Maersk Line - CRX [9]					
Cork, Ireland (IRL)	22	**1**	27	**1**	**2**
Tilbury, Great Britain (GBR)	21	**1**	25	**1**	**2**
Rotterdam, the Netherlands (NLD)	19	**1**	25	**1**	**2**
Bremerhaven, Germany (DEU)	16	**1**	25	**1**	**2**
Melfi Marine Corporation S.A. - Mediterranean/Canada Service [10]					
Naples, Italy (ITA)	21	**1**	35	**1**	**2**
Leghorn, ITA	20	**1**	35	**1**	**2**
Genoa, ITA	19	**1**	35	**1**	**2**
Barcelona, Spain (ESP)	17	**1**	35	**1**	**2**
Valencia, ESP	16	**1**	35	**1**	**2**
Lisbon, Portugal (PRT)	13	**1**	27	**1**	**2**
Halifax, Canada (CAN)	5	**3**	39	**1**	**4**
Melfi Marine Corporation S.A. - Mexico/Caribbean [10]					
Progreso, Mexico (MEX)	8	**2**	69	**2**	**4**
Altamira, MEX	5	**3**	69	**2**	**5**
Veracruz, MEX	3	**3**	69	**2**	**5**
Kingston, JAM	10	**2**	65	**3**	**5**
Rio Haina, Dominican Republic (DOM)	7	**2**	65	**3**	**5**
Melfi Marine Corporation S.A. - Caribbean Feeder [10]					
Manzanillo, Panama (PAN)	7	**2**	67	**2**	**4**
Cartagena, COL	4	**3**	67	**2**	**5**
Mediterranean Shipping Company - WEC Cuba Feeder [11]					
Caucedo, DOM	9	**2**	65	**3**	**5**
Rio Haina, DOM	8	**2**	65	**3**	**5**
Mediterranean Shipping Company - WEC Havana Service [11]					
Cristobal, PAN	2	**3**	67	**2**	**5**
Nirint Shipping - Europe/Cuba/Canada [12]					
Bilbao, ESP	23	**1**	35	**1**	**2**
Rotterdam, NLD	20	**1**	25	**1**	**2**
Willemstad, Curacao (CUW)	6	**2**	66	**2**	**4**
Oranjestad, Aruba (ABW)	4	**3**	66	**2**	**5**

(*) Routes as of August 13, 2016

FUTURE TRADE PARTNERS OF PORT MARIEL, CUBA						
Port, Country	Nautical miles (nmi) from Port Mariel [13]	Calculated Transit Time (days) [14]	Voyage Duration Score [2, 3, 4]	Ecoregion [5]	Ecological Similarity Score [4]	Total Donor Score
Tampa, USA [15, 16]	304.85	1	**3**	70	**2**	**5**
Houston, USA [15, 17]	795.77	2	**3**	43	**1**	**4**
Mobile, USA [15, 18]	622.42	2	**3**	43	**1**	**4**
New Orleans, USA [15, 19]	681.47	2	**3**	43	**1**	**4**
Norfolk, USA [20]	988.69	3	**3**	41	**1**	**4**

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Appendix 4. Threat Assessment Scores of Potential AIS of Concern

Species (common name)	Ecological Impact	Economic Impact	Human-Health Impact	Geographic Extent	Invasive Potential	Management Difficulty
Alexandrium minutum (Red tide phytoplankton) [5] (Total Score: **22** out of 22)	(4) *General:* Production of toxins during red tide blooms can be harmful to zooplankton and fishes and cause reductions in copepod reproduction [5, 10]	(4) *General:* Production of dense, red tide blooms can result in mass mortality of native species and temporary closure of shellfish farms [5, 10]	(2) Produces gonyauto-toxins that cause PSP [14]; Species (e.g., zooplankton, shellfish, crabs) can become contaminated through bio-accumulation of *A. minutum*; Human consumption of these species can result in PSP (symptoms: muscle paralysis, neurological symptoms, death) [7, 16] PSP events: Taiwan [11], France [13], S. Australia [1, 8], New Zealand [3]	(4) Cosmopolitan: found in enriched coastal zones globally, from temperate to tropical regions [4,6]; Inhabits a "wide range of coastal hydrographic regimes" [5, 10] ***Detection in Cuba*:** Cienfuegos Bay (2009) – unknown status; only record for Cuba- no documentation in App. 1 [12]	(4) Asexual (binary fission) and sexual (production of resting cyst) reproduction [15]; Resting cysts can remain dormant for years until conditions are favorable in relation to light, temperature, and nutrient concentrations [6] AIS risk in harbors with no known populations [8] Associated shipping vectors: ballast water [5, 8, 10]	(4) Monitoring waters is time consuming (requires microscopic examination and taxonomic expertise in identifying species) [5] Once established, impossible to eradicate [2] Ballast water management via mid-ocean exchange and on-board treatment of ballast tanks are the recommended preventative measures [8]

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Species (common name)	Ecological Impact	Economic Impact	Human-Health Impact	Geographic Extent	Invasive Potential	Management Difficulty
Phyllorhiza punctata (White-spotted jellyfish) [2] (Total Score: **20** out of 22)	(4) *General*: Predation (e.g., pelagic fish eggs, bivalve larvae, copepods); Dense blooms can alter ecosystem dynamics; Mucus production can alter abiotic conditions (i.e., increasing toxin production of species & viscosity of water that can affect zooplankton) [2, 3, 5, 8]	(4) *In Gulf of Mexico*: 2000 Bloom- Predation of important species (e.g., fish, shrimp, crab) larvae; Clogging of shrimp nets & damage to fishing gear & boat intake systems; Temporary closure of fishing industry that resulted in an estimated $10 million loss [5, 10]	(1) *General*: Mild sting could affect recreational activities (e.g., unpleasant to swim & unsightly with dead species littered on beaches); Stings can be treated with vinegar [2, 5]	(4) *Multiple ecoregions:* Western Atlantic (Puerto Rico, Mexico, Jamaica), Brazil, S. California, Hawaii, & Mediterranean [2] *General*: Most areas of introduction have not had large AIS populations &, thus, little impact [6-9]	(3) Alternation of generations may facilitate transportation via shipping pathways: ballast water (planktonic planula, ephyrae, or medusa) & bio-fouling (benthic scyphistoma or strobila) [1] Reemergence of blooms in Gulf of Mexico, Puerto Rico, and Brazil (subsequent blooms have not cause significant impacts) [2, 3, 6, 8]	(4) Unknown- information was lacking on successful control measures at the time of this study [3] Species' ability to remain within small polyp stage for years – difficult to detect and remove from established regions [9]

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Species (common name)	Ecological Impact	Economic Impact	Human-Health Impact	Geographic Extent	Invasive Potential	Management Difficulty
Perna perna (Brown mussel)[4] (Total Score: **18** out of 22)	(4) *General* [4]: Ecosystem engineers (changes to habitat structure; creation of space for other organisms, such as polychaetes, algae, barnacles) [2]; Competition with native species (e.g., displacement of native pearl oyster in S. Brazil) [3, 10]	(2) *General*: Fouling of artificial structures (e.g., buoys, ships, piers) [4,7]	(2) Secondary-vector of PSP; Linked to PSP outbreaks in Manzanillo & Guayacan (northwest coast of Margarita Island), Venezuela between August & October 1991 [1]	(4) *Multiple ecoregions:* Gulf of Mexico (Texas), Mexico, Brazil, Argentina, Venezuela, India, Sri Lanka [4]	(3) Sexual reproduction; Maturity at <1 year [6]; Capable of spawning year-round in tropical regions [6]; Larvae can settle on a wide variety of surfaces (e.g., vegetation, rocks, wood) [5] Relative of ECAIS *Perna viridis* [9] Forecasted to expand along the Yucatan Peninsula and Gulf of Mexico coast [8] but has not significantly expanded in the region since 1995 [6] Associated shipping vectors: ballast water & biofouling [4, 7]	(3) More sensitive to abiotic conditions than *P. viridis*- potential control in cooling systems with chlorine treatments [9]

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Species (common name)	Ecological Impact	Economic Impact	Human-Health Impact	Geographic Extent	Invasive Potential	Management Difficulty
Vibrio cholerae (Cholera) [6] (Total Score: **18** out of 22)	(1) *General:* Naturally occurs in marine environments; Does not seem to affect aquatic species (other sp.: V. *damsela* causes lesions in fishes & *V. shiloi* causes coral bleaching) [11]	(4) *General:* Cholera outbreaks can cause temporary closure of oyster cultures [2] *In other regions:* Detection of O1 serogroup strain in Mobile Bay, USA closed harvesting from May to November 1999 [2]	(2) Serogroups O1 & O139 are the toxigenic strains of the species- cause acute diarrhea in humans due to consumption of contaminated food (e.g., shellfish) or water (e.g., poor sanitation) [3, 4, 12,13, 14]; if untreated can result in death within hours [14] In non-endemic areas (majority of population is not immune), species can cause infection rates as high as 10% & affect all age groups [8] In other regions: ballast water was the proposed vector in the 1991 Latin America epidemic (1.2 million illnesses & 12,000 deaths) [9, 10]	(4) *Multiple (marine) ecoregions*: Gulf of Mexico, Chesapeake Bay, Peru, Colombia, Brazil, Venezuela, Germany, Bangladesh, Argentina [5]	(4) Asexual reproduction (binary fission) [1, 6] Tolerates both marine and freshwater habitats; Can remain dormant in algae & zooplankton until favorable conditions (e.g., climatic events) [8, 14] Associated shipping vectors: ballast water [4, 12]	(3) Treatment regimens are available for infected humans & can rapidly cure ailments [3] To prevent introduction via shipping pathways, conventions on ballast water management (mid-ocean exchange & on-board ballast tank treatments) have been enacted [4, 7]

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Species (common name)	Ecological Impact	Economic Impact	Human-Health Impact	Geographic Extent	Invasive Potential	Management Difficulty
Hydroides elegans (Fouling serpulid worm) [fof] (Total Score: **16** out of 22)	(3) *General:* Competition, Displacement of native species; Ecosystem engineers [2, 4] *In other regions:* Inhabits oyster beds in Florida, USA & Japan- competing with adult oysters for food & oyster spat for settlement space [1, 2, 4, 9]	(3) *General:* Common fouling organism of artificial structures (e.g., ships, piers, aquaculture cages) [2, 9] *In other regions* [2]: Problems in Italy (e.g., industrial intake pipes) [11], Japan (e.g., oyster beds) [3]	(0) No known effect [2]	(4) Widely distributed in tropics & subtropics, dominating polluted harbor communities [2, 3, 4]	(2) Sexual reproduction with a 4-8 day larval duration [6] Tolerant of hydrogen sulfide, low oxygen, & dense phytoplankton concentrations in polluted harbors [2, 7, 8] Calcareous shell produces minimal drag on vessel, allowing species to remain attached despite flow of water of vessel hull [5, 10]; Species can settle on natural (e.g., shells, mangroves, corals) & artificial structures (e.g., ship hulls, pilings) [2]	(4) Relatively tolerant of AIS treatments applied to vessels (e.g., wood preserves & tributyltin anti-fouling compounds) [2, 7, 8]

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Appendix 5. Temperature and Salinity Tolerances (minimum and maximum) of AIS

	Tolerance Range	
Species	**Temperature (°C)**	**Salinity (PSU)**
Established Cuban AIS (ECAIS)		
Perna viridis	12 - 32.5 [1-4]	6 – 58 [2, 5]
Gymnodinium catenatum	12.5 – 35 [6-7]	34.7 - 36.17 [8-13]
Tubastraea coccinea	22.14 - 28.47 [14-38]	25 – 45 [13]
Teredo bartschi	10 – 35 [39]	7 – 45 [39]
Teredo furcifera	4.91 – 33 [40, 44-47]	6 – 40 [41-43]
Teredo navalis	0 – 30 [48-50]	5 – 45 [48, 50, 51]
Lyrodus pedicellatus	11 - 26.8 [52]	21.6 – 40 [52-57]
Charybdis hellerii	22.67 - 28.39 [58-60]	26 – 40 [61-70]
Amphibalanus reticulatus	27.1 - 27.1 [71, 72]	10 – 40 [73-75]
Styela plicata	8.53 - 30.2 [76-91]	17.5 – 40 [92, 93]
Sphaeroma terebrans	26.8 - 27.05 [94-105]	0 – 38 [106-109]
Styela canopus	2.27 – 30 [110-132]	23 – 43 [132]
Ascidia sydneiensis	14.42 - 28.1 [133-142]	24 – 40 [143, 144]
Asparagopsis taxiformis	23.16 - 28.54 [145-165]	34.44 - 35.45 [145-165]
Sphaeroma walkeri	12 – 30 [166, 167]	24 – 40 [167-169]
Potential AIS of Concern (PAISC)		
Alexandrium minutum	10 - 30 [170, 171]	4 - 37.5 [172]
Phyllorhiza punctata	15 - 33 [173, 176]	15 – 40 [174, 175]
Perna perna	7.5 – 30 [177]	15 – 55 [178, 179]
Vibrio cholerae	10 – 43 [180]	2.5 - 45 [180]
Hydroides elegans	13 – 30 [181, 183]	15 - 42 [181, 182]

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