Ports and pests: Assessing the threat of aquatic invasive species introduced by maritime shipping activity in Cuba

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**A R T I C L E   I N F O**

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Maritime trade  
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Aquatic invasive species

**A B S T R A C T**

Aquatic invasive species (AIS) are biological pollutants that cause detrimental ecological, economic, and human-health effects in their introduced communities. With increasing globalization through maritime trade, ports are vulnerable to AIS exposure via commercial vessels. The Cuban Port of Mariel is poised to become a competitive transshipment hub in the Caribbean and the intent of this study was to evaluate present and potential impacts AIS pose with the likely future increase in shipping activity. We utilized previous assessment frameworks and publicly accessible information to rank AIS by level of threat. Fifteen AIS were identified in Cuba and one, the Asian green mussel *Perna viridis* (Linnaeus, 1758), had repeated harmful economic impacts. Five species associated with trade partners of Port Mariel were considered potentially detrimental to Cuba if introduced through shipping routes. The results presented herein identify species of concern and emphasize the importance of prioritizing AIS prevention and management within Cuba.

1. Introduction

Following successful introduction and establishment in host ecosystems, aquatic invasive species (AIS) impose ecological, economic, and health-related damages that can be severe and intensify over time (U.S. Federal Executive Order Number 13112, 1999). In the absence of natural predators and competitors, AIS can overtake available resources, disrupt the local food web, alter habitat structures, and act as secondary vectors of parasites and disease (Pyšek and Richardson, 2010). The breadth of AIS effects, however, is not limited to biological impacts on native marine species as physical damages to property can also result in monetary loss. For example, fouling of artificial structures - including ships, piers, and industrial intake pipes - can be safety hazards that require maintenance (i.e., time and money for service) and even replacement of damaged structures (GISP, 2008). Competition or extensive population growth can initiate mass mortality of economically important species or damage to fishing gear and aquaculture facilities, potentially causing closures of industrial activities (Graham et al., 2003; GISD, 2016b). Additionally, direct influences on human-health can occur, ranging from cuts and stings to life threatening ailments, such as paralytic shellfish poisoning (PSP) and cholera (Pyšek and Richardson, 2010; Cohen et al., 2012).

Increasing globalization and the dominant use of ocean-going vessels for international trade has resulted in commercial shipping being the primary unintentional vector of AIS, responsible for over two-thirds of known introductions (Molnar et al., 2008; Hulme, 2009). The main mechanisms of AIS dispersal via ocean-going ships are ballast water exchanges and biofouling. In order to become an AIS via this route, a species must endure a succession of stages from transportation and release to subsequent establishment (Wonham et al., 2001). First, the organism must become attached to the exterior wetted surface area of a ship or be taken in with ballast water (Carlton, 1985; Hewitt et al., 2009). 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 (Hewitt et al., 2009). 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 (Wonham et al., 2001). Although survival times during transit vary, the greatest loss of species abundance in ballast tanks occurs within the first five days (Cordell et al., 2009). With faster vessels and decreasing transit times, the likelihood of species survival between origin and recipient sites is increasing (Dunstan and Bax, 2008).

As sites that receive maritime trade goods, ports are at high risk of AIS introduction (Carlton and 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 the exotic species (Bax et al., 2003; Westphal et al., 2007). AIS that do become established may reflect connectivity of global and regional shipping networks (Seebens...
et al., 2016). 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 and Carlton, 1998). Consistent with the intermediate disturbance hypothesis, anthropogenic input can alter aquatic environments that may not be hospitable for native species and niches therefore become available for AIS (Hobbs and Huenneke, 1992; Hulme, 2009).

1.1. Port Mariel

Situated 46 km west of the capital of Havana on the northern coast of Cuba, Mariel Bay is economically and ecologically valuable (Morrison et al., 2008; Ruiz et al., 2008). The 7.8 km² 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; Morrison et al., 2008; Ruiz et al., 2008). Input from the adjacent thermolectric plant and cement factory as well as urban and agricultural runoff have polluted the water (Ruiz et al., 2008; González Díaz, 2010). Additionally, construction at Port Mariel has further disturbed natural conditions within the bay (Núñez et al., 2005). Alterations and pollution of Mariel Bay have diminished its environmental quality, impacting the health of native species and providing opportunities for AIS establishment (Cohen and 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 and artisanal fishers in the community, with some catches being sold 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 fish poisoning (Morrison et al., 2008). The introduction of toxigenic AIS in Mariel Bay could negatively affect individuals who consume the AIS or a species contaminated by the AIS.

The opening of the billion-dollar container terminal at Port Mariel in 2014 not only signifies Cuba's continued pursuit of national growth and international connectivity but also an opportunity for more frequent exposure to AIS via ocean-going vessels (Miller, 2016a). At the Port, container operations have been expanded and a special economic development zone has been designated, attracting industrial interests (Miller, 2016a). From these efforts, container throughput at Port Mariel has risen from 160,000 twenty-foot equivalent units (TEU) in 2014 to 330,000 TEU in 2015 (Miller, 2016b). Further, 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 (Miller, 2016a). Accommodation of these vessels will likely result in greater volumes of goods and enhance Port Mariel's potential of becoming a regional transshipment hub for the Caribbean. 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) if the U.S. trade embargo is lifted (Miller, 2016a). While it is important to draw attention to the economic gain and projected success associated with improvements to Port Mariel, it is also necessary to consider relevant outcomes—specifically, the potential biological consequences of AIS known to be associated with increased international trade.

1.2. Assessment of AIS threat

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. Once established, an invasive population is nearly impossible to eradicate and costly to control (Molnar et al., 2008; McGeoch et al., 2016). For example, in the United States, the estimated cost of the invasive freshwater zebra mussel Dreissena polymorpha (Pallas, 1771) between 1989 and 2000 was $775 million to $1 billion dollars (Carlton, 2001). The most effective strategies for reducing these unwelcomed species are prevention and early, rapid response (McGeoch and Squires, 2015). In order to take efficient and effective actions against AIS, however, it is first necessary to understand the current ecological, economic, and health-related impacts of these species as well as the risks of the AIS introductory pathways.

A risk assessment is a tested method to measure the potential establishment and impacts of AIS. AIS assessments can be conducted through a variety of methods and are dependent upon the availability and quality of data to parameterize such analyses (Mazzotti and 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 spread and donor regions (Washington Invasive Species Council (WISC), n.d.; Keller et al., 2011; Seebens et al., 2016; Verna et al., 2016). A categorical risk scoring system (hereafter referred to as a ‘threat assessment’) provides a standardized format for examining AIS with different taxonomies, impacts, and distributions (Leung and Dudgeon, 2008; Molnar et al., 2008). 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; McGeoch et al., 2016). Threat assessment formats produce standardized results that can be applied to national and global-scale analyses and 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 and Dudgeon, 2008). Results of a threat assessment are relatively easy to comprehend, allowing government officials, scientists, and citizens alike to understand the invasive potential of introduced species and the feasibility of removing present AIS, as well as preventing future AIS introductions (WISC, n.d.; McGeoch and Squires, 2015).

1.3. Study motivation

The motivation for this study was to identify those aquatic species that may pose the greatest threat to Cuba, particularly in anticipation of increased maritime trade at Port Mariel, and to emphasize the importance of AIS prevention and mitigation within the country.

2. Methods

The study was partitioned into three components, consistent with the application of previously utilized threat assessment frameworks. First, we conducted a scored threat assessment of established, ship-related AIS in Cuba (hereafter referred to as ‘Established Cuban AIS’) and abbreviated as ‘ECAIS’) in order to facilitate future prioritization of management resources of those species already present. Second, existing and projected container shipping routes to Port Mariel were examined to determine international ports (hereafter referred to as ‘Potential AIS Donor(s)’ and abbreviated as ‘PAIDS’) that could possibly introduce harmful AIS into Cuba. Third, a select number of species recorded within the PAISD and surrounding regions (hereafter referred to as ‘Potential AIS of Concern’ and abbreviated as ‘PAISC’) were assessed by invasion potential and the severity of impact the species may impose on Cuba. In addition, we compared recorded salinity and temperature values of the PAISC and the ECAIS to evaluate inter-species similarities in distribution capabilities due to environmental tolerances.

To efficiently identify and evaluate the risk of AIS, we examined information from publically accessible databases, published reports, and primary literature. 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 analyses.

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The AIS scoring system utilized in this study was based on the assessment frameworks proposed by Molnar et al. (2008) and the Washington Invasive Species Council (WISC, 2009). Our threat assessment was divided into six categories: ecological impact, economic impact, human-health impact, geographic extent, invasive potential, and management difficulty (Appendix A). Following Molnar et al. (2008), we classified the ‘ecological impact’ of AIS based on the magnitude that the species disturbed the native biotic and abiotic environments. ‘Economic impact’ characterized the severity of an AIS impact on natural and artificial maritime systems and activities that could result in monetary loss. This category was a combination and modification of the impact categories (e.g., aquaculture industry, physical infrastructure, and recreational sector) proposed by the WISC (2009). As part of our economic impact category, we also took into consideration other artificial structures exposed to AIS that not were mentioned by the WISC (2009), including piers and industrial intake systems. ‘Human-health impact’ was the direct effect an AIS posed on the human population. This category was also derived from WISC (2009), but we adjusted the scale from three to four classifications in order to have uniform category scoring scales within our framework. ‘Geographic extent’ represented the global distribution of an AIS and, in accordance with the classifications published by Molnar et al. (2008), was scored based on the number of non-native ecoregions in which the species was present. 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). From categories in both Molnar et al. (2008) and WISC (2009) frameworks, we classified the ‘invasive potential’ of AIS based on the rate in which the
species has recently been or projected to be established in new, non-native regions as well as on their life history traits, such as dormancy in ballast water tanks during vessel transit and reproduction frequency. Finally, ‘management difficulty’ described the relative predicted amounts of time and other resources required to eradicate or control an AIS population once established (Molnar et al., 2008).

Identification and scoring of AIS in this study was based on information derived from publicly accessible databases, published reports, and primary literature (Appendix A). Combined, the assessment categories described the current or potential threat AIS may or do cause upon introduction. Every category had a scoring system of zero to four in increasing order of threat. The highest threat score attainable was 24. Consistent with the terminology used by the International Maritime Organization (IMO) (2017), and modifying the methodology of Molnar et al. (2008), we defined AIS that had ecological, economic, or human-health related impact scores of three or four as ‘harmful’.

2.2. Established Cuban AIS

Established Cuban AIS (ECAIS) were defined as non-native, aquatic species recorded in Cuba at the start of the study and associated with transportation via ocean going vessels (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 (Bickford et al., 2007; Hulme, 2009). When available data on AIS in Cuba was limited, ECAIS were assigned high threat scores based on documented concern of species presence within the country or their known impact or spread in other countries. In fact, under favorable conditions (e.g., nutrient inputs, disturbances, habitat changes), these species may have the potential to expand their populations beyond their initial sites of establishment and cause serious damage in Cuba if they are left unmanaged (Sakai et al., 2001; Moreira-González et al., 2014).

In addition to ranking ECAIS by cumulative threat score (Appendix B), the strength and direction of covariation between the ECAIS threat assessment categories were examined via inter-category correlations (Ricciardi and Cohen, 2007; Gotthardt and Walton, 2011) using the R statistical software package (R Development Core Team, 2011) 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).

2.3. International trade partners as Potential AIS Donors

To understand the global connectivity and examine the risk of AIS via shipping pathways to Port Mariel, present and future trade partners were identified and ranked as Potential AIS Donors (‘PAISD’) (Fig. 1). Present international trade partners were determined from known container vessel routes to 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 (Suarez 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 (Kaluzza 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. International ports of call that preceded 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 C).

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 (Gollasch and Leppäkoski, 2007; Cordell et al., 2009; Keller et al., 2011). The scoring systems for PAISD were derived from 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 (a score of 3), 6 to 10 days as medium risk (2), and > 10 days as low risk (1) (Verna et al., 2016). For future trade partners, we calculated voyage duration as (Miller, 2013):

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

The distances between future ports and Port Mariel were determined from inter-port distances published on the website SeaRates.com (SeaRates, 2016). Vessel speed was set as the average speed in knots of container ships on active routes to Port Mariel, calculated from vessel data collected by marinetraffic.com (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.

2.4. Potential AIS of Concern

Subsequent to the analysis of PAISD, Potential AIS of Concern (‘PAISC’) were identified and assessed based on the invasion risk and the severity of impact the PAISC may impose on Cuba if introduced to Port Mariel. PAISC were selected with the following criteria:

1. Recorded in or near PAISD ports (i.e., surrounding waters, connected waterways, or in coastal habitats < 10 km away) as established AIS, harmful species of unknown origins, or native species that are invaders in other regions
2. Documented ecological, economic, or human-health related damages in non-native or cryptogenic regions
3. Recorded association with introduction via shipping vectors (e.g., ballast water or biofouling)
4. Capable of withstanding the physical conditions of Mariel Bay (Ruiz et al., 2008):
   a. Salinity (range: 35.73–36.18 PSU; mean: 36.11 PSU)
   b. Temperature (range: 27.2–29.9 °C; mean: 28.3 °C)

The threat assessment framework utilized for ranking ECAIS was also used for scoring the PAISC (Appendix D). For PAISC assessments, we took into consideration the species’ impact and population growth within the PAISD regions. When PAISD information was limited, we scored the AIS based on their threat on a wider geographic scale. Recorded salinity and temperature tolerances (i.e., minimum, maximum, and range) of the PAISC and the ECAIS (Appendix E) were compared to determine if PAISC could withstand similar environmental conditions, indicating that the potential AIS are capable of becoming established in Cuba (Barry et al., 2008). An independent, two-sample t-test was initially chosen to compare salinity and temperature tolerances. Due to relatively large difference in sample sizes of ECAIS (n = 15) versus PAISC (n = 5), however, 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 Development Core Team (2015).

3. Results

3.1. Threat assessment of Established Cuban AIS

Fifteen species were identified as ECAIS (Fig. 2). *Perna viridis* and *Gymnodinium catenatum* (Graham, 1943) had the highest threat assessment scores of 23 out of a maximum total score of 24. These two species also had invasive histories associated with human illnesses. *Perna viridis*, *G. catenatum*, *Teredo bartschi* (Clapp, 1923), *T. furcifera* (von Martens, 1894), *T. navalis* (Linnaeus, 1758), *Lyrodus pedicellatus* (Quaile, 1849), and *Charybdis hellerii* (Milne-Edwards, 1867) had both ecological and economic impact scores greater than or equal to three, classifying them as ‘harmful’ in these categories. Every species analyzed had known non-native or cryptogenic distributions in multiple marine ecoregions and, therefore, they all received the highest impact score (four) for geographic extent. The four highest-ranking species (*P. viridis*, *G. catenatum*, *T. navalis*, and *Tubastraea coccinea* (Lesson, 1829)) and *C. hellerii* scored three or greater for invasive potential. Management difficulty scores were greatest for *P. viridis*, *G. catenatum*, *T. coccinea*, and *Amphibalanus reticulatus* (Utinomi, 1967).

The average impact scores (± SEM) for ECAIS were 2.47 ± 0.32 for ecological, 2.73 ± 0.32 for economic, and 0.80 ± 0.35 for human-health. All ECAIS received an impact score of 4 for geographic extent. The mean impact score for invasive potential was 2.20 ± 0.26 and 1.66 ± 0.35 for management difficulty. Geographic extent varied the least with a score of 0 while human-health impact and management difficulty had the greatest disparities, measuring 1.89 and 1.81 respectively.

3.1.1. Correlation of Established Cuban AIS threat categories

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 - economic impact and human-health impact (rho = 0.903; p = 4.053e-06) - showed a very strong, significant correlation (i.e., rho = 0.80–1.00; p < 0.01). Correlations using Spearman’s rank (Weir, 2014) between ecological impact and invasive potential (rho = 0.788; p = 0.0005) and ecological impact and human-health impact (rho = 0.65; p = 0.009) were significantly strong (rho = 0.60–0.79). Ecological impact and economic impact (rho = 0.614; p = 0.015), and management difficulty and invasive potential (rho = 0.608; p = 0.016), were also found to be strong but not significantly correlated. The remaining correlations were classified as moderate relationships (rho = 0.40–0.59), including human-health impact and management difficulty (rho = 0.548; p = 0.034), human-health impact and invasive potential (rho = 0.496; p = 0.06), ecological impact and management difficulty (rho = 0.483; p = 0.068), and economic impact and invasive potential (rho = 0.472; p = 0.07544). The weakest correlation was between economic impact and management difficulty (rho = 0.433; sig = 0.107). Geographic extent was excluded from the correlation analyses because the ECAIS scores were invariant for this category.

3.2. Potential AIS Donors to Port Mariel

The port of Kingston, Jamaica received the highest PAISD score of six out of a maximum total score of six (Fig. 3). 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 (FL), USA) received a PAISD score of five, while the remaining future trade partners – Houston (TX), Mobile (AL), New Orleans (LA), and Norfolk (VA), USA - were assigned scores of four.
3.3. Threat assessment of Potential AIS of Concern

Five species were identified as PAISC (Table 1). The dinoflagellate *Alexandrium minutum* (Halim, 1960) had the greatest overall threat assessment score of 24 out of a maximum total score of 24 (Fig. 4). Additionally, *A. minutum* scored the highest value for invasive potential. *Alexandrium minutum, Phyllorhiza punctata* (von Lendenfeld, 1884), and *Hydroides elegans* (Haswell, 1883) had harmful ecological and economic impact scores as well as the highest management difficulty scores. *Alexandrium minutum, P. punctata, Perna perna* (Linnaeus 1758), and

<table>
<thead>
<tr>
<th>PAISD Scores</th>
<th>Port, Country</th>
<th>PAISC</th>
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<tbody>
<tr>
<td>3 3</td>
<td>Kingston, Jamaica (JAM)</td>
<td><em>Alexandrium minutum</em> (Ranston et al., 2007), <em>Phyllorhiza punctata</em> (NMNH, 2012)</td>
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<td>3 2</td>
<td>Oranjestad, Aruba (ABW)</td>
<td><em>Hydroides elegans</em> (NMNH, 2014), <em>Vibrio cholerae</em> (Cañon Páez et al., 2005)</td>
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<td>3 2</td>
<td>Cartagena, Colombia (COL)</td>
<td><em>Hydroides elegans</em> (Bastida-Zavala and ten Hove, 2002), <em>Perna perna</em> (Hicks and Tunnell, 1995), <em>P. punctata</em> (Ocaña-Luna et al., 2010)</td>
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<td>3 2</td>
<td>Puerto Limon, Costa Rica (CRI)</td>
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<td>3 2</td>
<td>Altamira, Mexico (MEX)</td>
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<td>3 2</td>
<td>Veracruz, MEX</td>
<td><em>H. elegans</em> (Bastida-Zavala and ten Hove, 2002), <em>P. perna</em> (Hicks and Tunnell, 1995), <em>P. punctata</em> (Ocaña-Luna et al., 2010)</td>
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<tr>
<td>3 2</td>
<td>Port Everglades, United States of America (USA)</td>
<td><em>H. elegans</em> (Ruiz et al. unpublished data as cited in Fofonoff et al., 2016a)</td>
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<tr>
<td>3 2</td>
<td>Tampa, USA</td>
<td><em>P. perna</em> (Hicks and Tunnell, 1995)</td>
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<td>3 2</td>
<td>Caucedo, Dominican Republic (DOM)</td>
<td><em>V. cholerae</em> (Depaola et al., 1992)</td>
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<tr>
<td>3 2</td>
<td>Rio Haina, DOM</td>
<td><em>P. punctata</em> (USGS Nonindigenous Aquatic Species Program, 2006 as cited in Fofonoff et al., 2016b)</td>
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<td>3 1</td>
<td>Mobile, USA</td>
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<td>Houston, USA</td>
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<td>Port Everglades, United States of America (USA)</td>
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<td>Halifax, Canada (CAN)</td>
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<td>2 1</td>
<td>Norfolk, USA</td>
<td><em>V. cholerae</em> (Louis et al., 2003)</td>
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<td>2 2</td>
<td>Willemstad, Curacao (CUW)</td>
<td><em>H. elegans</em> (Bastida-Zavala and ten Hove, 2002)</td>
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<td>2 2</td>
<td>Barranquilla, COL</td>
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<td>Manzanillo, PAN</td>
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<td>Progreso, MEX</td>
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<td>Tilbury, Great Britain (GBR)</td>
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<td>Rotterdam, the Netherlands (NLD)</td>
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<td><em>V. cholerae</em> (Böer et al., 2013)</td>
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<td>Cork, Ireland (IRL)</td>
<td><em>A. minutum</em> (Touzet et al., 2007)</td>
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<td>1 1</td>
<td>Lisbon, Portugal (PRT)</td>
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<td>1 1</td>
<td>Naples, Italy (ITA)</td>
<td><em>H. elegans</em> (Guerriero et al., 2007)</td>
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<td>1 1</td>
<td>Leghorn, ITA</td>
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<td>1 1</td>
<td>Genoa, ITA</td>
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<td>1 1</td>
<td>Barcelona, Spain (ESP)</td>
<td><em>A. minutum</em> (Bravo et al., 2008), <em>H. elegans</em> (Zibrowius, 1973)</td>
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<tr>
<td>1 1</td>
<td>Valencia, ESP</td>
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<td>1 1</td>
<td>Bilbao, ESP</td>
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<tr>
<td>1 1</td>
<td>A. minutum (Butrón et al., 2011)</td>
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* 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.
Vibrio cholerae (Pacini, 1854) were associated with human-health impacts; all except P. punctata were considered harmful. All of the species had non-native distributions in multiple ecoregions and therefore scored four for geographic extent.

3.3.1. Temperature and salinity tolerances of AIS

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) (Fig. 5). 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).

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) (Fig. 6). Mean maximum salinity tolerances of ECAIS (41.71 ± 1.39 PSU) and PAISC (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. Discussion

4.1. Established Cuban AIS

Threat assessments revealed a range of ECAIS, their impacts, and management potential. Seven of the fifteen ECAIS received total threat assessment scores of 12 or below. Overall, these lower-ranking species did not pose a risk to human health and in their non-native ranges most have caused relatively minor ecological and economic impacts such as competition for resources with native species that did not result in local mass mortality (e.g., Asparagopsis taxiformis (Delile) Trevisan de Saint-León, 1845) and Styela plicata (Lesueur, 1823)) and fouling of artificial structures without causing closure of maritime activities (e.g., Ascidia sydneiensis (Stimpson, 1855), Sphaeroma walkeri (Stebbing, 1905), and Styela canopus (Savigny, 1816)) (da Rocha and Kremer, 2005; Katsanevakis et al., 2014; NIMPIS, 2017a; NIMPIS, 2017b). The exception was Sphaeroma terebrans (Bate, 1866), which was classified as ecologically harmful due to its documented boring of mangroves (i.e., species of wider ecosystem importance) (Molnar et al., 2008). Additionally, unknown active spread rates in Cuba, fewer invasive characteristics, and limited information on management difficulty contributed to their ranking.

In comparison, the remaining eight ECAIS ranked higher in their threat assessments due to combined harmful ecological and economic impact scores, association with human illnesses, and greater invasive potential (i.e., recent spreading, future projections, and advantageous life history traits). Further, the study results suggest 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 discharge in harbors that may contribute to toxic blooms of the naked dinoflagellate Gymnodinium catenatum, and
potentially eradicating isolated occurrences of orange-cup coral, *Tuaboras coccinea* (Guerrero-Moreno as cited in Lopeztegui-Castillo et al., 2014; Moreira-González et al., 2014; Mantelatto et al., 2015; FGBNMS, 2017).

The Asian green mussel received one of the two highest threat scores and was the only invader that was recorded as having repeated economic impacts in Cuba. Recognized by the Cuban government as one of the worst invasive species in the nation, the Asian green mussel has been detected in three separate bays: Cienfuegos, Mariel, and Santiago de Cuba (Fernández-Garcés and Rolán, 2005; UNDP, 2011; Lopeztegui Castillo et al., 2013; Fernández, 2015). In Cienfuegos Bay, high mussel densities have obstructed cooling channels of the local thermoelectric plant, causing temporary closures and associated monetary costs for their removal (Guerrero-Moreno as cited in Lopeztegui-Castillo et al., 2014). In addition to economic impacts, the species is an ecological and human health cause for concern due to its invasive history of fouling natural habitats and competing with native species as well as acting as a secondary vector for paralytic shellfish poisoning (PSP) (Rajagopal et al., 2006; UNDP, 2011). The population expansion and impact of the Asian green mussel in Cuba is an example of what can occur if an AIS is successfully introduced into the country.

### 4.1.1. Correlation of Established Cuban AIS threat categories

Inter-category variations in threat scores showed positive correlations, with the most significant and strongest relationships among five of the six categories: invasive potential, ecological impact, economic impact, human-health impact, and management difficulty. 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 naval shipworm, *Teredo navalis*) may take advantage of niches in non-native locations that lack the predation and competition present in indigenous regions (Grave, 1928; Figueira de Paula et al., 2014; NIMPIS, 2016a). Therefore, AIS may be capable of outcompeting native species and dominating local habitats and resources, causing ecological and economic impacts on the host environment (e.g., displacement of native coral species by orange-cup coral and destabilized wooden structures from boring naval shipworms) and concern of illness outbreaks in the local community (e.g., fishery closures due to naked dinoflagellate toxic blooms) (Creed, 2006; Fofono et al., 2016c; NIMPIS, 2016a). The greater the invasive potential and distribution spread, the harder it is for local managers to control the AIS population and their impacts. Strong, positive covariations between invasive potential, ecological, economic, and human-health impacts, and management difficulty categories demonstrate the accuracy of the scoring system that can be used in prioritizing ECAIS management in Cuba.

### 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. Due to ecoregional separation (Atlantic Ocean) and relatively long voyages (more than ten days to Port Mariel), European and Mediterranean ports (i.e., Great Britain, Ireland, the Netherlands, Germany, Portugal, Spain, and Italy) received the lowest scores (two) as PAISD. With the exception of Tampa (a score of five), the United States 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 due to their lower ecological similarity scores. International trade partners that ranked the highest as donor ports — i.e., Kingston, Jamaica (a score of six), Caucedo, Dominican Republic (five), and Cartagena, Colombia (five) — not only were found at regional risk of AIS expansion but also demonstrate Port Mariel’s direct connection to major trans-shipment ports of the Caribbean. Kingston, Cartagena, and Caucedo as well as Limon, Costa Rica (five) and Veracruz, Mexico (five) were ranked in the top 20 Latin ports by volume of container throughput in 2015 (ECLAC, 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). Due to relatively similar ecosystems, short transit durations, and high maritime activity, trans-shipment ports of the Caribbean, such as those highlighted above, could feasibly expose Port Mariel to PAISC. Monitoring invasions in these locations could forewarn Cuban officials of the potential spread of AIS to Cuba from these transshipment hubs, that, therefore, should be treated as AIS prevention 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 introduced to 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 cause a multitude of serious impacts to the ecosystem (e.g., *Hydroides elegans* competition with native species for resources and space (NIMPIS, 2016b)), economy (e.g., fouling of artificial structures by *Perna perna* and damages to fishing gear by *Phyllorhiza punctata* (Hicks and Tunnell, 1995; Graham et al., 2003)), and health of the local community (e.g., life-threatening outbreaks due to toxigenic strains of *Vibrio cholerae* and PSP-causing *Alexandrium minutum* (Cohen et al., 2012; GISD, 2016a)). The assessment ranking reflects the capabilities and concerns of these species as AIS. The threat of the PAISC underscores 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.

### 4.4. 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. Presently, AIS management initiatives in Cuba are improving.

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 and Rey Santos, 2006). Invasive species were identified in the 2007–2010 Cuban National Environmental Strategy as a serious threat (CITMA and EDF, 2009) and in collaboration with the Global Environmental Facility and the United Nations Development Programme, the Cuban Ministry of Science, Technology, and Environment led a five-year (2011–2016), $15 million (USD) project focused on enhancing invasive species control and prevention efforts within the country (UNDP, 2011). From this project, Cuba has improved monitoring programs by developing a national invasive database (Sistema de Información sobre Especies Exóticas Invasoras, SIMEEI) and strengthening the early warning and rapid response system (Sistema de Alerta Temprana y Respuesta Rápida, SATRR) and format for invasive impact assessments (UNDP, 2011; Ziller and Arellano Acosta, 2016). To control AIS, the Directorate of the Maritime Security and Inspection at the Cuban Ministry of Transport was assigned the role of establishing new quarantine systems and legislation (“Instrucción DSIM No. 05-2011”), maintaining regulations regarding shipping pathways, and training 50 ballast water and biofouling control specialists (UNDP, 2011; Alexander Lopeztegui-Castillo, personal communication, August 2016). The implementation of these programs has already made a difference: the impact of the Asian green
mussel has been minimized in the invaded bays and SATRB has been utilized in controlling the species’ expansion within the country (Fernández, 2015; Lopeztegui Castillo et al., 2015; Alexander Lopeztegui-Castillo, personal communication, August 2016; Ziller and Arellano Acosta, 2016).

Regarding additional AIS control measures, the timing of the CITMA project intersects with the September 2016 ratification of the International Maritime Organization’s “International Convention for the Control and Management of Ships’ Ballast Water and Sediments” (the “Convention”; IMO, 2017). 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 (IMO, 2017). 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 (UNEP, 2010). 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, 2014). 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 AIS. It is essential that the AIS 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 AIS management.

4.5. Recommendations for 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. Multiple additional shipping and environmental parameters should be examined to better understand the propagate pressure from AIS introduced via ocean-going vessels at Port Mariel. A model integrating all vessel arrivals, ballast discharge records, docking periods, and abiotic conditions, for example, could offer quantitative forecasts of AIS risk and aid to Cuban officials in improving the regulation and monitoring of in-coming, AIS-contaminated vessels (Davidson et al., 2009; Keller et al., 2011; Seebens et al., 2016; Verna 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 ocean condition data in the region, such as coastal and oceanic influences around Mariel Bay, could be utilized to forecast AIS expansion not only in Cuba but also in the Caribbean region (Johnston and Purkis, 2015). Previous research of larval dispersal from northwestern Cuba demonstrated self-recruitment within Cuba as well connectivity to the Bahamas and to Florida, USA (Johnston and Purkis, 2015; Kough et al., 2016). For the studied AIS, better biological parameter estimations from field observations would improve the modeled projections as well as further refine our conceptual assessment framework. Identifying possible national and regional AIS expansion from Port Mariel could assist in prioritizing AIS monitoring in Cuba and prompt collaborative efforts among countries whose PAISC are already established in Cuba. With the help of a recent memorandum of understanding to protect shared marine resources and the improvement of diplomatic relationships, both the United States and Cuba would benefit from this information to reduce the threat of AIS and jointly preserve the valuable marine assets (NOAA, 2015).

5. Conclusion

Information from this study provides insight into the established and possible 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. This information can be utilized by: (1) the Cuban government to allocate resources for preventing AIS; (2) Cuban citizens for educational purposes and to encourage community participation to help control AIS population densities and dispersal; (3) other countries as a threat assessment template for AIS analyses; and (4) scientists for further research. Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.marpolbul.2017.07.071.

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