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Research Article

A baseline survey to document the distribution and abundance of native and non-native barnacle species in Port Canaveral, Florida

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Abstract

Global shipping is a common vector of non-native species (NNS), especially biofouling organisms. Ports with heavy ship traffic can contain a higher proportion of NNS compared to surrounding coastal habitats. Port Canaveral, Florida, is a busy and growing port, but little is known about the native and non-native species present. A baseline survey was designed to identify barnacle species, conspicuous members of the biofouling communities growing on structures in the port, and determine their origin status (i.e., native or NNS). In addition to species identity, the relative abundance of each species and NNS at five different sites during two time periods was assessed. Eight species of barnacles were identified, three of which are non-native in Florida. Barnacle communities differed by site and time period, with a significant interaction between the two factors. During both time periods, NNS comprised more than 90% of the barnacles at four of the sites. While this survey provides a baseline for barnacles present at Port Canaveral, Florida, expanding the surveys to include other organisms will give a more complete view of the number of NNS present at the port, supporting managers' future assessment of community changes.

Key words: Barnacles, spatial and temporal changes, Cirripedia, biofouling survey, NNS prevalence

Introduction

There has been a long history of ship-mediated invasions worldwide (Davidson et al. 2018; Ojaveer et al. 2018), with ships accounting for more than half of the initial introductions in some regions (Davidson et al. 2018). Busy commercial harbors are often hotspots for non-native species (NNS) (Ferrario et al. 2017). NNS introduced by ships can then be spread further into local waters as biofouling on recreational vessels (Megina et al. 2016; Ferrario et al. 2017; Ojaveer et al. 2018). Once established, NNS can have significant economic and ecological impacts (Katsanevakis et al. 2014; Ojaveer et al. 2018). For example, NNS are reported as reducing aquaculture production by fouling equipment such as nets and cages (Katsanevakis et al. 2014), decreasing the abundance of native seaweeds through epibiotic growth (O'Brien et al. 2013), and transmitting diseases that can increase mortality of native species (Katsanevakis et al. 2014).

Surveys of ports and marinas have been a key tool in generating species lists of both native and NNS on local, regional and country-wide scales (Cohen et al. 2005; Arenas et al. 2006; Schwindt et al. 2014; Bishop et al. 2015; Nall et al. 2015; Azevedo et al. 2020). A species list establishes a baseline for future assessments to determine if any changes have occurred over a specified time frame (Cohen et al. 2005; Arenas et al. 2006) or to aid in the development of monitoring programs (Schwindt et al. 2014). By repeatedly conducting surveys in a similar manner at the same location, surveyors can detect increases in NNS (Bishop et al. 2015), as well as record patterns of distribution or abundance (Lawson et al. 2004; Bishop et al. 2015). Monitoring provides an essential knowledge base for guiding management practices and for the early detection of new introductions (Olenin et al. 2010). For example, in the Shetland Islands, an initial area-wide NNS survey and literature review of NNS surveys in the islands and the UK were conducted to help develop a biosecurity plan (Collin and Shucksmith 2022).

Port Canaveral is a multifunctional port on the east coast of Florida. It is the second busiest embarkation cruise port in the world (Kodzi and Saeed 2021) and is a growing international cargo hub with trade partners from all over the world (Port Canaveral 2019). Port Canaveral also regularly receives traffic from US Coast Guard and Navy vessels, charter fishing vessels, recreational boaters and even spacecraft.

In Port Canaveral, only two studies have generated a species list which included the relative abundance of biofouling organisms (Sweat 2016; Johnson and Roberts 2017). While these studies identified several cryptogenic or NNS to Florida, they focused on one location and/or one time point. Performing surveys at regular intervals will be a useful tool to keep track of the diversity and abundance of NNS being introduced to Port Canaveral, which could be one indicator of how increased ship traffic is impacting the port. Therefore, a baseline survey was designed to identify and quantify the relative abundance of barnacles, a prominent taxon of the biofouling community on ship hulls (Ashton et al. 2016). Because species abundance can change seasonally (Weiss 1948) and spatially, even between sites less than 5 km away (Lawson et al. 2004), this study included multiple sites within the port conducted at two sampling periods. The feasibility of conducting surveys within the port was tested by focusing on a single taxon, especially one that is more noticeable and more easily identified compared to other members of the biofouling community. In addition to compiling a list of native and non-native barnacle species, the goals of the survey were to determine if community structure differed temporally and spatially and to assess the prevalence of NNS within the port.

Materials and methods

Five publicly accessible locations distributed throughout Port Canaveral, Florida were selected as survey sites: Jetty Park, Freddie Patrick Park Boat

Table 1. Description of the surfaces that biofouling communities were sampled from at each survey site. Survey sites are listed based on distances from the port entrance starting with the furthest site.

Survey Site	Latitude	Longitude	Distance from Inlet (km)	Sampling Surface Description
Canaveral Lock	28°24′33.5″N	80°38′5.8″W	4.7	Wooden dolphins wrapped in high density polyethylene; nearly vertical
Rodney S. Ketcham Boat Ramp	28°24′31.4″N	80°37′52.3″W	4.3	Formed concrete wall; vertical
Cape Marina	28°24′31.0″N	80°37′38.7″W	4	Formed concrete pilings; vertical
Freddie Patrick Park Boat Ramp	28°24′30.4″N	80°35′40.8″W	0.7	Formed concrete walls; vertical
Jetty Park	28°24′30.4″N	80°35′31.0″W	0.4	Granite rocks; orientation varied from horizontal to vertical

Ramp, Cape Marina, Rodney S. Ketcham Boat Ramp and the port side of Canaveral Lock. Different surfaces were sampled based on location (Table 1), including formed concrete, granite and wood dolphins (a mooring point consisting of several piles connected above the water) wrapped in high density polyethylene. All surfaces are part of human-built structures and had a well-developed biofouling community. While different surfaces can lead to different biofouling communities, longer periods of immersion tend to decrease this effect (Anderson and Underwood 1994).

Two surveys were conducted, one during warmer months (August–October 2019) and one during cooler months (February–March 2020) with mean (± standard deviation) water temperatures of 28.1 ± 0.3 °C and 20.0 ± 0.5 °C, respectively. Herein these time points will be referred to as the "warm" and "cool" sampling periods. At each survey site, the intertidal biofouling community was randomly sampled using a 25 × 25 cm quadrat (n = 5 per time period) at spring low tide. The location of the five quadrats at each site were noted during the warm sampling period to ensure that they were not sampled again during the cool sampling period. The entire biofouling community within the quadrat was photographed and then removed using a metal scraper. Each sample was bagged separately, labeled and frozen for later processing. It was not possible to remove the entire community without fracturing some barnacles, therefore only whole individuals were identified. Barnacle identification was conducted using a stereomicroscope at up to 80X magnification. Online and printed guides (Henry and McLaughlin 1975; Southward 1975, 1976; Zullo 1979; Gittings et al. 1986; Riley 2002; Gomez-Daglio and González 2006; Skinner et al. 2007; Lozano-Cortés and Londoño-Cruz 2013; Fofonoff et al. 2021) were utilized to identify individuals to the lowest taxonomic level possible and to classify each species as native, cryptogenic or non-native for Port Canaveral.

Data Analysis

There were a number of individuals that could not be identified due to small size, poor quality or lack of identifying features and were not included in analyses. The number of individuals per m^2 were estimated for each species based on the counts of identified barnacles for each sample. A two-way permutational multivariate analysis of variance (PERMANOVA) was used to see if there were differences in community composition by site

Table 2. Two-way PERMANOVA results to assess the effect of survey site (five levels), sampling period (two levels) and the interaction between the two factors. Analysis was based on Bray-Curtis dissimilarity of square root transformed barnacle abundance data. The p values were obtained using 9999 permutations.

Source	df	SS	MS	F	p
Survey site	4	5.21	1.30	19.13	< 0.001
Time	1	0.30	0.30	4.45	0.004
Site × Time	4	0.85	0.21	3.13	< 0.001
Residual	30	2.04	0.068		

and sampling period for each taxon followed by a pairwise PERMANOVA when appropriate. The PERMANOVAs were based on Bray-Curtis dissimilarity matrices created from square root transformed data to reduce the influence of highly abundant species. Nonmetric multidimensional scaling (nMDS) plots were used to visualize any differences in community composition and similarity percentages (SIMPER) were used to determine which species contributed the most to differences in community composition.

The relative proportion of NNS (percent of identified individuals) were determined for each sample. A two-way ANOVA was used to determine if the mean proportion of NNS differed by site and sampling period followed by a Tukey's HSD test for pairwise comparisons. The data sufficiently met the assumptions of normality and equal variances. All statistical analyses were conducted in R (R Core Team 2020) using the RStudio environment (RStudio Team 2020). The multivariate analyses were conducted with the vegan package (Oksanen et al. 2020).

Results

Eight different barnacle species were identified at the survey sites (*Amphibalanus amphitrite* (Dariwn, 1854), *A. eburneus* (Gould, 1841), *A. improvisus* (Darwin, 1854), *A. reticulatus* (Utinomi, 1967), *Balanus trigonus* (Darwin, 1854), *Chthamalus fragilis* (Darwin, 1854), *C. stellatus* (Poli, 1791) and *Tetraclita stalactifera* (Lamarck, 1818). Three of the species (*A. amphitrite, A. reticulatus* and *B. trigonus*) are non-native (Fofonoff et al. 2021), while the remaining five are native (Skinner et al. 2007; Fofonoff et al. 2021) to Florida. The barnacle community structure varied significantly with survey site and sampling period. There was also a significant interaction between survey site and sampling period (Table 2). Visually, the survey sites group similarly on nMDS plots when separated by sampling period (Figure 1), with Jetty Park separate from the remaining survey sites. Therefore, the significant interaction between survey site and sampling period indicates that differences in community composition due to sampling period are not consistent among the survey sites. Comparison between sampling periods, run separately for each survey site, indicated that barnacle community composition was significantly different at the Cape Marina ($F_{1,9} = 23.8$, $p = 0.01$) and the Canaveral Lock ($F_{1,9} = 18.5$, $p = 0.02$) survey sites, while there was no community composition difference between sampling periods for the other three survey sites ($p > 0.05$).

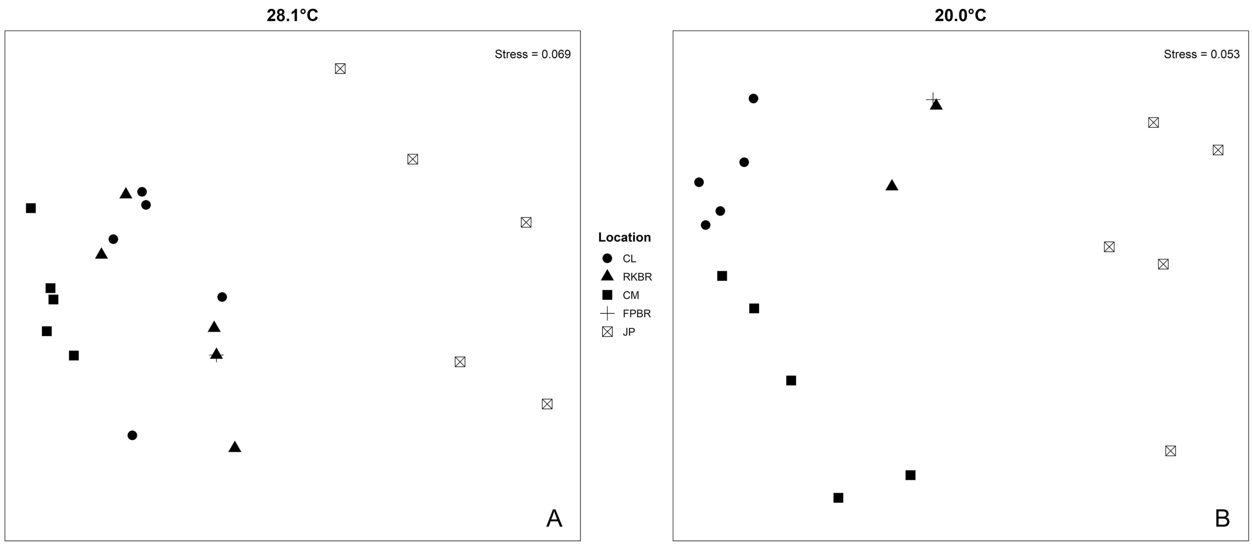

Figure 1. Nonmetric multidimensional scaling plots showing differences in barnacle community composition among survey sites during A) the warm sampling period (August–October 2019) and B) the cool sampling period (February–March 2020) labelled by the mean temperature during the survey. The plots were constructed using the Bray-Curtis index based on square root transformed data of barnacle species abundance. CL: Canaveral Lock; CM: Cape Marina; FPBR: Freddie Patrick Park Boat Ramp; JP: Jetty Park; RKBR: Rodney S. Ketcham Boat Ramp.

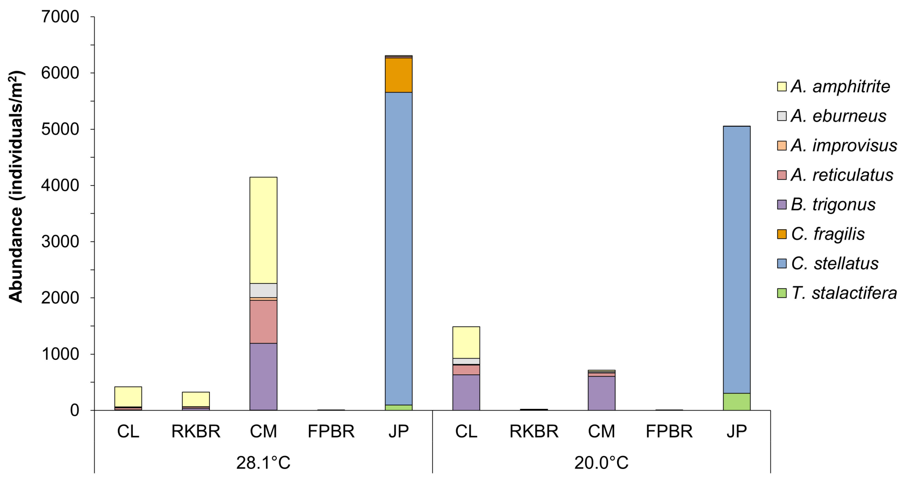

Figure 2. Mean abundance (individuals/m^2) of the eight barnacle species found at each survey site for the warm (August–October 2019) and cool (February–March 2020) sampling period labelled by the mean temperature during the survey. Survey sites are arranged furthest to closest (left to right) to the port entrance. CL: Canaveral Lock; RKBR: Rodney S. Ketcham Boat Ramp; CM: Cape Marina; FPBR: Freddie Patrick Boat Ramp; JP: Jetty Park.

The overall barnacle abundance was much higher during the warm sampling period compared to the cool sampling period at Cape Marina, while barnacle abundance was higher during the cooler sampling period at Canaveral Lock (Figure 2). At Cape Marina, *A. amphitrite* contributed the most (44.5%) to differences in community composition, while at Canaveral Lock, *B. trigonus* contributed the most (49.9%) to differences. During the warm sampling period, *A. amphitrite* was the dominant species at both Canaveral Lock and Cape Marina. At Cape Marina, the mean abundance (± standard deviation) of *A. amphitrite* decreased from 1891 ± 747 to 26 ± 42 leading to *B. trigonus* being the dominant species during the cool sampling

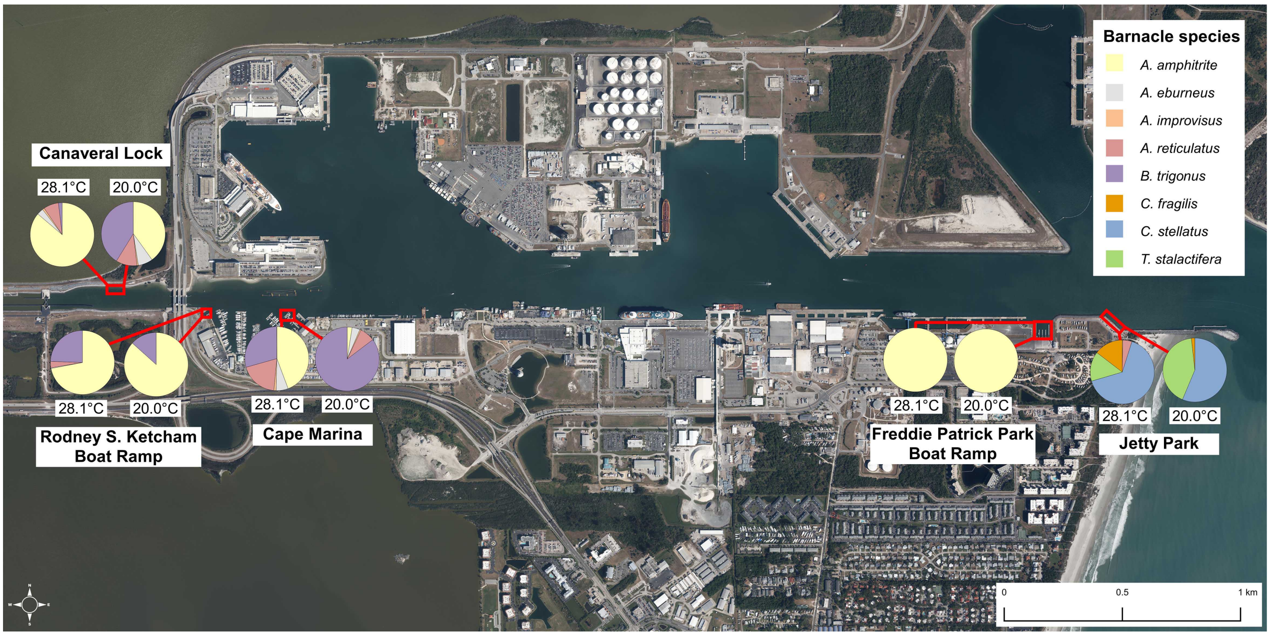

Figure 3. Relative abundance (percentage) of each barnacle species at the five survey sites for the warm (August–October 2019) and cool (February–March 2020) sampling period labelled by the mean temperature during the survey.

period. At Canaveral Lock, *B. trigonus* increased between the warm and cool sampling period (10 ± 14 vs. 634 ± 310) giving it a similar proportion of the identified barnacles as *A. amphitrite* (Figure 3).

The mean proportion of NNS was significantly different by survey site ($F_{4,30}$ = 721.6, p < 0.001), but not by sampling period ($F_{1,30}$ = 0.9, p = 0.3). Post hoc analysis indicated that Jetty Park was significantly different from all other survey sites (p < 0.001 for all comparisons). Although there were relatively few, all barnacles identified at Freddie Patrick Park Boat Ramp during both sampling periods and Rodney S. Ketcham Boat Ramp during the cool sampling period were NNS. NNS comprised more that 90% of the identified barnacles at all survey sites during both sampling periods, except Jetty Park. Only 4.9 ± 9.7% of the barnacles at Jetty Park were non-native during the warm sampling period, and all barnacles were native during the cool sampling period at Jetty Park (Figure 4).

Discussion

Three of the barnacles (*Amphibalanus amphitrite*, *Chthamalus stellatus* and *Tetraclita stalactifera*) identified during this study were previously recorded for Port Canaveral (Sweat 2016; Johnson and Roberts 2017). The remaining five species have a known distribution in Florida (Karlson and Osman 2012) with some having a recorded distribution in the nearby Indian River Lagoon (Mook 1976, 1983). Although not found during this study, the non-native *Megabalanus coccopoma* (Darwin, 1854) has been noted before in Port Canaveral (Johnson and Roberts 2017) but has not been observed in recent years (KZH, personal observation). The native range of *A. amphitrite* is difficult to determine (Henry and McLaughlin 1975),

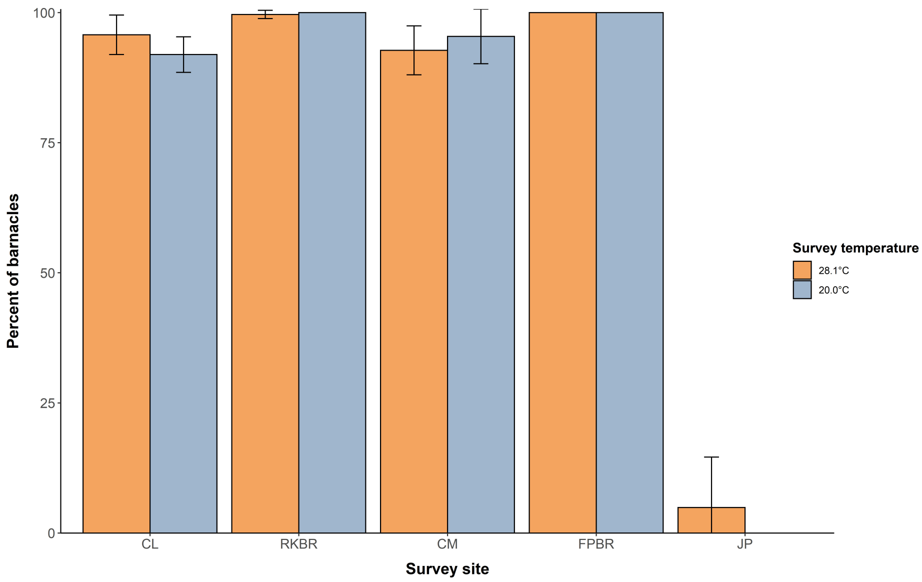

Figure 4. Mean (± standard deviation) percentage of barnacles identified as NNS (*Amphibalanus amphitrite*, *Amphibalanus reticulatus* or *Balanus trigonus*) for the five survey sites during the warm (orange; August–October 2019) and cool (blue; February–March 2020) sampling period labelled by the mean temperature during the survey. Survey sites are arranged furthest to closest (left to right) to the port entrance. CL: Canaveral Lock; RKBR: Rodney S. Ketcham Boat Ramp; CM: Cape Marina; FPBR: Freddie Patrick Boat Ramp; JP: Jetty Park.

but it is likely native from southeastern Africa to southern China in the west Pacific and Indian Oceans (Fofonoff et al. 2021). *Amphibalanus reticulatus* is native to the Indo-Pacific, while *Balanus trigonus* has a wide native distribution in the Pacific and Indian Oceans (Fofonoff et al. 2021). All three barnacle species were introduced to the east coast of Florida by ship hulls and were first noted in abundance in Biscayne Bay, Florida (Fofonoff et al. 2021). *Amphibalanus amphitrite* had established, although not dominant, populations in Biscayne Bay in the 1940s (Weiss 1948). *Balanus trigonus* was noted in south Florida by 1961 (Moore and McPhearson 1963), while *A. reticulatus* became established by 1969 (Moore et al. 1974). All three barnacles are now common throughout Florida (Fofonoff et al. 2021).

The barnacle community composition at Jetty Park differed the most from the rest of the survey sites. The community was mostly composed of both chthamalid barnacles, and *T. stalactifera*, species that are often found on rocky coasts exposed to wave and tidal action (Southward 1975; Crisp et al. 1981). The barnacles at Jetty Park were removed from granite rocks which make up the portion of jetty located just inside the entrance to Port Canaveral in the most exposed location sampled. The only seasonal differences in barnacle composition were detected at the Cape Marina and Canaveral Lock survey sites predominantly driven by the difference in abundance of *A. amphitrite* and *B. trigonus*, respectively. This difference is likely tied to the temperature tolerances and peak recruitment periods of these two species. Maximum recruitment rates in Florida occur in early summer for *A. amphitrite* (Weiss 1948) and spring and fall for *B. trigonus* (Werner 1967). Additionally, *B. trigonus* is less tolerant of heat compared to *A. amphitrite* (Werner 1967) and high temperatures (around 28 °C) decreases the successful attachment of recruits (Thiyagarajan et al. 2003). Lower temperatures can decrease reproductive rates and settlement of *A. amphitrite* (Qiu and Qian 1999) with a critical breeding temperature of around 20 °C (Weiss 1948), which was reached in the months preceding the cooler collection period (AW, unpublished data) when *A. amphitrite* abundances were low. Copper concentrations may also influence the distribution of *A. amphitrite* that is often found at higher prevalence on surfaces coated with copper paint (Tribou and Swain 2015). Dissolved copper concentrations are often high around marinas and harbors (Srinivasan and Swain 2007) that could at least partially explain the higher prevalence of *A. amphitrite* in the interior of Port Canaveral.

While there are more native species than NNS within Port Canaveral, two of the native species (*C. fragilis* and *T. stalactifera*) were restricted to Jetty Park and a third (*C. stellatus*) only had one individual identified outside of Jetty Park, where hydrodynamic conditions are the most severe (AW, *personal observation*). At the four most interior sampling sites, NNS abundance was much higher compared to the native species, comprising at least 90% of the barnacles. Surveys of ports and surrounding areas typically find most species are native, but NNS can often dominate the biofouling communities (Cohen et al. 2005; Schwindt et al. 2014). The same is true for an introduced barnacle, *Elminius modestus* (Darwin, 1854), on the shores of Lough Hyne Marine Nature Reserve in Ireland which went from occasional observations in 1988 to replacing all other barnacle species in some parts of the Lough in 2001 (Lawson et al. 2004).

This study provides a baseline for barnacle species present at Port Canaveral, demonstrating there is spatial and temporal variation in community structure within the port. Given that NNS comprise the majority of the barnacle community in the port's interior, future work should expand the survey to include all biofouling taxa to generate a more complete list of NNS at Port Canaveral, an essential first step in developing NNS management plans (Olenin et al. 2010; Collin and Shucksmith 2022). Future surveys should consider sampling on submerged portions of surfaces using SCUBA (e.g., Schwindt et al. 2014), which could give a more encompassing view of the biofouling community as not all organisms flourish in the intertidal zone. Genetic techniques could also be used to help increase sampling effort to detect NNS in the port but will likely need to be used alongside traditional morphological identification. A survey in Portugal found that metabarcoding detected a high overall diversity of organisms, but while 27 of 29 NNS observed during the study were detected with metabarcoding, the other two were only found visually on settlement plates (Azevedo et al. 2020). Continued documentation of the biofouling community at Port Canaveral will provide managers with a deeper understanding of invasion pathways and aid in developing and evaluating port management policies and best practices for reducing invasions. If abundances of new or current NNS increase, they could cause functional (e.g., clogging intake pipes) or ecological (e.g., disrupting food webs) problems within the port (Katsanevakis et al. 2014). Additionally, Port Canaveral serves as one of several entrances into the Indian River Lagoon, and new species transported on recreational boats could further disrupt the already disturbed ecosystem.

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Author's contribution

AW: research conceptualization, sample design and methodology, investigation and data collection, data analysis and interpretation, writing – original draft; KZH: research conceptualization, sample design and methodology, funding provision, writing – review and editing; GS: research conceptualization, sample design and methodology, funding provision, writing – review and editing.

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