Managing human mediated range shifts: understanding spatial, temporal and genetic variation in marine non-native species

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Abstract

The use of molecular methods to manage natural resources is increasingly common. However, DNA-based methods are seldom used to understand the spatial and temporal dynamics of species' range shifts. This is important when managing range-shifting species such as non-native species (NNS), which can have negative impacts on biotic communities. Here we investigated the range-shifting NNS *Ciona robusta, Clavelina lepadiformis, Microcosmus squamiger* and *Styela plicata* using a combined methodological approach. We first conducted non-molecular biodiversity surveys for these NSS along the South African coastline, and compared the results with historical surveys. We detected no consistent change in range size across species, with some displaying range stability and others showing range shifts. We then sequenced a section of cytochrome c oxidase subunit I (COI) from tissue samples and found genetic differences along the coastline but no change over recent times. Finally, we found that environmental DNA metabarcoding data showed broad congruence with both the non-molecular biodiversity and the COI datasets, but failed to capture complete incidence of all NSS. Overall, we demonstrated how a combined methodological approach can effectively detect spatial and temporal variation in genetic composition and range size, which is key for managing biodiversity changes of both threatened and NSS.

1. Introduction

Biodiversity is undergoing a global redistribution as a result of human influence, with species increasingly found in environments outside their previously reported geographic range [1]. Contemporary climate change is causing species to shift their ranges to accommodate novel environmental conditions [2, 3], and human-mediated species introductions dramatically increase the range of non-native species (NNS) [4-6]. This exposes species to abiotic conditions and biotic interactions that are different to those experienced in native habitats. Such changes in distribution can result in a dramatic increase or decrease in population size, or may have a limited detectable immediate effect [1, 7]. Understanding these responses is important to answer fundamental ecological and evolutionary questions about changing biotic communities, but also for natural resource managers when predicting changes in ecosystem services and natural capital [1, 8].

Global biodiversity loss has consistently been shown to reduce ecosystem function, and in turn affects the provision of ecosystem services [9, 10]. A key driver of biodiversity loss is the introduction of NNS [11], which also imposes a substantial global economic cost [12] and has a dramatic impact on public health [13, 14]. In the marine environment the majority of NNS introductions are associated with transoceanic shipping [5, 15, 16] and therefore, major ports and harbours are hotspots for NNS. Once a species is introduced to these sites, secondary spread can be facilitated by smaller recreational vessels, marinas and marine infrastructure surrounding these major harbours [17, 18]. Considering the increasing number of yearly NNS introductions [6, 19], improving our understanding of how range shifts of NNS occur through time and space is critical in the design of effective management and mitigation responses.

Natural resource managers have finite budgets and limited information when making decisions simultaneously on a number of NNS with variable or unknown impact [20, 21]. For each NNS, managers can attempt to eradicate a population, make efforts to avoid any further expansion into new areas, or acknowledge that control is not possible and work on mitigation strategies [22, 23]. These limited options are compounded by the vast costs associated with control or eradication, and even when control methods may be possible, they might be politically or publicly unacceptable [24, 25]. Furthermore, control measures can be unsuccessful because of incomplete eradication of the target species or ongoing species reintroductions [20, 26, 27]. Consequently, managers frequently take no action to control NNS or act only when evidence for both presence and substantial impact has been gathered [28]. It is therefore beneficial to develop tools that provide researchers and

managers with information to facilitate decision-making. Genetic tools can complement existing methods for assessing NNS range shifts by providing information that would be unfeasible or impossible to produce otherwise [29].

Even when NNS can be unambiguously identified, it can be difficult to determine when and where they were first introduced into a region, (for example see Hudson *et al.* [30]). Since eradication or control efforts are improved by early detection [31], methods with high sensitivity are needed to increase the likelihood of successful management outcomes. One such method is the isolation of DNA from environmental samples (environmental DNA or eDNA) such as water or sediment for the detection of organisms. Studies have demonstrated that the amplification of DNA barcode regions from eDNA (i.e. eDNA metabarcoding) can be used to detect marine NNS [32-35] and that it is a sensitive and accurate method for biomonitoring [36, 37]. However, eDNA surveys are rarely used in conjunction with existing methods to detect NNS range shifts, and eDNA metabarcoding can validate, endorse, or highlight flaws, in current biodiversity management strategies.

During a range expansion, understanding if there was a single NNS introduction event or multiple simultaneous introductions is valuable for managers to target possible source regions, and to effectively manage introduction vectors (e.g. ballast waters). As NNS spread across the new region, understanding if expansions are due to local spread or introductions from distant regions is useful to target containment efforts. Finally, after eradication efforts have been conducted, understanding if the reappearance of NNS is due to incomplete eradication or a secondary reintroduction is of value for effective management into the future. The sequencing of DNA isolated from NNS has previously identified the source of NNS [38, 39], provided evidence of multiple introductions [40] and tested if post eradication invasions are a result of incomplete eradiation or reinvasion [41]. Cumulatively, these studies have demonstrated the value of DNA evidence for the management of NNS. Furthermore, observations from both laboratory [42, 43] and field studies [44-49] have shown that eDNA can provide population genetics inference, but very little work has used this approach to study NNS [50].

Here we combined eDNA metabarcoding, mitochondrial gene sequencing, and non-molecular biodiversity surveys to study four NNS that are directly relevant to marine natural resource managers. First, we evaluated if the NNS shifted their ranges over decadal time scales and compared each range shift to historical data. Secondly, we evaluated changes in genetic diversity and haplotype composition for each NNS between two sampling occasions across the sampled coastline. Finally, we examined how spatial genetic variation data can

inform the management of range shifting species by comparing eDNA metabarcoding data to biodiversity survey and mitochondrial DNA sequence datasets.

2. Methods

(a) Fieldwork and historical biodiversity data

The coastline of South Africa is an ideal system to study range shifting species and their management. South Africa has been subject to intense human impact and many species invasions have been documented across the three environmentally varied coastal ecoregions [51-53]. Moreover, rapid assessment survey (a non-molecular biodiversity survey technique) [54] data has been previously collected and mitochondrial sequence data have been generated for NNS along the entire coastline [52]. Furthermore, historical data are available for a range of relevant species [55-57] providing an insightful opportunity to conduct a spatial and temporal analysis of range expansions. Here, we selected twelve human impacted sites and conducted surveys (see details below) between October and November 2017. The sampled sites were the 11 sites previously sampled in 2007 and 2009 by Rius *et al.* [52], which included all major harbours and a number of marinas, and a new marina constructed post 2009 (Figure 1a with full details in Supplementary Table 1). Collectively, the sites encompass the main introduction points for marine NNS into the South African coastline.

At each sampling site a rapid assessment survey was conducted following Rius *et al.* [52], targeting non-indigenous ascidian species (Class: Ascidiacea). Ascidians are unique species for studying range expansions as they are successful invaders [58] and have a relatively short pelagic larval phase, meaning that long-distance dispersal can only be achieved through anthropogenic transport of species [59]. For each site, species abundance was ranked as absent (0%), scarce (< 10%), common (10-50%), or dominant (> 50%) based on observations of substrate coverage as in [52].

Rapid assessment survey data from 2007 and 2009 was sourced from Rius *et al.* [52] for the species of interest. Additionally, historical incidence data was extracted from several taxonomic publications [55-57, 60, 61]. These investigations are not an exhaustive survey of the coastline, but they provide valuable historical species incidence data over the last century and are therefore of value in gaining a broad understanding of range shifts over time.

(b) Sample collection, DNA extraction and Sanger sequencing

Tissue samples were collected for species for which genetic data were available from the 2009 surveys (*Ciona intestinalis, Clavelina lepadiformis, Microcosmus squamiger* and *Styela plicata*). Samples were collected where sufficient numbers of individuals per species were present at a site to provide a reasonable estimate of genetic diversity (minimum 10 individuals), with 30+ individuals per species being the target at each sampling site. Organisms were sampled by hand, with no adjacent (within 0.3m) individuals collected, and dissected within six hours (see details of research permit in the Acknowledgements). For each sampled individual, approximately 10mm^2 of tissue from around the syphons was dissected using tools decontaminated with 10% bleach solution (3.5% chlorine), except in the case of *C. lepadiformis* for which a single zooid was removed from the tunic and stored. Tissue samples were preserved in 100% ethanol and stored at ambient temperature during transportation, and then stored at -80°C in the laboratory until later DNA extraction.
DNA from ascidian tissue samples was extracted using the Qiagen (Hilden, Germany) DNeasy Blood and Tissue Kit (96 Well Format) following manufacturer's recommended protocol with one blank control per extraction run. The final DNA elution was performed using 200μl of Qiagen Buffer ATL. A section of the cytochrome c oxidase subunit I gene (COI) was sequenced for all tissue samples aiming to cover the entire section previously analysed in Rius *et al.* [52]. Each PCR contained 6μl of Applied Biosystems (Foster City, Califonia, USA) AmpliTaq GOLD 360 Mastermix, 1.8μl of oligonucleotide mix (5 μm concentration per primer), 1.2μl of undiluted template DNA and PCR quality water up to 12μl total reaction volume. The reaction conditions varied by primer set and are listed in Supplementary Table 2a. During preliminary trials a set of primers were designed and validated for *M. squamiger* (sequence details in Supplementary Table 2b), existing primer sets [62-64] were optimised for the remaining three species. Successful amplification was confirmed using gel electrophoresis and PCR products were cleaned using Applied Biosystems ExoSAP-IT Express following the manufacturer's recommended protocol. Cleaned products were normalised to approximately 50ng/μl and 5μl of sample was added to each of 5μl of the forward or reverse primers (5μm) used in the initial PCR. These samples were sent for sequencing using the Macrogen Europe (Amsterdam, Netherlands) EZ-Seq service. Resultant chromatogram files were analysed using Geneious Prime (v2020.2.4) (Biomatters Ltd, Auckland, New Zealand). For each sequence the forward and reverse traces were aligned and sequences with ambiguities or failed reactions were re-sequenced from the initial PCR once and subsequently discarded if poor results persisted. The 764 COI sequences from Rius *et al.* [52] were added to the analysis and trimmed,

truncated and aligned with the experimental data as follows. For each species, sequences were trimmed to remove primer binding and poor-quality regions and aligned using the Geneious Alignment Tool. Subsequently each alignment was manually checked to confirm complete alignment, and short sequences that did not overlap at all polymorphic regions or had ambiguous base calls were discarded.

(c) Environmental DNA metabarcoding

Before each rapid assessment survey, surface seawater was sampled from the top 10cm for eDNA metabarcoding following Holman *et al.* [65]. Briefly, three replicate 400ml water samples were filtered on site with a 0.22 µm polyethersulfone enclosed filter. Filters were preserved with Longmire's solution until DNA extraction following Spens *et al.* [66]. Data generated from these samples is presented in Holman *et al.* [65] with the aim of conservatively characterising whole community diversity. COI and ribosomal RNA (18S) data targeting metazoans [67, 68] was reanalysed as follows for accurate ascidian species detection. Primer regions were removed from forward and reverse reads using the default settings of Cutadapt (v2.3) [69]. Sequences were denoised and an ASV (amplicon sequence variant) by sample table generated using DADA2 (v1.12) [70] in R (v3.6.1) [71] with parameters as in Holman *et al.* [65]. Recent work has highlighted that different bioinformatic methods have an effect on the resolution of intra-specific variation of eDNA metabarcoding data [42, 45, 72]. Therefore in addition to the sequenced tissue samples and DADA2 methods outlined above, we reanalysed the COI data using the *unoise3* algorithm [73] as follows. Raw COI paired-end fastq data from Holman *et al.* [65] was merged using usearch (v11.0.667) [73] with the following parameters *-fastq_maxdiffs 15 -fastq_pctid 80*. Primer sequences were then stripped from each merged read using Cutadapt (v3.1) [69] under the default parameters and reads longer than 323 and shorter than 303 base pairs (±10 from the expected size of 313) were discarded. Reads from all samples were pooled, and singletons and reads with an expected error greater than 1 were discarded using vsearch (v2.15.1) [74]. The *unoise3* algorithm from usearch was then used to generate ASVs with *-unoise_alpha* set at 5 as recommended for resolving metazoan intraspecific variation with a COI fragment of 313 base pairs in length [45]. Sequences were then mapped back to the ASVs using the *-usearch_global* function of vsearch with an *-id* parameter of 0.995 to produce an ASV by sample table.

To provide an initial taxonomic assignment all ASVs were compared using a BLAST (v2.6.0+) search with no limits on sequence similarity or match length to the NCBI *nt* database (downloaded 16th May 2019). Taxonomic assignments were then parsed using a

custom R function (*ParseTaxonomy*, DOI:10.5281/zenodo.4671710) with the default settings. The taxonomic assignments were subset to include only those with a hit to species in the class Ascidiacea. The following quality control steps were then applied to each dataset. The data was filtered to only retain ASVs that appeared in more than one replicate sample. For any ASVs detected in both the negative and experimental control samples, the maximum number of reads in the negative controls were subtracted from the experimental control samples. Reads were then divided by the total number of reads per sample and relative proportions were used in all subsequent analyses, technical replicates per site were averaged. The remaining ASVs were then taxonomically checked manually using the online National Centre for Biotechnology nucleotide BLAST search function against the *nt* databases (last accessed on 1st October 2020) under default megablast parameters. For each ASV in the COI dataset, taxonomy was only assigned at species level if multiple, independent sequences had a match greater than 97% identity (with 100% coverage) with no other species within 97% of the target ASV. For the 18S dataset a 100% match (with 100% coverage) between the subject ASV and database sequences was required for taxonomic confirmation. Additionally, as some taxa within the same genera have near 100% similarity at the 18S region, taxonomy was only assigned to species if organisms from the same genera were in the database with at least 1 base pair between the query and species from the same genera. Following taxonomic annotation, ASVs assigned to the same species were merged for the distribution datasets. ASVs were kept separate for the haplotype reconstruction of the COI data.

(d) Data manipulation and statistical analyses

Distances between sites along the coast were estimated by drawing a transect 1 km parallel to the coastline in Google Earth Pro (v7.3.2.5776) and calculating the distance between each pair of sites. The study area was plotted using the function *map* from the package *maps* (v3.3.0). Sequenced COI regions from 2009 and 2017 were aligned separately for each species using the Geneious aligner in Geneious Prime; alignments were truncated to include only overlapping regions. Sequences were manipulated using the *SeqinR* package in R (v4.2-5) [75]. Nucleotide and haplotypic diversity were calculated using the *nuc.div* and *hap.div* functions from the *pegas* package (v0.14) [76]. For each species an alignment was created between the tissue sampled COI sequences and the eDNA metabarcoding derived haplotypes. The region of overlap was extracted and used in subsequent analyses. Haplotype frequencies were calculated per site for the tissue derived sequences and the different bioinformatic analyses of eDNA metabarcoding data. Minimum spanning network

haplotype maps [77] were created using the default settings of PopArt (v1.7) [78]. Analyses of molecular variance (AMOVA) were performed using the function *poppr.amova* from the *poppr* package (v2.8.6) [79]. AMOVA models were structured to analyse the effect of sampling year and sites for each species. All data analyses were conducted in R (v4.0.3) unless otherwise stated.

3.Results

(a) Range shifts

Rapid assessment surveys found that non-native ascidians known to be broadly restricted to warmer waters (*M. squamiger* and *S. plicata*) [52] showed distributions principally limited to the southern and eastern coastlines (Fig.1b). In contrast *C. robusta* and *C. lepadiformis* were found along most of the coastline. We found no change across years in range extent for *M. squamiger* and *S. plicata*, a decrease in easternly range for *C. robusta* and an expansion of range both westerly and easterly for *C. lepadiformis* (Fig.1c). Historical total range extent data (Fig.1d) showed more recent increases in range for *C. lepadiformis* and *S. plicata* compared to *C. robusta* and *M. squamiger*. The COI and 18S eDNA metabarcoding data showed mixed results. There was good agreement between detections from eDNA and rapid assessment surveys in *M. squamiger* and *S. plicata* (see Fig.1b). However, 18S entirely failed to detect *C. robusta* or *C. lepadiformis*, and COI demonstrated a number of false-negative metabarcoding detections in these species (Fig.1b). For sites sharing detections from eDNA metabarcoding and rapid assessment surveys, eDNA metabarcoding data and field density estimates showed a non-significant relationship (18S $p = 0.052$, COI $p = 0.297$) (see Supplementary Note 1 for details).

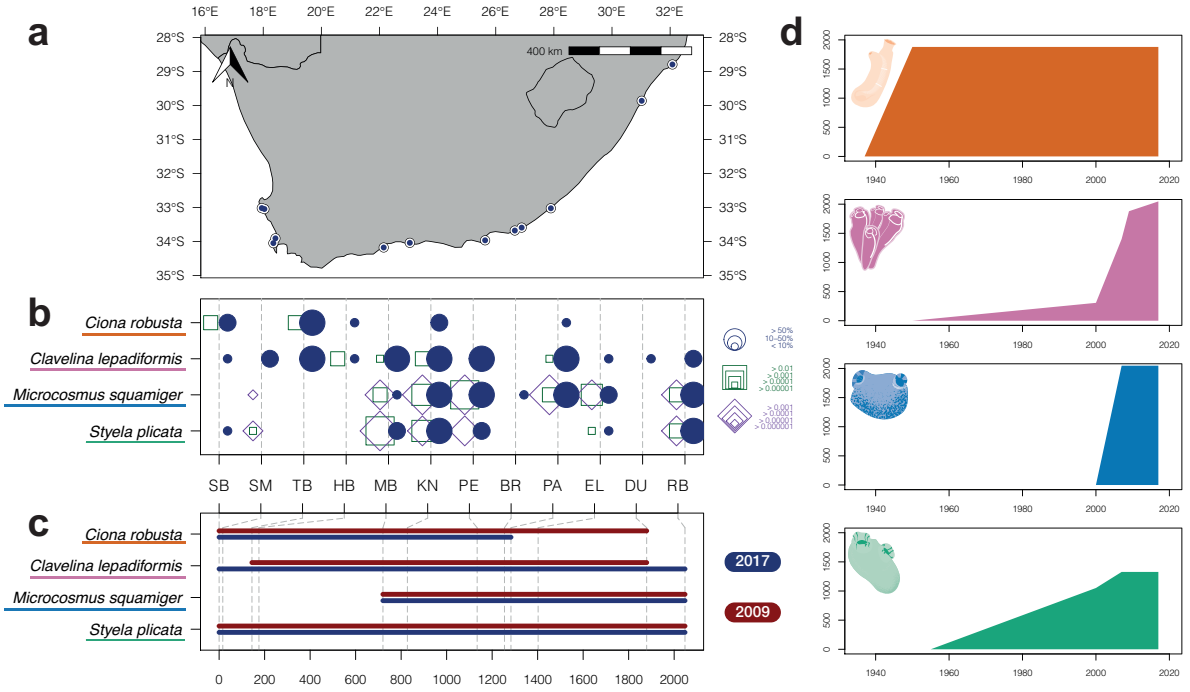

Figure 1 a Map depicting the coastline of South Africa, sampling sites are shown as blue points, full details in Supplementary Table 1. **b** Bubble plot showing incidence of four non-native ascidians across the sampling sites shown in the map from west to east. Blue bubbles show results of rapid assessment surveys and square outlines show the results of eDNA metabarcoding surveys conducted concurrently. Results from COI are shown with green squares and 18S shown with purple squares, the size of each point or square shows the comparative density. Site codes correspond with sites as detailed in Supplementary Table 1. **c** Line plot showing range extent over the surveyed coast for 2009 (dark red) rapid assessment surveys from Rius *et al.* [52] and surveys conducted in 2017 presented here (blue). The location of each site across the coastline is shown with grey dashed lines. **d** Historical maximum range extent for each of the featured species across the coastline of South Africa, y axis is kilometres of extent, x axis is year, colour indicates each of the species indicated according to labels in b and c.

(b) Changes in genetic composition

A total of 1,320 sequencing reactions generated 660 bi-directionally sequenced COI sequences. After alignment and quality control, 541 samples remained with complete alignment and no missing site information, 88 for *C. robusta,* 261 for *C. lepadiformis,* 90 for *M. squamiger* and 102 for *S. plicata*. After combining the COI sequences with previously sequenced samples from 2009 [52], alignments were 626, 440, 635 and 599 base pairs in length for *C. robusta*, *C. lepadiformis*, *M. squamiger* and *S. plicata* respectively. Observed haplotype richness across both sampling years and all sites was highest in *M. squamiger* followed by *C. robusta, S. plicata and C. lepadiformis* (Fig. 2). There was no statistically significant difference between nucleotide or haplotype diversity between sampling years across all species ($p > 0.05$ in all cases, see Supplementary Note 2 for full model output and

details). Additionally, AMOVA models found no significant differences between sampling years across all species ($p > 0.05$ in all cases, see Supplementary Table 3 for full model outputs), but significant differences between sampling sites within years ($p < 0.05$ in all species, see Supplementary Table 3 for full model outputs). In all species, the greatest proportion of the genetic variance was found between samples, then within sampling sites, followed by variance between sampling sites (Supplementary Table 3). As shown in Figure 2, haplotype frequencies agreed with the AMOVA analyses, showing stable patterns of genetic variation occurring between years and variation in haplotype frequencies across the study system (Figure 2).

After aligning the shorter sequences derived from eDNA metabarcoding data to the sequenced COI region, alignments were 191, 258, 289 and 286 base pairs in length for *C. robusta*, *C. lepadiformis*, *M. squamiger* and *S. plicata* respectively. Regardless of bioinformatic method and across species, the eDNA metabarcoding data did not recover all the haplotype sequences derived from tissue (Figure 3).

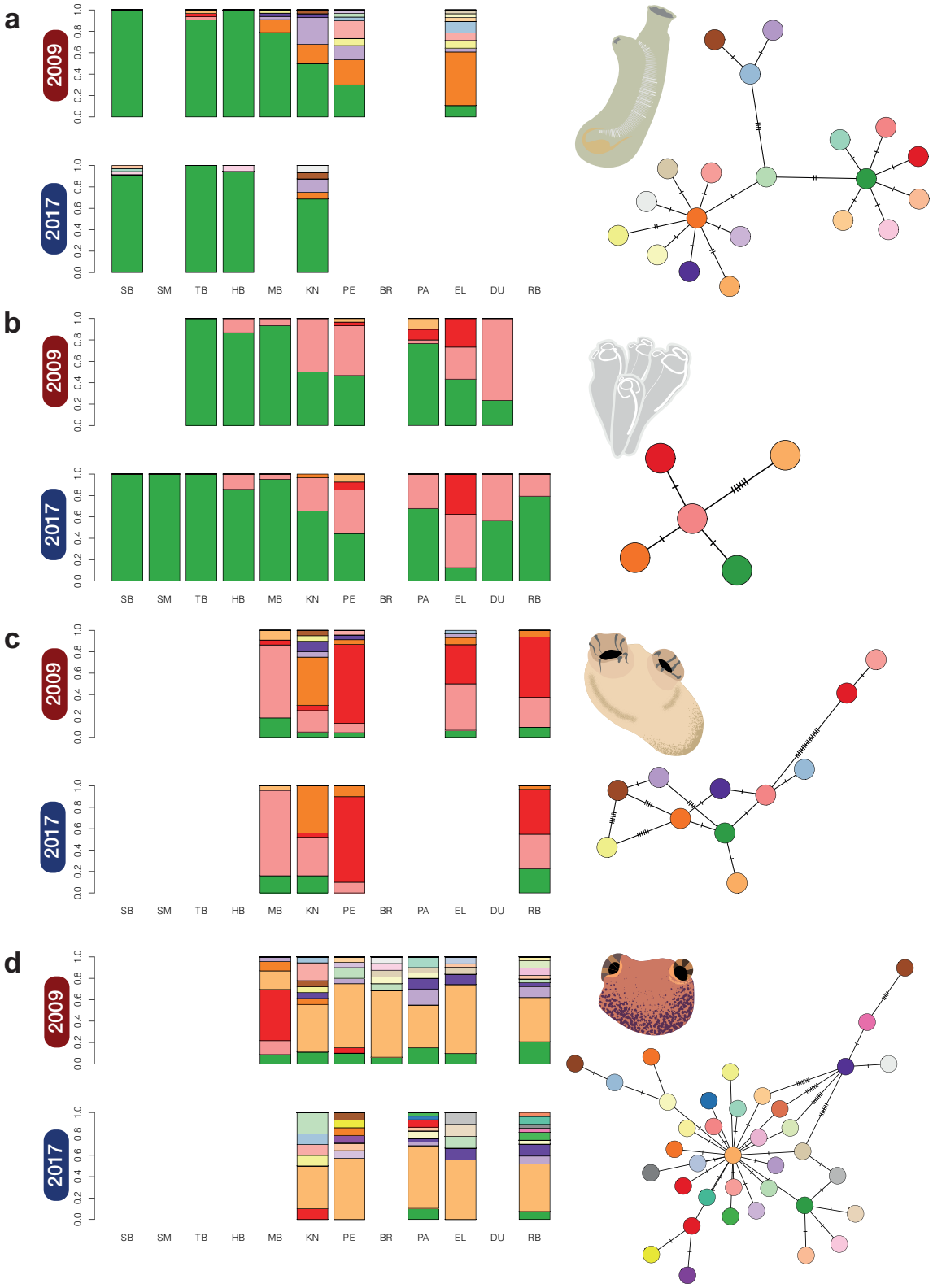

Figure 2 Mitochondrial DNA COI haplotype proportions for **a** *Ciona robusta* **b** *Clavelina lepadiformis* **c** *Styela plicata* **d** *Microcosmus squamiger* across the South African coastline. Results are shown for surveys conducted in 2009 and 2017 for each species, site abbreviations follow supplementary Table 1. Haplotype networks based on minimum spanning distance are shown for each species with colours matching the bar plot within species, the number of cross-hatches indicates the mutation steps between haplotypes.

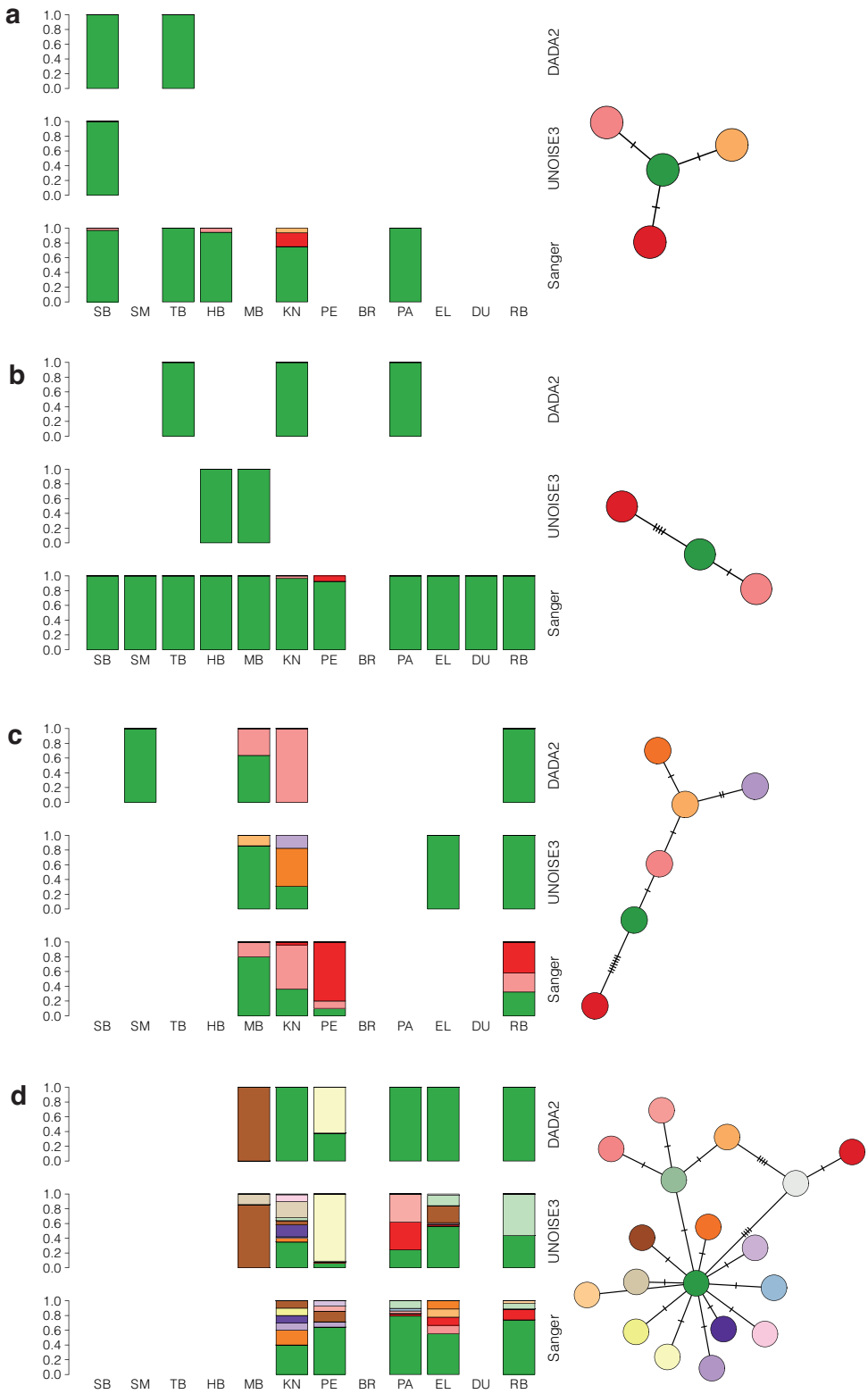

Figure 3 Haplotype proportions recovered using eDNA metabarcoding for **a** *Ciona robusta* **b** *Clavelina lepadiformis* **c** *Styela plicata* **d** *Microcosmus squamiger* across the South African coastline. Results are shown for analysis of COI eDNA metabarcoding data using the denoising software DADA2 and UNOISE3 for each species, site abbreviations follow supplementary Table 1. Haplotype networks based on minimum spanning distance are shown for each species with colours matching the bar plot within species, the number of cross-hatches indicates single nucleotide mutation steps between haplotypes.

4. Discussion

Here we found both losses and gains in range size across sampling years for four non-native ascidian species, with no consistent pattern emerging when introduction dates were compared. For all species we found haplotype variability across the study region but no significant change in genetic variation for almost a decade. Finally, eDNA metabarcoding data recovered broad NSS incidence trends and for some species was as accurate as non-molecular surveys. Most dominant haplotypes from tissue samples were detected with eDNA metabarcoding but fine scale genetic patterns could not be resolved using the eDNA metabarcoding data. Cumulatively, the evidence demonstrates that a suite of tools, including DNA and non-DNA biodiversity surveys can be used in combination to evaluate the role of genetic variation on range shifts and to inform natural resource managers.

Non-DNA biodiversity surveys found that *C. lepadiformis* expanded its range by 168.4 km since surveys in 2009, for an assumed rate of 21.1 km per year. This is in line with previous studies that found an average marine non-native spread rate of 44.3 km per year [80], with values of 16 km per year for tunicates, 30.0 km per year for barnacles and 20 km per year for a bryozoan species [81]. In contrast, we observed a range contraction for *C. robusta*, which was unexpected. There are limited studies showing range contraction in the introduced range for marine species. However, previous work has identified biotic resistance for invasions of several species in the genus *Ciona* [82, 83], and so it might be feasible for local species to have begun predating on *Ciona robusta* during the 80+ years it has been documented in South Africa (Fig.1d). A lack of any western increase in range for *M. squamiger* might be explained by the species inability to mature to reproductive age in the colder sea temperature on the western coast [52]. Further range expansions or contractions (eastwards for *M. squamiger* and east or westward for *S. plicata*) cannot be ruled out as observations of these species extended to the margins of the sampled area. It is important to note that the harbours and marinas in this study act as islands of suitable habitat, and the frequency of introductions outside these areas is relatively uncertain. Further surveys of surrounding hard benthic environments are required to understand the role of artificial environments across the coastal ecosystem. Overall, these patterns demonstrate that the spread of marine non-native species is not characterised by a continuous expansion of range, but rather by a complex picture of expansions and contractions in response to dynamic abiotic and biotic conditions.

A consistent pattern of genetic differentiation emerged across the studied species; significant differences across sampling sites and persistence of similar haplotypes across

time (Fig. 2). Previous studies of temporal changes in genetic diversity of non-native ascidians have found some evidence for genetic differences over time [84, 85]. In contrast other work has found relatively stable genetic diversity over several years [86, 87]. In our study, the time between sampling occasions (i.e. 2009 and 2017) represents between four and 24 generations depending on the species [88-90]. Therefore, dramatic changes in haplotype frequencies could only be as a result of anthropogenic transfer of haplotypes between sites or changes in site frequencies in response to high mortality events (for example extreme weather events). These types of changes have been documented in ascidian species elsewhere [85, 91, 92], and a large number of NNS introductions have been documented in South African marinas and harbours supporting regional transfer of these organisms [52, 53]. It is therefore somewhat surprising that across four different species, all of which are known to be transported anthropogenically, there was little evidence of shifts in haplotype composition. Consequently, our results demonstrated that the studied NSS are well-established and are not subject to high levels of mortality or genetic bottleneck that may affect population viability. It may be that these well-established haplotypes impede newcomers to succeed and ultimately change the haplotype composition of the site.

We found that eDNA metabarcoding captured similar incidence data as rapid assessment surveys for some species, and performed poorly for others. Previous work has identified that NNS can be detected using eDNA metabarcoding [32, 34], but these surveys aimed at detecting any NNS rather than a specific set of target taxa. Several studies have identified that general target metabarcoding primers show lower reliability and sensitivity compared to species-specific quantitative PCR assays [93, 94]. Additionally, previous work has identified that in some cases different bioinformatic methods carry variable sensitivity [95], although this effect is fairly minimal in this dataset (see Supplementary Note 3). Managers should therefore be aware that general metabarcoding primers will perform well in the detection of some important NNS but others may be missed due to poor sensitivity. In cases when a list of priority species can be assembled, mixed DNA positive control samples or trials with aquaria of known composition (for example Holman *et al.* [96]) would provide information on which NNS might be overlooked by eDNA metabarcoding. Inevitably, there will be a cost-benefit trade-off between using imperfect broad metabarcoding assays for monitoring unknown invaders, and expending resources on the development and application of eDNA tools targeting specific known NNS.

In some cases, resource managers might be interested in tracking invasions using haplotype data [97]. Here, we showed that eDNA metabarcoding with broad-target primers resolves broad scale patterns of haplotype diversity (Fig. 3). However, fine-scale genetic variation

was not recovered in our study, indicating that targeted eDNA amplicon sequencing [48] might be more appropriate when this level of genetic data is required. As with biodiversity incidence data, the management objectives for a given NNS determine how haplotype sequencing should be implemented. If large numbers of tissue samples can be easily collected and there are sufficient resources, then sequencing the tissue directly might be more appropriate. In contrast if a broad scale analysis across a larger or difficult-to-sample area is preferred resolving haplotype data from eDNA metabarcoding might be preferable. Overall, eDNA based techniques show great potential for NNS detection but for our target taxa, we demonstrated that current biodiversity surveys and direct tissue sequencing are more reliable for the detection of NNS and genetic composition. It is important to note that there are several key advantages of eDNA-based methods compared to the other tools used in this work. Firstly, eDNA samples can be collected with minimal training and the sequenced DNA provides an unambiguous identification, provided reference data is available [33, 34]. Secondly, eDNA-based methods can be automated and can scale to a much greater survey effort at reduced cost compared to other methods [98]. Third, the limitations described above concerning the sensitivity of eDNA-based incidence data and lack of resolution of eDNA-based haplotype data can be attributed to the use of metabarcoding with broad-target primers. Reanalysing the samples with metabarcoding primers for more specific groups or using species specific qPCR assays [94] would provide increased sensitivity and accuracy.

Overall we demonstrated how our combined methodological approach can effectively detect spatial and temporal trends of range shifts and genetic differentiation, but also to monitor biodiversity changes of both threatened and NSS. The strengths of eDNA or DNA-based biomonitoring demonstrated here for the detection of range shifting species make them a pragmatic choice for natural resources managers. These tools provide managers with greater sensitivity and accuracy when monitoring biodiversity in human impacted environments.

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Authors Contributions

All authors contributed to the initial study design. L.E.H. collected the samples, generated and analysed the data, prepared all figures and wrote the first draft of the paper. S.P.N. and M.R. advised on the sampling design and subsequent laboratory work. All authors substantially contributed to further manuscript drafts and provided final approval for publication.

Data Availability

Raw and processed data are available online via Zenodo with the following DOI 10.5281/zenodo.5046379. Raw sequence data associated with analyses can be found under European Nucleotide Archive Project Accession PRJEB38452

Code Availability

Associated R scripts and intermediate files are available online via Zenodo with the following DOI 10.5281/zenodo.5046379.

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