

Research Article**Tracking the settlement patterns of marine biofouling communities**

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Co-Editors' Note: This study was first presented at the 11th International Conference on Marine Bioinvasions held in Baltimore, Maryland, USA, May 15–19, 2023 (<http://www.marinebioinvasions.info>). Since their inception in 1999, the ICMB meetings have provided a venue for the exchange of information on various aspects of biological invasions in marine ecosystems, including ecological research, education, management and policies tackling marine bioinvasions.



Citation: Peters K and Meyer S (2024) Tracking the settlement patterns of marine biofouling communities. *Management of Biological Invasions* 15(4): 549–566, <https://doi.org/10.3391/mbi.2024.15.4.06>

Received: 13 November 2023

Accepted: 8 April 2024

Published: 8 December 2024

Handling editor: Tammy Robinson

Thematic editor: Amy Fowler

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OPEN ACCESS**Abstract**

Monitoring programs or surveys for marine alien species in biofouling assemblages often focus on surveying after a specific period of settlement, with few regions being able to implement ongoing monitoring systems. Whilst these programs are beneficial, they provide only a snapshot in time and may miss key information about the temporal settlement patterns of alien fouling species and the associated management implications. The current study initiated a temporal settlement study that could 1) track the settlement patterns of fouling species with a focus on detecting alien species and 2) highlight the change in community structure across different months, providing an insight into the ecological succession. The study was conducted in a marina in Cape Town, South Africa, using PVC settlement plates, and serves as a baseline for a longer-term monitoring settlement study. The findings indicate that the species richness and abundance differed significantly across the months (Wald test: $p < 0.001$), with a rapid increase in both measures within a month and a gradual decline in species richness after two to three months. A total of 12 alien species were detected accounting for 55% of the community structure. Within the first month, eight of those species had already settled. Overall, four species (*Corella eumyota*, *Diplosoma listerianum*, *Neodexiospira brasiliensis* and *Balanus trigonus*) were particularly dominant throughout the study. The ascidian, *Corella eumyota* currently listed as native, was one of the first species to settle and demonstrated strong competitive traits, raising concerns about its current status in the region. The findings highlight the value of temporal settlement studies revealing patterns that may have been overlooked when sampling at only one point in time. Thus, a longer-term monitoring program that tracks the temporal settlement patterns of alien fouling species could start to provide valuable insights to management authorities.

Key words: non-indigenous, alien species, invasive species, monitoring, ecological succession

Introduction

Managing biological invasions in the marine environment poses several challenges. This is due to the fluid nature of the system, where the transfer and spread of species is far more complex to monitor and control, compared to terrestrial counterparts (van Wilgen et al. 2020). Ports, harbours and marinas act as both hotspots and stepping-stones for marine alien species (López-Legentil et al. 2015) as shipping is the dominant pathway through which these species are transferred, either in ballast water or as biofouling (Ruiz et al. 1999; Ashton et al. 2006; Costello et al. 2022).

These manmade coastal systems act as both sources and sinks of such species (Kinlan and Gaines 2003; Molnar et al. 2008). The presence of marine alien species in ballast water and in biofouling assemblages is well documented (Carlton 1996; Ruiz et al. 2000; Hewitt et al. 2009; Clarke Murray et al. 2011; Chan et al. 2015; Pinochet et al. 2023). Management interventions to address ballast water as a vector for alien species transfer, has been implemented in the form of the ballast water management convention (IMO 2004, 2017). However, there is currently no international, legally binding management standard to address the issue of biofouling. There are, however, biofouling guidelines developed through the GloFouling project in order to address the spread of aquatic species in biofouling assemblages (IMO 2023) and encourage voluntary implementation of these practices.

While larger vessels play an important role in the transfer of marine alien species, often responsible for primary introductions, biofouling on smaller, recreational vessels is recognised as an important vector for both primary introductions and secondary spread (Inglis and Floerl 2002; Clarke Murray et al. 2011; Ashton et al. 2014; Peters and Robinson 2017). Many recreational vessels have long layover periods in marinas, inadequate maintenance regimes and inefficient anti-fouling paint applications for the usage type of the vessels (Floerl et al. 2005; Ashton et al. 2006; Srinivasan and Swain 2007; Peters et al. 2019a). Further, the movement of recreational vessels is relatively unrestricted (Clarke Murray et al. 2011), with vessels being able to travel to any marina in the world, including regions that are considered as Marine Protected Areas (Iacarella et al. 2020). Recreational marinas, therefore, act as hubs for marine alien species (Ferrario et al. 2019; Peters et al. 2017; Ulman et al. 2019). The artificial infrastructure in marinas such as floating jetties and pontoons, recreational vessels that often remain stationary for long periods of time, and chains and ropes present in the water, house substantial biofouling communities that consist of both native and alien species (Ferrario et al. 2019; Outinen et al. 2021; Gittenberger et al. 2023), with some cases demonstrating that alien species dominate the space (Oricchio et al. 2019). However, this is context and region-specific (Spagnolo et al. 2019) and may, therefore, vary depending on when these communities are observed and at which scales. Thus, it highlights the need to better understand biofouling communities in order to better manage biofouling as a vector.

Monitoring biofouling communities is a practiced method for detecting marine alien species (Azevedo et al. 2020; Loureiro et al. 2021; Tamburini et al. 2021). In many cases, surveys of existing biofouling communities are a good starting point to gain baseline information and subsequent surveys are ideal to detect any potentially new introductions. These surveys include the collection of samples from existing marina infrastructure as well as the vessels within those marinas (Ulman et al. 2019; Tamburini et al. 2021). To

gain an understanding of how biofouling can be managed more efficiently as a vector, it is important to consider the settlement patterns of these communities. Several studies have highlighted the use of settlement plates to monitor marine alien species or to assess ecological effects such as predation (Giachetti et al. 2019; Loureiro et al. 2021; Tamburini et al. 2021; Diem et al. 2023). However, in many regions, ongoing or long-term monitoring is not feasible, due to a lack of resources and capacity (Egoh et al. 2020; Loureiro et al. 2021). In developing nations, such as South Africa, baseline surveys and monitoring for alien species are often undertaken by academic institutions and are rarely practiced by government institutions for the purposes of biosecurity. Studies that focus on monitoring for marine alien species in such regions often focus on detecting alien species after a given time period (i.e. after a few weeks or months) (e.g. Loureiro et al. 2021; Diem et al. 2023), with very few regions being able to implement ongoing monitoring systems to assess temporal patterns. While these programs are beneficial, they provide only a snapshot in time and may miss key information about the settlement of alien fouling species that could inform management interventions, particularly for biofouling communities on vessels that could be transferring species. Thus, tracking settlement patterns of biofouling over shorter periods of time could provide crucial insights into the ecological succession of biofouling communities thereby supporting more effective evidence-based management. The current study sought to 1) track the settlement patterns of biofouling species with a focus on detecting alien species and 2) highlight the change in community structure across different months, providing an insight into the ecological succession of a biofouling community.

Materials and methods

Study site

The study took place in False Bay Yacht Club marina (34.192; 18.435), within False Bay, South Africa (Figure 1). False Bay is a southward facing bay of approximately 1,000 km², bounded by the Cape Peninsula to the west and Cape Hangklip to the east (Pfaff et al. 2019). Due to False Bay's unique location, its shelf oceanography exhibits a range of oceanic conditions from seasonally pulsed upwelling events to periodic warm-water intrusions from the Agulhas Current system (Jury 2020). Thus, this system influences the hydrodynamic processes within False Bay, and the ranges in oceanic conditions allow for immense biological productivity to occur. Sampling occurred during April, May, June, July, and August 2022. The False Bay Yacht Club marina is adjacent to a larger naval port and both the port and marina receive local and international vessels. In addition, the naval port, along with the marina, holds the highest number of alien species in the region (Peters et al. 2017), making this marina an ideal study site to investigate the settlement of marine alien species.

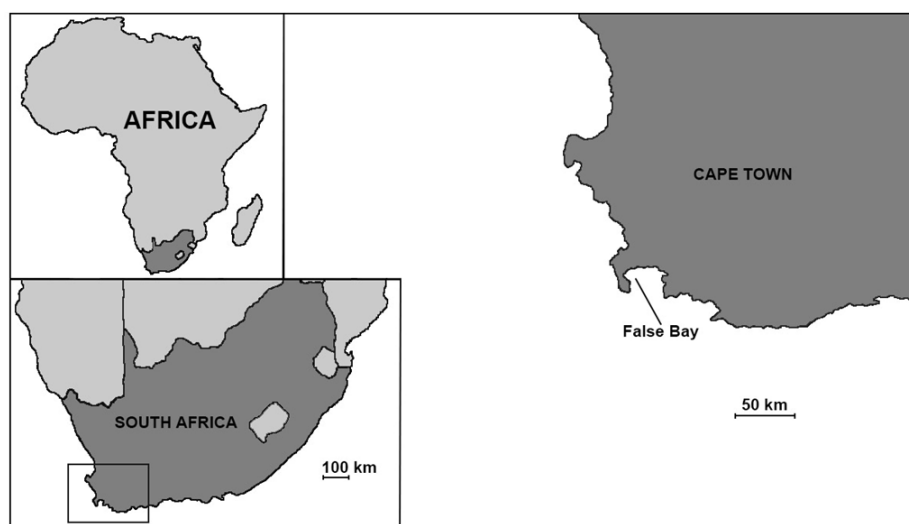


Figure 1. Location of the False Bay Yacht Club marina, in the Western Cape Province of South Africa.

Data collection

To allow for the settlement of organisms, a total of 24 grey, sprayed Polyvinyl Chloride (PVC) plates of 10×10 cm were used to assemble settlement arrays. The pre-treatment and assembly of the arrays followed the approach by Loureiro et al. (2021), where prior to the assembly of the arrays, the plates were sanded, and soaked in distilled water for 10 days to allow for chemicals to leach. Arrays were assembled according to the same method applied by Loureiro et al. (2021) where two PVC plates were fixed to a rope, in the centre of the plate, and separated by 1 m in a vertical plane (Figure 2). The arrays were suspended from a floating jetty, with the top plate positioned at a 1 m depth and the bottom plate at a 2 m depth from the surface of the water. Each array had a single weight fastened to the end of the rope which ensured stability and prevented the ropes from posing a safety risk to vessels.

Twelve arrays were installed along a single jetty, with 2 m between arrays (Figure 2). Arrays were deployed in March 2022 and monitored monthly from April 2022 to August 2022. This sampling period aligned with the Austral Autumn and Winter seasons. During each monitoring period, arrays were removed from the water and photographs were taken of the underside (i.e. only one side) of each plate. In addition, in situ identification of only sessile organisms occurred to the lowest taxonomic level to improve the accuracy of identification when processing the photographs. The percentage cover of each species was recorded using the photographs with a 10×10 grid overlaid to estimate cover as a measure of abundance. The identification of specimens were confirmed using the works of Picker and Griffiths (2011), Rocha et al. (2012), Branch et al. (2022), and local taxonomic experts were consulted when uncertainty occurred. Each species was assigned a classification of either native or alien according to Robinson et al.

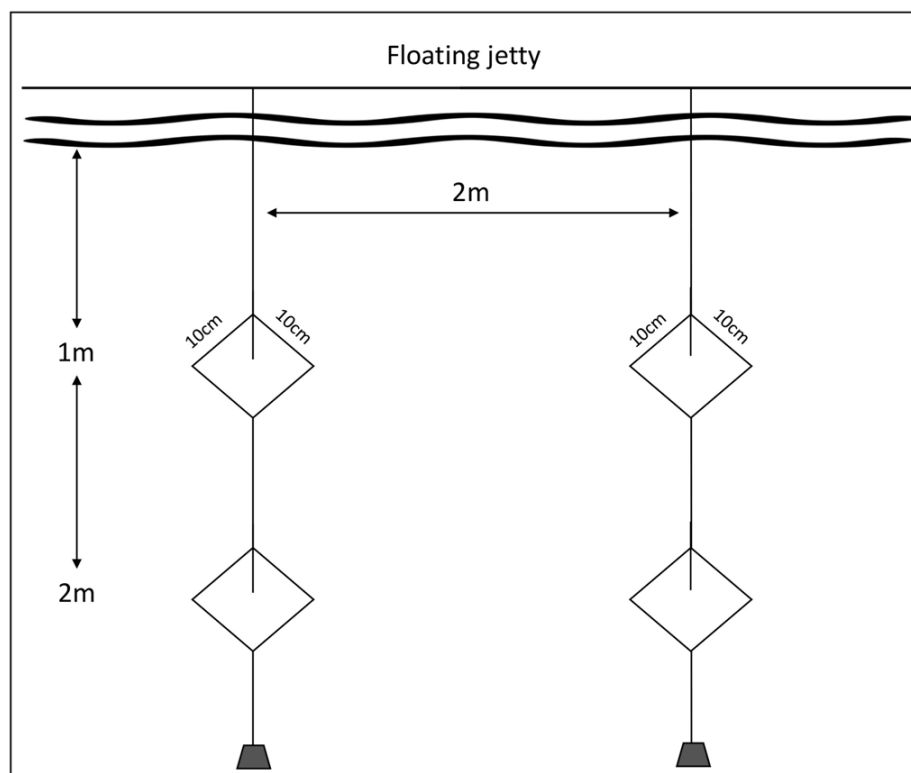


Figure 2. Illustration of the settlement arrays deployed using exposed plates, with each array being 2 m apart and each array consisting of a plate at 1 m below the surface and 2 m below the surface.

(2016). Where organisms were not successfully identified to species level, they were classified as uncertain.

Statistical analyses

All univariate analyses were undertaken in R (Version 4.2.3). Prior to testing for differences, the data were tested using Shapiro-Wilk and Levene's tests for normality and homogeneity of variances, respectively. Differences in species richness and abundance of biofouling organisms were analysed using a general mixed effects model where depth (two levels: 1 m and 2 m) and month (five levels: April, May, June, July, August) were considered as fixed factors and plate was considered as a random factor. The Akaike Information Criteria were used to select the best fit model and a Wald test was used to consider the significance of the factors (Bolker et al. 2009). Posthoc analyses were run to determine where significant differences occurred. Multivariate analyses were undertaken in Primer (Version 7). To assess whether the communities differed across the months and between the depths as well as the effect of depth $\times$ month, abundance data were square root transformed and a PERMANOVA was performed. A SIMPER was then undertaken to determine which species contributed to the differences detected.

Results

Overall, 22 species were detected, the majority of which were ascidians. Of the 22 species, 12 were alien (55%) while 18% were considered as native species ($n = 4$). Species that could not be identified to species level were classified as uncertain, making up 23% ($n = 5$) and one species from the genus *Didemnum* is likely to be alien. However, further studies are required to confirm this. The native species consisted of three ascidian species, *Botrylloides magnicoecum* (Hartmeyer, 1912), *Botryllus gregalis* (Sluiter, 1898) and *Corella eumyota* (Traustedt, 1882) and one barnacle species, *Notomegabalanus algicola* (Pilsbry, 1916). The species that could not be identified to species level were three ascidians, one bivalve and a sponge species, all of which occurred in relatively low abundances. Of the 12 alien species, eight had settled within the first month (April), an additional three by the second month (May) and by the fourth month (July) the last alien species was detected (Table 1).

Table 1. List of marine alien species and the months in which they were first detected on settlement plates in False Bay Yacht Club marina, Cape Town, South Africa, for a period of five months during 2022.

Taxonomic group	Species name	April	May	July
Ascidacea	<i>Ascidella aspersa</i>		X	
	<i>Asterocarpa humilis</i>	X		
	<i>Botryllus schlosseri</i>	X		
	<i>Ciona robusta</i>	X		
	<i>Clavelina lepadiformis</i>		X	
	<i>Diplosoma listerianum</i>	X		
Bryozoa	<i>Bugula neritina</i>	X		
	<i>Bugulina flabellata</i>		X	
	<i>Cryptosula pallisiana</i>			X
	<i>Watersipora subtorquata</i>	X		
Cirripedia	<i>Balanus trigonus</i>	X		
Polychaeta	<i>Neodexiospira brasiliensis</i>	X		

The best fit model for species richness and abundance included all factors and thus the saturated model was applied. There was a significant effect of month on species richness (Wald test: Chi square = 73.59, $df = 4$, $p < 0.001$) but no significant effect of depth (Wald test: Chi square = 0.86, $df = 1$, $p = 0.354$) (Figure 3A). The species richness of communities in April differed significantly to those in May ($p < 0.001$), June ($p < 0.001$), and July ($p = 0.004$). Further, the suite of species in May differed significantly to those in July ($p = 0.003$) and August ($p < 0.001$). The suite of species detected in June also differed significantly to those found in August ($p < 0.001$). While no significant differences were detected for depth, it should be noted that at 1m depth, the species richness increased in the first two months (April to May) after which a steady decline occurred (Figure 3A). At the 2 m depth, there was a rapid increase in species richness in the first two months as well, which continued steadily by month three and a rapid decline occurred a month later when compared to the plates at the 1 m depth.

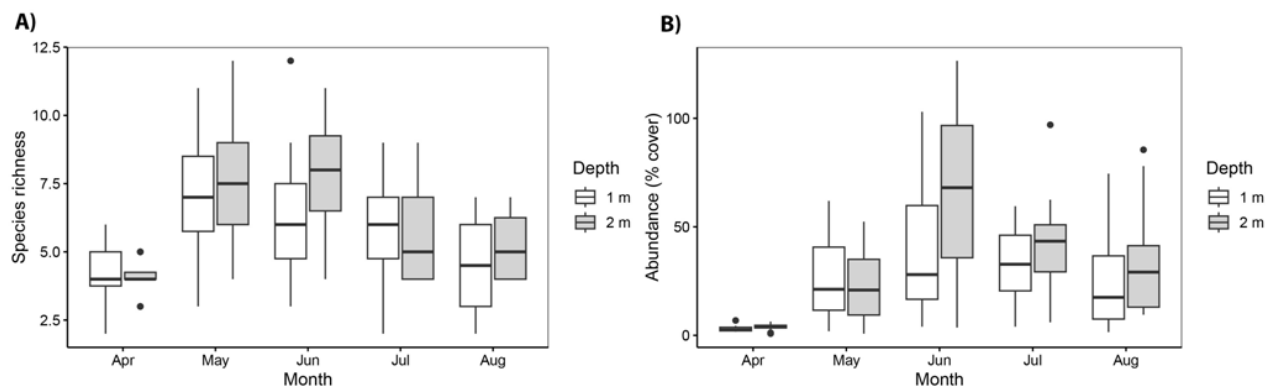


Figure 3. The species richness (A) and abundance (B) of biofouling organisms settling on plates across a four month period (April – August) and between two depths (1 m and 2 m).

For the abundance of species, there was also a significant effect of month (Wald test: Chi square = 72.24, $df = 4$, $p < 0.001$) and no significant effect of depth (Wald test: Chi square = 1.60, $df = 1$, $p = 0.205$) (Figure 3B). The abundance of the biofouling community in April was significantly lower than the abundance detected in the rest of the months ($p < 0.001$). In addition, the abundance of the community in June differed significantly from the community in May ($p < 0.001$) and August ($p = 0.001$). The abundance of biofouling organisms increased from April to June after which a reduction was observed. While no significant differences were detected for depth, it is interesting to note that from June to August the abundance was relatively higher for the plates at the 2 m depth.

The community analysis revealed a significant difference in community structure, as a result of both month and depth, with no effect of month $\times$ depth (Table 2). The biofouling community in April differed significantly compared to the communities that established in the months thereafter (Figure 4). Communities that had the highest dissimilarity (72.88%) were those found in month 1 and month 4 (i.e. April and July, respectively) with the species contributing most to this difference, in order of highest abundance, being the solitary ascidian *Corella eumyota* (currently listed as native), colonial invasive ascidian *Diplosoma listerianum* (Milne Edwards, 1841), invasive tubeworm *Neodexiospira brasiliensis* (Grube, 1872), alien barnacle *Balanus trigonus* (Darwin, 1854) and native colonial ascidian *Botrylloides magnicoecum* (Table 3). It should be noted that by July, the abundance of both *C. eumyota* and *D. listerianum* declined considerably, even though they were still the most dominant species. Communities that

Table 2. Results derived from the PERMANOVA indicating the variables considered (month, depth and month $\times$ depth), degrees of freedom (df), sum of squares (SS), mean sum of squares (MS), Pseudo-F and significance values (P) and number of permutations (Perms).

Source	df	SS	MS	Pseudo-F	P	Perms
Month	4	38701	9675.3	8.3381	0.001	998
Depth	1	4473.6	4473.6	3.8554	0.001	998
Month $\times$ Depth	4	4871.5	1217.9	1.0496	0.405	999
Res	110	1.2764E5	1160.4			
Total	119	1.7569E5				

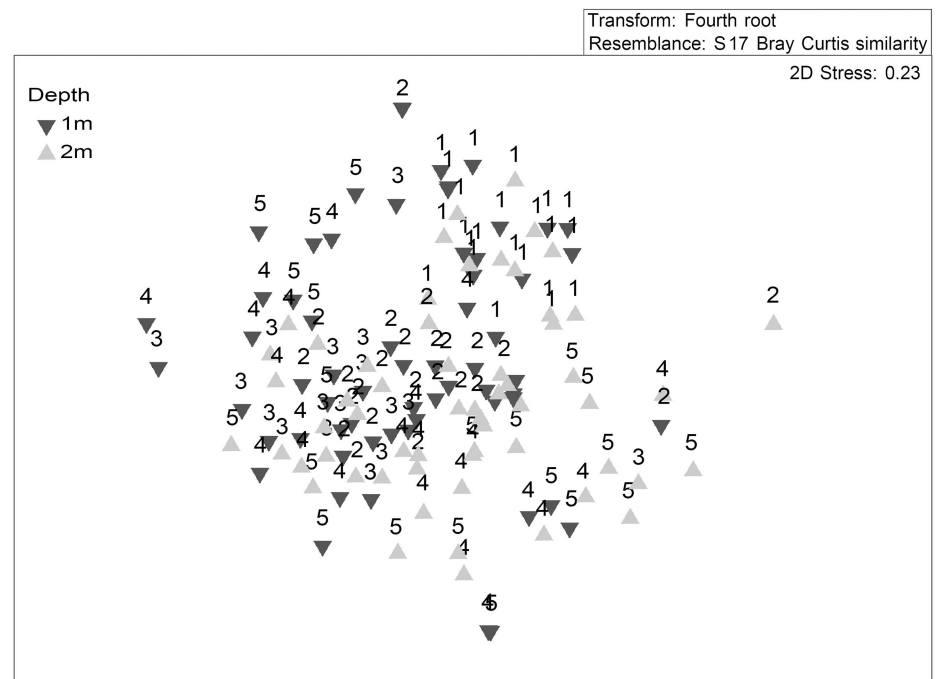


Figure 4. An MDS ordination depicting the difference in biofouling community structure for a period of five months (April–August, depicted by the numbers 1 to 5 in the diagram) and at two depths (depicted by the light grey and dark grey triangles), in the False Bay Yacht Club marina.

Table 3. A subset of the SIMPER analysis of the biofouling community composition for months one and four, months two and three, and between the 1 m and 2 m settlement plates, in False Bay Yacht Club, Cape Town, South Africa. Only the five species that contributed most to the community composition are listed.

Species name	Average abundance		Contribution %
	Month 1: April	Month 4: July	
	Average dissimilarity between months: 72.88%		
<i>Corella eumyota</i>	1.43	2.79	19.43
<i>Neodexiospira brasiliensis</i>	0.45	1.99	16.21
<i>Diplosoma listerianum</i>	0.50	1.70	13.81
<i>Balanus trigonus</i>	0.1	0.77	7.15
<i>Botrylloides magnicoecum</i>	0.00	0.66	5.25
	Month 2: May	Month 3: June	Contribution %
	Average dissimilarity between months: 51.67%		
<i>Corella eumyota</i>	2.84	3.95	20.27
<i>Diplosoma listerianum</i>	1.55	2.61	20.17
<i>Asterocarpa humilis</i>	0.72	0.78	6.16
<i>Neodexiospira brasiliensis</i>	1.67	1.72	5.67
<i>Balanus trigonus</i>	0.70	0.84	5.64
	1 m plates	2 m plates	Contribution %
	Average dissimilarity between plates: 60.69%		
<i>Corella eumyota</i>	2.15	3.17	20.98
<i>Diplosoma listerianum</i>	1.58	1.40	15.53
<i>Neodexiospira brasiliensis</i>	1.64	1.40	9.67
<i>Balanus trigonus</i>	0.71	0.61	7.02
<i>Asterocarpa humilis</i>	0.35	0.62	5.64

were the least dissimilar (51.67%) to one another were the communities at months 2 and 3 (i.e. May and June, respectively) where the presence and abundances of species were similar to the previously mentioned. However, instead of the native ascidian *Botrylloides magnicoecum*, the alien ascidian *Asterocarpa humilis* (Heller, 1878) appeared in the list of species that

contributed most. When we consider the two depths, communities at the 1 m and 2 m plates differed with an average dissimilarity of 60.69% where once again the species mentioned previously, contributed most to these patterns. *Corella eumyota* was more abundant at the 2 m depth (3.17 vs 2.15), whereas *D. listerianum* was relatively even in its abundance between the two depths (1.58 vs 1.40), with similar findings for the other alien species (Table 3).

Considering the total number of species detected across the months, it is notable that the number of alien species remained high relative to the number of native species and species of uncertain identity (Figure 5). Alien species that were initially detected but were no longer found by the third month (i.e. June) were the ascidian *Clavelina lepadiformis* (Müller, 1776) and the bryozoan *Bugula neritina* (Linnaeus, 1758). As the community analysis indicated, the abundances of specific alien species defined those communities along with two native ascidian species, *Botrylloides magnicoecum* and *Corella eumyota*. While *C. eumyota* occurred throughout the five months, being one of the opportunistic species, *B. magnicoecum* only appeared from month two (i.e. May) onward. This is also demonstrated in Table 3 considering the comparison between communities observed in the first month and the fourth month.

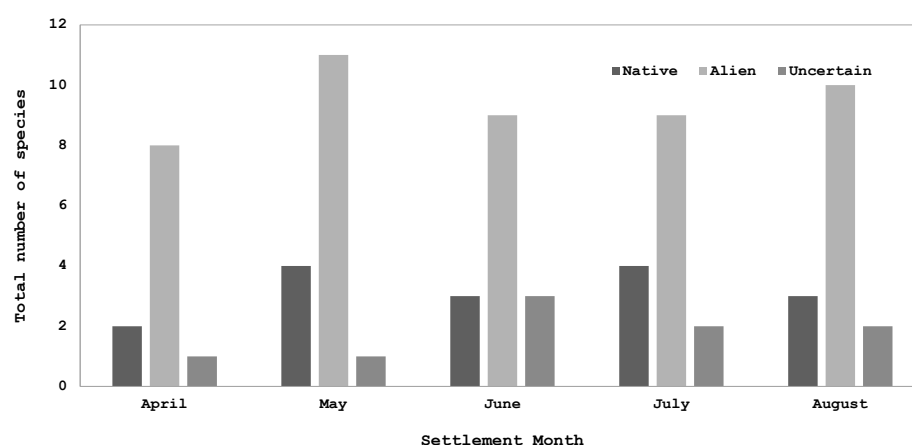


Figure 5. The total number of alien, native and species of uncertain identity detected across the five month settlement period in the False Bay Yacht Club marina.

Discussion

Understanding the ecological patterns of biofouling communities becomes a crucial part of being able to manage biofouling as a vector, particularly when vessels associated with varying levels of biofouling pose a risk of transferring species to new regions (Floerl et al. 2005; Willan et al. 2000; Ulman et al. 2019). The present study demonstrated changes in biofouling communities over a period of five months within a marina, in a temperate region, providing insights into the kinds of communities that could settle on available surfaces, including the hulls and niche areas of vessels. It was evident that alien species persisted throughout the study duration. While

the abundances fluctuated, at least four species were of particular concern throughout the settlement period.

Prior to implementing management strategies, it is important to understand the settlement patterns of marine biofouling species and their population dynamics. Therefore, the varying aspects of ecological succession have to be assessed in order to fully comprehend the nature of biofouling communities, to support management interventions. Studies on ecological succession of such communities often focus on microbial organisms (Watson et al. 2015; Abed et al. 2019). The assessment of ecological succession of macrofouling communities is less studied, particularly those that focus on marine invasive species (Giachetti et al. 2019; Fortič et al. 2021; Diem et al. 2023). Often, the focus is rather to understand the settlement dynamics of particular species to inform prevention mechanisms of biofouling and not necessarily the settlement dynamics and timing of marine alien species in particular, to inform management (Agostini et al. 2019; Muthukrishnan et al. 2022). The typical ecological succession pattern was adhered to in the present study with predominantly alien species taking on the role of pioneer species during primary succession observed in the first month of settlement. By the second month, a significant increase in species richness and abundance was observed and at the 1m depth, signs of competition are observed earlier (i.e. by month 3) than those observed at 2 m (i.e. by month 4), where a substantial decline in species richness was observed along with changes in abundances. Evidently, numbers of alien species remained high throughout the study period when compared to native species, and species of uncertain status, with relative increasing abundances for alien species. The increasing abundance of native species was driven largely by the solitary ascidian *Corella eumyota*.

As competition for space plays a vital role for sessile species, amongst other resources (Comerford et al. 2020), studies have demonstrated that a higher abundance of alien species settle on artificial substrata compared to native species (Tyrrell and Byres 2007; Clarke Murray et al. 2014). This was supported by the results of the present study, as native species could settle on artificial substrata, however, a higher percentage of alien species was found settling on the plates compared to those native species. Two potential outcomes for these results include substrate recognition and selection (Tyrrell and Byres 2007). Native species are less likely to settle on artificial substrata and actively avoid these substrates as they either do not recognise the available space, or it is considered as a suboptimal resource (Anderson and Underwood 1994; Tyrrell and Byres 2007). It is assumed that native species should be able to outcompete and have a competitive advantage on natural substrata compared to artificial surfaces, therefore, finding a higher abundance of alien species settling on artificial surfaces compared to native species is expected (Tyrrell and Byres 2007). However, patterns in community structure may change as settled individuals begin to

grow, intensifying the competition and further pushing the boundaries of limited space. Therefore, in these cases, factors such as, relative adhesion strength, relative growth rate, and additional competition will strongly influence the community structure over time (Tyrrell and Byres 2007). This is evident in the species richness pattern seen across the five months in the present study. It should also be noted that predation is likely to play a role in the population dynamics (Giachetti et al. 2019, 2022). For example, the predation of particular species could provide space for an already dominant species to make use of the newly available space, or it could allow for the settlement of new opportunistic species to occur. Assessing predation was outside of the scope of the current study, however, the disappearance of certain species, such as the invasive ascidian *Clavelina lepadiformis* and bryozoan *Bugula neritina*, was noted. As predatory species (such as crabs, fish, shrimp, polychaetes and nudibranchs) were observed on the settlement plates it can be inferred that those species were feeding on the sessile organisms.

Further, it is likely that the timing of the study could also play a role in shaping the communities observed, given that certain species spawn only in certain months or certain seasons (Fortič et al. 2021). As a result, it would be beneficial to undertake a study that considers this aspect, focusing on species that settle at particular time periods and across different seasons, within the region. Further, Lezzi and Giangrande (2018) found that deploying plates during each season of the year influenced the types of communities that settled, thus emphasising that the starting time of plate deployment and immersion time plays a crucial role in the development of biofouling communities. In the current study, although two seasons were taken into account, the timing of deployment was not staggered and thus it is likely that different communities could have developed if deployments were staggered (i.e., some plates deployed in Autumn and some in Winter). Overall, however, the findings indicated that although species richness and abundances started to decrease over time, alien species still dominated the space, posing associated biosecurity risks both within the invaded region as well as the risk associated with transfer.

The pioneer or opportunistic species in the current study were the invasive tubeworm *Neodexiospira brasiliensis* and the invasive colonial ascidian *Diplosoma listerianum*, as well as the currently listed native solitary ascidian *Corella eumyota*. These were also the species that occurred throughout the settlement period of five months, demonstrating their competitive nature within biofouling communities in the marina. By settling first and establishing their dominance, these species appeared to have a competitive advantage over other species that settled later. The colonial ascidian *D. listerianum* is able to overgrow other species giving it an additional competitive advantage, a trait that has been demonstrated for

other epibiotic invasive species (Leonard et al. 2017). Another species that appeared to be persistent was the barnacle *Balanus trigonus*, although this species occurred in lower abundances, it is known to be quite a hardy and successful invasive species (Spagnolo et al. 2019) which is evident in the current study as well. The initial opportunistic nature and persistent dominance of the currently listed native species, *C. eumyota* (e.g., Loureiro et al. 2021) brought into question whether the classification of this species should be reassessed in the region. The species demonstrated characteristic traits that were more in alignment with invasive species. Although the species is assumed to be native to the southern hemisphere (Monniot et al. 2001; Lambert 2004), one study has suggested that the populations in South Africa and New Zealand may be introduced (Dupont et al. 2007). However, the genetic analyses focused on microsatellite markers which may not provide a clear enough conclusion regarding the status of the species. The status of this species is currently being assessed in the region through genetic analyses that focus on mitochondrial DNA, which may provide improved insights into the classification of the species. It should also be noted that some studies have listed *C. eumyota* as a cryptogenic species (e.g. Primo and Vázquez 2004) and others have listed it as introduced (e.g. Arenas et al. 2006) which aligns with the characteristics of the species being demonstrated in the current study. What is evident from the literature is that the morphological identification of *Corella* species in particular are raising concerns as genetic analyses are revealing the complexity of the current status of such species, including that of *C. eumyota* (Alurralde et al. 2018). The species highlighted in the current study, therefore, provide important information that may have been overlooked, particularly in the case of *C. eumyota*, when gaining information from only one period in time, as opposed to tracking their settlement patterns.

The high numbers of alien species that are able to settle within the first two to three months raises concerns regarding the risk of transferring species. Within one month, eight of the 12 alien species (67%) detected, had already settled and although they occurred in low abundances initially, it raises concerns considering the risk of transfer at such an early stage of the settlement period, particularly if these organisms were to settle in the niche areas of vessels, or if they have morphological adaptations, such as dwelling in tubes, that may increase their likelihood to survive transit (Ros et al. 2020). With recreational vessels highlighted as important transfer mechanisms of marine alien species (Peters et al. 2019a; Ulman et al. 2019; Ashton et al. 2022) the findings of the current study are relevant to the risk associated with this transfer, particularly as vessels with seemingly no biofouling or low levels of biofouling, could host high numbers and abundances of alien species in the niche areas (Zabin et al. 2014; Peters et al. 2019b). It is notable that the plates used in the current study did not

have any anti-fouling coats. However, studies have shown that the age of anti-fouling paint and the time since a vessel was last cleaned has not deterred the settlement of marine alien species over short periods of time (Floerl et al. 2005; Peters et al. 2019b). While the numbers of alien species detected on the settlement plates in the current study cannot be directly comparable to vessel hulls, it demonstrates the settlement on plates as a proxy for the potential risk associated with fouling of vessels. Recreational vessels were previously studied in the False Bay Yacht Club where 88% of vessels supported alien species and the numbers of alien species ranged from one to nine species per vessel (Peters et al. 2019b). It is important to note that the age of these biofouling communities were not known for all vessels and the majority of yacht owners (70%) indicated that they do not clean their vessels before sailing, posing a risk of transfer, depending on the types of species present and the distance and speed at which the vessels travel (Davidson et al. 2008; Coutts et al. 2010; Pelletier-Rousseau et al. 2019).

Considering previous surveys undertaken in the marina on existing marina infrastructure, a total of 15 alien species were detected. However, this included the larger area of the Simon's Town harbour (Peters et al. 2017) and thus is not directly comparable to the current study. Further, a total of 16 alien species were previously detected on recreational vessels in the marina (Peters et al. 2019b), eight of which were not detected in the current study. In addition, three species detected in the current study were not recorded on the recreational vessels sampled previously. It should also be noted that the previous studies in the marina were once off sampling events, at different times (i.e., months, and thus seasons) and included a combination of both scrape and visual sampling techniques. This combination of techniques enhances the identification and detection of alien species within the biofouling communities (Peters et al. 2019a). Nevertheless, these differences highlight some of the constraints encountered with temporal settlement studies since the aim is to assess the change in communities and thus samples cannot be collected for more detailed investigation. It further reiterates the importance of assessing settlement patterns across different seasons and that the timing of sampling, the age of the biofouling communities and the type of artificial structure being assessed may influence the findings.

One of the largest constraints of the current study was in the identification of organisms that could not be identified to species level. Since the morphological characteristics of the species could not be identified based on current local identification guides, they could either be new introductions or undescribed species. It could also be a case of visual sampling not being as accurate as microscopic and genetic analyses that could be attained when making use of scrape samples. Since the aim of the current study was to track the settlement patterns and ecological succession of species, the aforementioned methods were beyond the ambit of the

study. It has previously been highlighted that the accurate identification of species, and thus their status, in the field of marine invasion biology is one of the greatest obstacles that hinders our ability to better understand the patterns that we find (Alurralde et al. 2018). It is, therefore, important for taxonomic guides to incorporate an integrated taxonomic approach to ensure accurate identification of species (Duarte et al. 2021), particularly in studies where the identification of species becomes a crucial part of the findings. Further recommendations for the present approach is to extend the tracking period (which is currently underway in the region) and to expand this approach to other regions to determine whether similar patterns occur. It is also quite crucial to monitor the settlement patterns on an annual basis to gain a clearer pattern of what is being observed on a monthly basis and to determine whether there are any seasonal patterns in the settlement (Lezzi et al. 2018).

In conclusion, the findings of the current study provide a case study for a marina in a temperate region and whilst the study was based in South Africa, the approach can be applied elsewhere to provide in-depth insights into the change in biofouling communities, with a particular focus on marine alien species. This research has demonstrated the complex nature of biofouling communities and the need for further studies to disentangle patterns that may occur across longer and varying timescales, taking seasons into account and across different regions. It was also clear in the current case that tracking the settlement patterns and ecological succession of biofouling communities revealed concerning patterns that could change the status of species. These are patterns that may have been overlooked when sampling only in a single time period as opposed to tracking temporal settlement patterns. The findings of the current study, therefore, demonstrate two key elements. Firstly, that a longer-term monitoring program, across different seasons that tracks the temporal settlement patterns of alien fouling species could provide valuable insights to the field and thus the management of invasives. Secondly, since the highest numbers of alien species were already detected within the first two months in the current study, this shorter time period could be used as a favourable monitoring option when resources are limited, and this could also be implemented seasonally. This finding should, however, be further investigated across different sites and during different seasons. Overall, implementing these approaches could assist management authorities considerably, particularly when prioritising regions to monitor, or targeting particular time periods to monitor and manage marine invasions.

Authors' contribution

KP conceptualised the research, study design and methodology. SM and KP played a role in the investigation, collection, analysis and interpretation of the data. KP applied for ethics clearance. Funding was provided via KPs departmental fund. KP wrote the original draft and SM contributed to parts of the draft. SM and KP both reviewed and edited the draft.

Acknowledgements

Salizwa Ntlabathi, Sibabalwe Tono and Juanita van Baalen are thanked for their assistance in the field and the collection of data. The marina staff at the False Bay Yacht Club are thanked for their continued support and assistance with this research. Mogamat Sedick Gallie is thanked for his assistance with purchasing and collating equipment and the Department of Conservation and Marine Sciences is thanked for the funding to purchase equipment. Charles Griffiths and Melissa Boonzaaier are thanked for their taxonomic expertise and Grea Groenwald for her statistical expertise. Finally, two anonymous reviewers are thanked for their valuable input.

Funding declaration

Funding for equipment was provided by the Department of Conservation and Marine Sciences in the Applied Sciences Faculty of the Cape Peninsula University of Technology.

Ethics and permits

An ethics exemption was granted for the current study by the Faculty of Applied Sciences, Research Ethics Committee, Reference no: 219190127/06/2022. Whilst no collections took place in the current study, a site permit was obtained from the marina.

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