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Research Article

Encapsulation as a biosecurity tool for managing fouling on recreational vessels

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Abstract

Hull fouling is a dominant vector in marine systems, with recreational vessels playing an important role in intra-regional transfer of biota. Encapsulation (i.e. the wrapping of a structure in plastic to deprive biota of oxygen and food, ultimately causing their death), offers promise as a tool for treating vessel fouling *in situ*. However, there is currently no standardised approach with detailed field application recommendations. In recognition of this gap this study aimed to: 1) use laboratory experiments to establish a timeframe for the effective encapsulation of yachts, 2) test this approach in the field and 3) consider the practicalities associated with implementing an encapsulation program. Laboratory experiments exposed the ascidian *Ciona robusta*, the mussel *Semimytilus algosus* and fouling communities to four treatments: aerated control in seawater, encapsulation in seawater, aerated seawater with 4% acetic acid and encapsulation in seawater with 4% acetic acid. All biota in acetic acid died in 24 hours regardless of encapsulation, while in encapsulated seawater mortality of all taxa occurred within three days. In the field four yachts and five pontoons with high (80–100%) and low (30–50%) fouling cover were encapsulated. It took more than three days to achieve mortality on all structures (pontoons high cover 3.7 days (± 0.48 SD); pontoons low cover 3.8 days (± 0.42 SD) and yachts 4.3 days (± 0.5 SD)). The discrepancy between laboratory and field results likely reflects an unavoidably higher water to fouling biomass ratio in field systems. These results suggest that five days may be sufficient for successful encapsulation of yachts. However, in recognition of the limited sample size of yachts in this study, it is recommended that these findings be used as a basis for further developing region specific protocols through adaptive management. Logistical considerations around the implantation of national encapsulation programs are also discussed.

Key words: *Ciona robusta*, fouling communities, intra-regional spread, invasion management, *Semimytilus algosus*

Introduction

Hull fouling is a dominant transport-related vector for the introduction of non-indigenous species in marine systems (Peters et al. 2017). Due to the high number of commercial ships and the frequency with which they move among international ports, a large body of research has considered the role of these vessels as vectors of fouling biota (Hopkins and Forrest 2010; Davidson et al. 2016). In contrast, smaller recreational vessels which predominantly move intra-regionally have been largely overlooked as a

vector (Clarke Murray et al. 2011; Peters et al. 2019), but the biosecurity risk they pose is becoming increasingly evident (Martinez-Laiz et al. 2019). In response, approaches for identifying yachts likely to support diverse fouling communities have been developed (Floerl et al. 2005), rapid assessment tools have been evaluated (Clarke Murray et al. 2013) and methods for accurately detecting alien biota on hulls have been assessed (Peters et al. 2019). Regardless of vessel type, biofouling is largely unregulated (Zabin et al. 2018) with a recent review highlighting that only three agencies, the Department of Conservation, New Zealand, the Western Australia Department of Primary Industries and Regional Development and the Galapagos Marine Reserve, have formal guidelines and protocols for assessing biofouling levels of vessels upon entry. Currently, biofouling regulations exist or are being investigated only in New Zealand, Australia, Canada, and certain states in the USA (Martinez-Laiz et al. 2019).

Management of vessel fouling has two foci i.e. prevention and removal. Antifouling paint is the primary tool used to prevent fouling (Floerl 2003). Antifouling paints containing copper have been used since the 1860s but these were replaced with TBT-containing paints after the Second World War (Yebra et al. 2004). However, due to TBT having deleterious impacts on non-target organisms (Oehlmann et al. 1996; van Gessellen et al. 2018), the use of TBT-containing paints was globally banned in early 2003 (IMO 2001). Since this time there has been a reversion to copper-based paints (Chen et al. 2013) although new generation paint formulations, including organic booster biocides, self-polishing copolymer paints and silicone fouling-release coatings are increasingly in use (Thomas 2001; Callow and Callow 2002; Konstantinou and Albanis 2004). Fouling removal tends to be labour-intensive, inconvenient and costly, as it most often entails the removal of vessels from the water and can thus only happen when dry docks are available (Inglis et al. 2012). Importantly, this process can result in the release of organisms into the water column, an important consideration from a biosecurity perspective (Hopkins and Forrest 2008).

South Africa has a coastline of 3650 km which spans multiple ecoregions, ranging from the cool temperate Benguela system on the west coast to the tropical Delagoa region on the east coast (Griffiths et al. 2010). Despite the length of this coastline, the country has only nine ports of entry of which seven receive foreign commercial vessels and only six have marinas that receive yachts (Figure 1). An additional six marinas service recreational vessels outside of official ports. This relatively low number of ports and marinas reflects the exposed nature of the coast. Despite this, coastal sailing is common in South Africa and the 12 marinas support an average of 140 (range 50–350) sailing vessels (hereafter referred to as yachts) (Robinson *unpublished data*). Notably, in South Africa motorised yachts are not common as the rough and exposed coastline is generally not

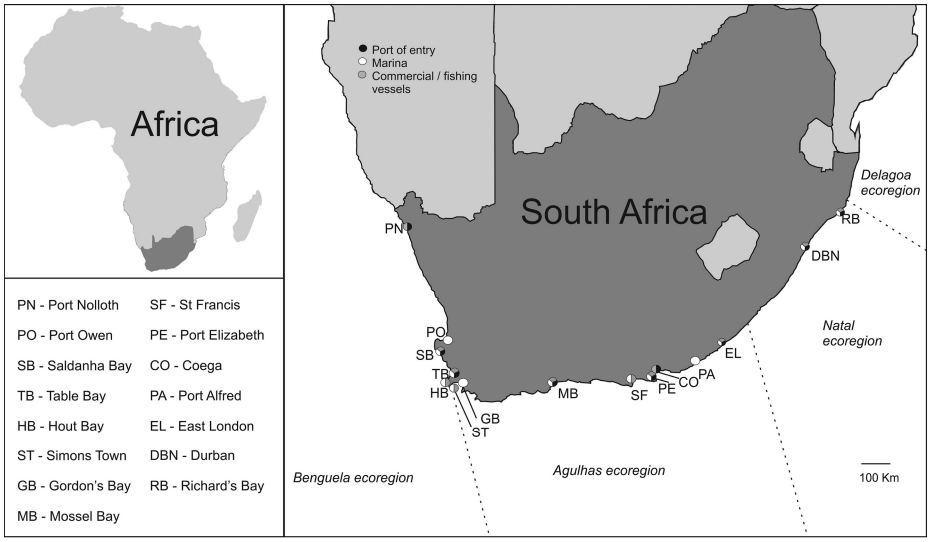

Figure 1. A map of South Africa showing the various ports and marinas along the coast. The vessels serviced at each are also indicated. The straight lines indicating the various coastal ecoregions are for illustrative purposes only.

conducive to their use. Because of the orientation of the South African coastline international yachts arrive either in Richard's Bay on the east coast or in Table Bay or Saldanha Bay on the west coast. However, as more than 85% of yachts arriving are cruising vessels (as opposed to racers taking part in international events), most travel with prevailing winds and arrive on the east coast. Between 2007 and 2016 an average of 31 international yachts entered the country via this route (Robinson *unpublished data*). Presently there are no regulations in place to manage yacht fouling in South Africa and despite the recognition of the bioinvasion risk they pose (Peters et al. 2017) international yachts move freely in South African waters. There is thus a clear need to address this gap in biosecurity. However, in this region resources allocated to marine biosecurity are limited and no personnel are allocated explicitly to this portfolio. As such, any measures developed need to be easily implementable with relatively little additional demand on resources. Measures should also be effective, so as to prevent wasting already sparse resources.

In-water encapsulation of vessels is a relatively new and potentially viable approach to managing the biosecurity risk associated with fouling (Coutts et al. 2010). Encapsulation refers to the wrapping of a structure, usually in plastic, to deprive fouling organisms of light, oxygen and food (Coutts and Forrest 2007). The mechanisms through which the encapsulation process likely acts are induced hypoxia, the build-up of waste products such as ammonia and sulphides and the interactions of these adverse conditions. Respiration by organisms within the encapsulation system results in the rapid depletion of oxygen (Coutts and Forrest 2007) and ultimately the development of anoxic conditions inside the encapsulation system (Atalah et al. 2016). Temperature affects the rate of dissolved oxygen depletion, as both respiration and decomposition occur faster at

high temperatures (Theede et al. 1969). The implication of this for the encapsulation process has been highlighted before, with encapsulation at high temperatures resulting in considerably lower dissolved oxygen levels than at lower temperatures (Atalah et al. 2016). Under anoxic conditions, the conversion of sulphur to hydrogen sulphide is favoured during decomposition (Atalah et al. 2016). In addition, the bacterial reduction of sulphides along with the putrefaction of proteins results in high levels of hydrogen sulphide (Theede 1973). Hydrogen sulphide can be toxic as it forms sulphides with ions of heavy metals, resulting in the interrupting of cellular respiration (Theede 1973). As such, high sulphide and low oxygen conditions have the same effect on organisms, ultimately resulting in suffocation (Bagarinao 1992). Hydrogen sulphide toxicity can also be affected by temperature, as under high temperatures metabolic rate is enhanced, which in turn elevates oxygen demands, heightens the production of hydrogen sulphide and hastens the suffocation of biota. Additionally, pH affects sulphide toxicity as it affects the form in which sulphide can occur. Ammonia is an unusual toxic substance in that it is produced by and yet poisonous to animals (Ip et al. 2001). Ammonia can be especially dangerous to aquatic organisms as it is taken up easily through gills and cell membranes (Boardman et al. 2004). It is toxic to organisms as a result of the formation of nitrates, which enhance the conversion of oxygen-carrying pigments to forms that are incapable of carrying oxygen, thus resulting in oxygen deficiency and ultimately suffocation (Camargo et al. 2005). When ammonia is present in water at high enough levels, it is difficult for aquatic organisms to sufficiently excrete the toxicant, leading to a toxic build up and ultimately death (Boardman et al. 2004). Ammonia toxicity increases as nitrate concentration increases (Camargo et al. 2005). Environmental factors, such as pH and temperature, can affect ammonia toxicity in aquatic animals, as ammonia toxicity increases with water pH (Randall and Tsui 2002) and temperature (Wurts 2003). Because of the effects of pH on various aspects of encapsulation, the use of acetic acid and chlorine as additives to alter pH has been considered (Forrest et al. 2007; Roche et al. 2015; Creed et al. 2019).

Encapsulation offers promise as a management approach as it enables vessels to be treated *in situ*, negating the expense of removing boats from the water (Roche et al. 2015). This could make it particularly useful in regions with limited resources. However, few studies have tested this technique on yachts in the field (but see Roche et al. 2015; Atalah et al. 2016; Morrisey et al. 2016), resulting in the lack of a consolidated evidence-based approach to applying encapsulation in the field. In recognition of the importance of yacht fouling as a vector of marine alien invasive species and the need to develop an effective management strategy to dealing with foreign yachts arriving in South African waters this study aimed to: 1) use laboratory experiments to establish a timeframe for the effective encapsulation of yachts, 2) test this approach in the field and 3) consider the practicalities associated with implementing an encapsulation program.

Materials and methods

Laboratory experiments

Laboratory experiments were used to determine a timeframe for encapsulation in the field. Model species likely to represent the extremes of vulnerability to encapsulation were chosen. The hard-shelled invasive mussel *Semimytilus algosus* (2–3 cm) was collected from Gordon's Bay Yacht Club (34°09′52″S; 18°51′42″E), while the soft-bodied ascidian *Ciona robusta* (tunic length 2–4 cm measured out of the water), were collected from Yacht Port Marina (33°01′36″S; 17°57′40″E) in Saldanha Bay (Figure 1). Biota were transported back to the laboratory at Stellenbosch University (33°93′28″S; 18°86′44″E) and were immediately exposed to experimental conditions.

In addition to individual taxa, fouling communities were also considered. These were allowed to settle on 20 cm × 20 cm PVC plates deployed at depths of 1.5–2.5 m in Yacht Port Marina for sixteen weeks. When they were retrieved percentage fouling cover was estimated visually using a grid. Fouling cover ranged from 85% to 100% (92.3 ± 4.2 SD). These communities were diverse with a mean of 8 (± 5 SD) species present per plate, representing a mix of indigenous and alien species (see Supplementary material Table S1).

Experimental containers measuring 45 cm × 30 cm × 15 cm were used for all laboratory work. A single organism or fouled plate was placed in each container and water was added to achieve a 1:3 ratio of biomass to water. Both individuals and fouling communities were exposed to four different treatments in the laboratory: a control containing aerated seawater, encapsulated seawater (hereafter referred to as encapsulation treatment), an aerated 4% acetic acid solution (hereafter referred to as aerated acetic acid treatment), and an encapsulated 4% acetic acid treatment (hereafter referred to as encapsulated acetic acid treatment). For experiments considering *S. algosus* and *C. robusta* six replicates were applied per treatment while five replicates were considered for fouling assemblages. All laboratory experiments were undertaken at 13 °C which is representative of the areas from which the biota were collected (Smit et al. 2013).

Water quality was assessed for each treatment prior to the commencement of the experiments and daily thereafter, until all biota died. Parameters considered included pH, sulphide, ammonia and dissolved oxygen. These were chosen to confirm desired treatments (i.e. effective encapsulation as reflected in low dissolved oxygen levels and the effect acetic acid as reflected in lowered pH) as well as to gain a measure of the effects of the treatments (e.g. increasing sulphide and ammonia concentrations). Prior to the experiments, a hole of 10 cm × 10 cm was cut in each lid and a 2 L plastic access bag was secured in this space. This access bag allowed the sampling of water and the checking of mortality (described below) without disturbing the airtight seal. These water samples

were collected through the access bag using a needle and syringe, and the resulting holes were immediately sealed with waterproof tape. Changes in these parameters were statistically assessed by means of mixed effects models, where each outcome variable (ammonia, sulphide, dissolved oxygen and pH) was assessed in relation to treatment (control aerated sea water, encapsulated sea water, aerated sea water with 4% acetic acid and encapsulated sea water with 4% acetic acid) and time as fixed factors. Replicates were considered a random factor to account for repeated measures through time. For all analyses assumptions were considered through visual inspection of residuals in R version 3.4.2. Mixed effect models were conducted using the lme4 and car packages.

Mortality was assessed daily using two complementary methods. Firstly, by touching biota with a needle through the plastic bag, where organisms were considered dead if no movement occurred when prodded (Floerl et al. 2005). Secondly, once all biota in a replicate were thought to be dead due to a lack of response, loss of physical integrity or colouration, they were removed and placed in control conditions for 24 hours after which mortality was visually confirmed (Hopkins et al. 2016). Mortality was assessed at the same time as the collection of water samples so as to minimise the number of times that encapsulation bags were punctured. Treatments were terminated when 100% mortality was reached in all replicates. Time to mortality was statistically analysed by means of mixed effects models, where treatment, ammonia and sulphide concentrations were considered fixed effects. Again, replicate was applied as a random factor to account for the repeated measures through time.

Field experiments

In total four yachts were encapsulated, one at Port Owen Marina and three in Table Bay. At the latter site, encapsulation of pontoons underneath marina walkways provided an opportunity to assess the effect of fouling cover on the efficacy of the process. Ten pontoons with high fouling cover (80–100%) and ten with low cover (30–50%) were considered. Communities on these pontoons were three years old (Dipenaar *pers. comm*., marina manager). These pontoons were deemed appropriate proxies for yachts as they accounted for one third of submerged surface area of yachts, reached a depth of at least 1.5 m (the depth within which most fouling occurs on yachts in South African marinas (Robinson *pers. obs.*)), and supported the same invasive fouling species as recorded on the yachts (see results). Nonetheless, it is acknowledged that pontoons do not possess niche areas. During the field tests ambient water temperatures were 17.1 (± 1.9) Prior to encapsulation, pontoons and yachts were surveyed by an experienced scientific diver for the presence of alien fouling species. In order to test the assumption that pontoons offer good proxies for yachts,

alien fouling community composition was compared between yachts and pontoons with low and high levels of fouling. This was done by applying an ANOSIM test in Primer 6 based on dissimilarities of the presence / absence data gained during the diver surveys.

After surveying the vessel, the diver pulled the encapsulation device up over the hull of the vessel and ropes were used to secure the device onto the vessel. Water was then pumped out of the encapsulation device at 15 l/sec using a SalFlo submersible pump. Water was removed until it was not possible run the pump any longer without potential damage to the device. This served to minimise the biomass to water ratio within the device. The same process was carried out for pontoons, where ropes were attached to the marina walkways. All pontoons were encapsulated at the same time. Due to legal considerations acetic acid could not be used in the field.

Water samples were taken daily from six randomly selected positions inside and outside of the encapsulation device. For each sample, dissolved oxygen, ammonia and sulphide levels were measured. These variables were measured through time to track the depletion of oxygen and the build-up of waste products (i.e. ammonia and sulphide) through time. Water quality was assessed inside and outside of encapsulation device for both yachts and pontoons. Dissolved oxygen, ammonia and sulphide levels on the initial day of experiments were compared to those on the day on which total mortality occurred using t-tests or Mann-Whitney tests, depending on the nature of the data. Separate analyses were conducted for samples collected inside and outside of the device as interactions between these positions were not of interest.

Mortality was assessed daily in the same way as in the laboratory experiments, where organisms were considered dead if no response was observed after being prodded through the encapsulation device. Once all biota appeared dead, the device was removed and after 24 hours organisms were visually assessed to confirm mortality. The time taken to reach total mortality was analysed using a Generalised Least Squares model. Structure (yachts, pontoons with high coverage and pontoons with low coverage), dissolved oxygen, ammonia and sulphide levels inside of encapsulation device on the day on which total mortality was recorded were investigated as potential predictors of time to total mortality with the best fit model being selected using AIC values.

Results

Laboratory experiments

For all laboratory experiments, there were significant effects of treatment and time on all four water quality measures (Table 1). For all experiments controls were deemed effective as no decrease in dissolved oxygen and pH, nor increase in waste products were observed. In all other treatments, dissolved

Table 1. Main effects Wald test results from generalised mixed effects models considering the fixed effects of treatment and time on (a) dissolved oxygen, (b) pH, (c) ammonia and (d) sulphide during experiments considering *Semimytilus algosus*, *Ciona robusta* and fouling communities.

	Semimytilus algosus		*Ciona robusta*		Fouling communities	
	χ	p	χ	p	p	χ
(a) Dissolved oxygen						
Treatment	102.41	< 0.001	23.06	< 0.001	70.52	< 0.001
Time	48.72	< 0.001	14.98	< 0.001	16.33	< 0.001
(b) pH						
Treatment	931.74	< 0.001	300.99	< 0.001	1106.77	< 0.001
Time	19.16	< 0.001	51.54	< 0.001	7.39	< 0.001
(c) Ammonia						
Treatment	113.89	< 0.001	26.70	< 0.001	73.59	< 0.001
Time	79.57	< 0.001	81.95	< 0.001	27.25	< 0.001
(b) Sulphide						
Treatment	28.48	< 0.001	9.70	< 0.001	32.79	< 0.001
Time	39.73	< 0.001	14.75	< 0.001	31.46	< 0.001

oxygen and pH decreased significantly through time, while ammonia and sulphide increased, although the magnitude of these changes varied among taxa (See Table S2 for details). The encapsulated and encapsulated acetic acid treatments had significantly lower dissolved oxygen levels than the aerated control and acetic acid treatments ($p < 0.05$ in all cases, see Tables S3 (*Semimytilus algosus*), S4 (*Ciona robusta*), S5 (fouling communities)). Both treatments containing acetic acid had a lower pH level when experiments commenced, as expected. In all experiments, the pH of the encapsulation treatment decreased through time, but was still significantly higher than the pH of the treatments containing acetic acid ($p < 0.05$ in all cases). The levels of both ammonia and sulphide increased through time in all treatments besides controls, with the encapsulated treatment having the highest levels of both these waste products.

No mortality of individuals or communities was observed in control units for the entire duration of the experiments (Figure 2). Encapsulation and acetic acid treatments were deemed effective as 100% mortality was recorded for organisms and communities exposed to these treatments. All *C. robusta* individuals in all other treatments besides the control died within 24 hours negating any statistical analyses. There were significant effects of treatment, ammonia and sulphide on time to mortality for both *S. algosus* and the fouling communities (Table 2). *Semimytilus algosus* individuals in aerated acetic acid (coefficient = −1.45, t = 14.67, $p < 0.001$) and acetic acid (coefficient = −1.50, t = 15.42, $p < 0.001$) treatments had significantly shorter time to mortality (24 hours ± 0 SD in both cases) than those in the encapsulated treatment (72 hours ± 0 SD). There was no significant difference between the acetic acid treatments (coefficient = −0.41, t = −48, $p > 0.05$). The same was observed for fouling communities, where those in both the aerated acetic acid (coefficient = −1.31, t = −6.55, $p < 0.001$) and the encapsulated acetic acid (coefficient = −1.11, t = −6.71, $p < 0.001$) treatments died significantly faster than those in the encapsulation treatment. There was no significant difference in time to mortality between

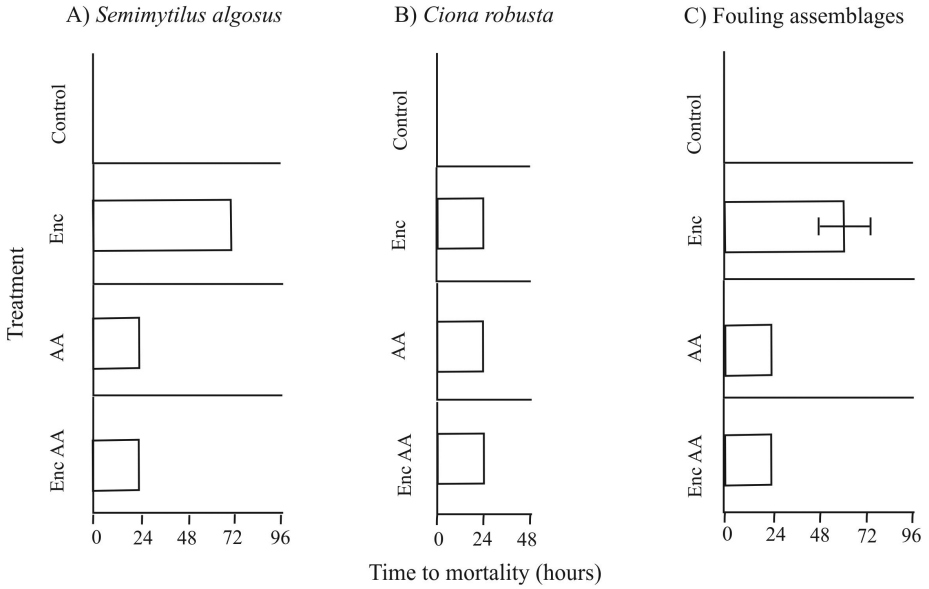

Figure 2. Time to mortality (hours) of a) *Semimytilus algosus*, b) *Ciona robusta* and c) fouling assemblages through time in different treatments. Enc: Encapsulation; AA: Aerated acetic acid; Encapsulated acetic acid.

Table 2. Main effects Wald test results from generalised mixed effects models considering the fixed effects of treatment, ammonia and sulphide on time to mortality (days) for *Semimytilus algosus* and fouling communities. Mortality of all *Ciona robusta* individuals by 24 hours precluded statistical analyses of this species.

	S. algosus		*C. robusta*	
	χ	p	χ	p
Treatment	1.5	< 0.001	62.71	< 0.001
Ammonia	1.64	< 0.001	0.99	< 0.001
Sulphide	3.26	< 0.001	7.44	< 0.001

the acetic acid treatments (coefficient = 0.07, t = 0.41, p > 0.05). Notably, *S. algosus* in the encapsulated treatment took the longest to die of all biota (i.e. 72 hours). Based on these results, it was suggested that encapsulation for a period of three days be trialled in the field.

Field experiments

In total eight alien fouling species were recorded on yachts and pontoons. These were the mussels *Mytilus galloprovincialis* and *Semimytilus algosus*, as well as the ascidians *Ciona robusta, Clavelina lepadiformis, Microcosmus squamiger, Botryllus schlosseri, Ascidiella aspersa* and *Styela plicata*. Notably all species were recorded on both yachts and pontoons and community composition did not vary between the structures (ANOSIM, R = −0.085, p = 0.91).

The average time taken to reach total mortality on pontoons with high and low cover was 3.7 (± 0.48 SD) days and 3.8 (± 0.42 SD) days respectively, whereas for yachts this time was 4.25 (± 0.5 SD) days (Figure 3). The best fit GLS model was significant and included structure, ammonia, sulphide and dissolved oxygen as predictors for time to mortality (Table 3). Structure was the only significant main effect (p < 0.001), where yachts had

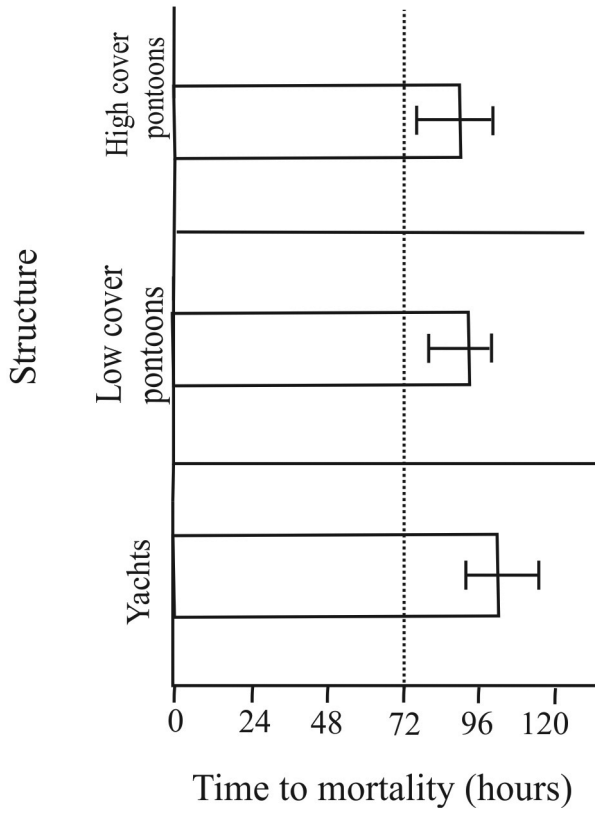

Figure 3. Mean ± SD time taken to reach total mortality for yachts and pontoons with high and low fouling cover in the field. Dotted line represents the expected effective encapsulation period of three days based on laboratory experiments.

Table 3. Statistical results from the generalised least squares model considering predictors of the time taken for fouling communities to reach total mortality.

Factor	F-value	p-value
Structure	21.34	< 0.001
Dissolved oxygen	140	0.24
Ammonia	2.81	0.10
Sulphide	0.002	0.96

a longer time to mortality than pontoons with either a high (coefficient = 0.50, t = 6.28, $p < 0.001$) or low fouling cover (coefficient = 0.43, t = 5.73, $p < 0.001$). Notably there was no difference in time to mortality between pontoons with high and low fouling ($p > 0.05$).

Water quality within encapsulation device declined through time but did not change outside of the encapsulation system (see Tables S6 (pontoons high cover), S7 (pontoons low cover), S8 (yachts) for full statistical results). Ammonia and sulphide levels were significantly higher on the final day of experiments than the initial day, while dissolved oxygen levels and pH were both lower on the final day of encapsulation (Mann-Whitney, $p < 0.001$ in all cases, Figure 4).

Discussion

Encapsulation is recognised as a potential management tool for hull fouling on vessels (Coutts et al. 2010; Roche et al. 2015; Atalah et al. 2016). This study considered the potential of this technique for treating fouling

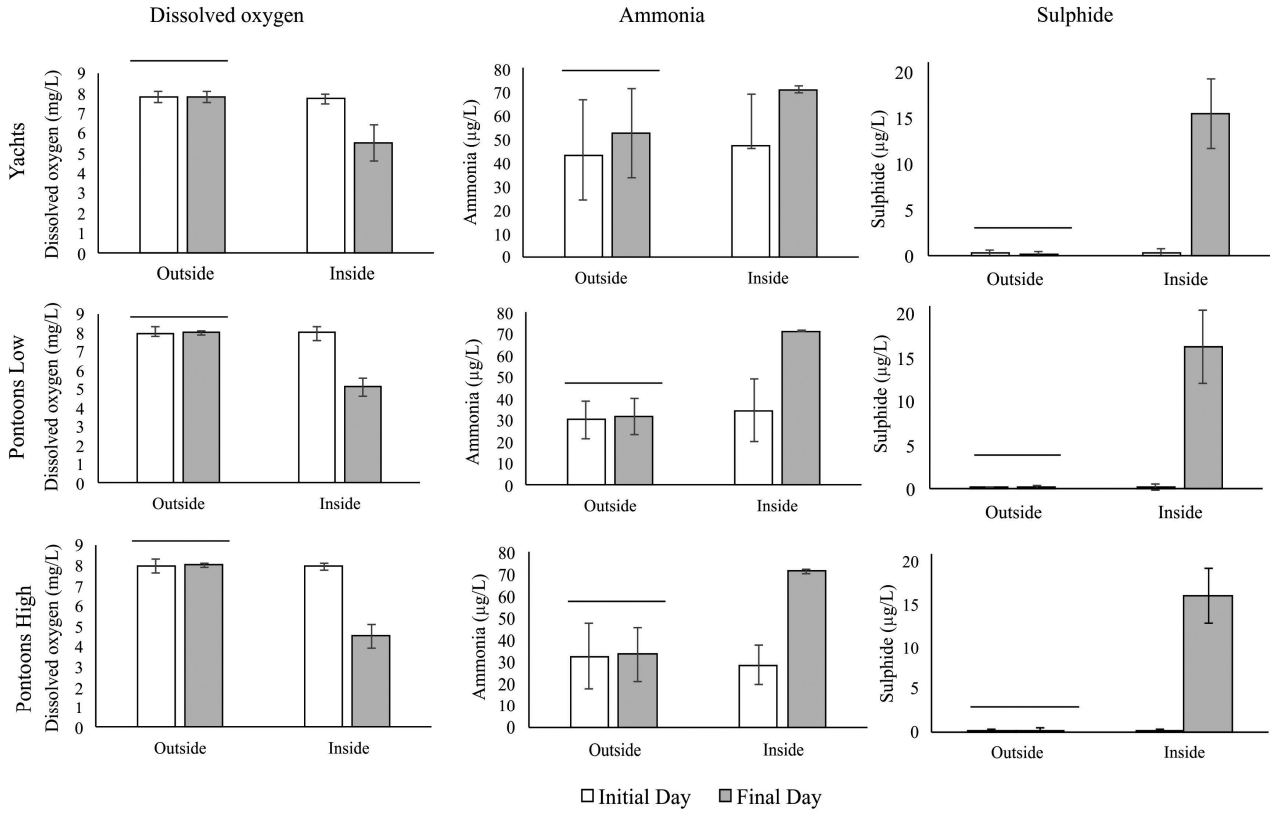

Figure 4. Mean ± SD levels of dissolved oxygen, ammonia and sulphide inside and outside the encapsulation device on the first and final day of encapsulation. Lines connect bars that do not differ significantly ($p < 0.05$).

on recreational vessels with a view to minimising their biosecurity risk. While laboratory experiments suggested that three days of encapsulation would be effective in achieving total mortality of all taxa, this was found to be too short when tested in the field. Instead, the field study demonstrated that up to five days of encapsulation was required to kill all fouling biota on vessels. This approach was effective at killing all fouling, including all alien species.

Laboratory results in this study confirmed that encapsulation results in total mortality of fouling biota, including soft-bodied ascidians, hard-shelled mussels and fouling communities as a whole. The alien ascidian, *Ciona robusta*, was found to be more sensitive to encapsulation than the hardy invasive mussel *Semimytilus algosus*. This finding is likely a result of the differences in morphology between these taxa. Soft-bodied biota such as ascidians and cnidarians have vulnerable soft, fleshy tissues that offer little barrier to the environment and leave them exposed directly to the marine environment (Pawlik 1993). In contrast, taxa such as mussels, have protective calcium carbonate shells (Falini et al. 1996) that offer a physical barrier between the surrounding environment and the animal. Molluscs utilise this as a defence against adverse conditions, sealing up their valves for extensive periods of time when the environment becomes unfavourable (Shumway 1977; Atalah et al. 2016), explaining why *S. algosus* survived longer than *C. robusta*. In a previous encapsulation study considering the

mussel *Mytilus galloprovincialis* and the ascidian *C. robusta* (Atalah et al. 2016), the time to mortality for the ascidian was the same is in the current study, but much longer for the mussels (i.e. 18 days *vs* three days). It is likely that these differences reflect the larger sized mussels (5–7 cm) used by Atalah et al. (2016) compared to the 2–3 cm sized mussels used in the current study. When mussels are found on yachts in South Africa they tend to be less than 3 cm in length (Robinson *pers. obs.*) but it is acknowledged that foreign vessels arriving may support larger mussels in which case encapsulation duration may need to be extended. It is also possible that this difference in time to mussel mortality reflects the effect of temperature on encapsulation as Atalah et al. (2016) conducted their laboratory work at 10 °C while the current study was conducted at 13 °C.

Previous studies have demonstrated that the addition of acetic acid to encapsulation systems decreases the treatment time of a variety of taxa (Forrest et al. 2007; Roche et al. 2015; Atalah et al. 2016). The addition of a 4% acetic acid solution during laboratory experiments in this study reduced the treatment time significantly for both the mussels and fouling communities, but this effect of acetic acid was not observed for the ascidians. However, this apparent lack of an additive effect on soft-bodied biota is simply a reflection of their sensitivity to adverse conditions and the fact that they had a short survival time even without the addition of acetic acid. However, a sampling regime with a finer temporal resolution may well detect such an effect. In contrast to *C. robusta*, the survival time of both the mussels and the fouling communities was reduced to less than 24 hours when acetic acid was present, regardless of encapsulation. For fouling communities this may be explained by the dominance of soft-bodied taxa whose membranous fleshy bodies would make them susceptible to chemical additives. The death of *S. algosus* within 24 hours when placed in acetic acid reflects the breakdown of the bivalves' carbonate-based shells. These shells consist of an inner calcified shell and an outer proteinaceous layer (Breesan et al. 2014) which is broken down when exposed to acetic acid, resulting in the shells crumbling after just 24 hours. Despite the obvious efficacy of acetic acid in encapsulation, legal ambiguity around the use of acetic in the field in South Africa precluded this approach being extended to the field study. Overall the results of the laboratory study suggested that encapsulation for three days should be sufficient to kill all fouling biota. While chlorine has been suggested as an additive in encapsulation systems (e.g. Roche et al. 2015; Morrisey et al. 2016), its use on yachts is a challenge, at least in South Africa. This is due to concerns by yachts people that their hulls may be damaged or discoloured by the use of chlorine. In fact, chlorination was not considered in this study as it was not possible to secure vessels upon which to run field trials.

Despite the predictions founded on the laboratory results, in the field it took an average of 4.25 (± 0.5 SD) days for fouling communities to reach

total mortality on yachts, suggesting that encapsulation systems should be left in place for at least five days to be effective. Interestingly, despite pontoons appearing to offer good proxies for yachts, communities on these structures died faster than on yachts regardless of their cover (i.e. 3.7 ± 0.48 SD days for high cover and 3.8 ± 0.42 days for low cover). The difference is likely related to the ratio of fouling biomass to water trapped within the encapsulation system. As encapsulation works by the creation of a closed system in which existing oxygen is depleted by fouling organisms and waste products build up, the lower the ratio of biomass to water, the faster mortality will be achieved. The encapsulation device made for the pontoons were cube shaped like the pontoons and thus ensured tighter fit and the easy exclusion of most of the water from the system. In contrast, structures on yachts, such as keels and propellers, make having a tight-fitting device difficult, especially when one device is made for use on several yachts of different sizes. As a result, a large amount of water is unavoidably encapsulated with the yacht and the ratio of water to fouling biomass is increased, resulting in a longer encapsulation period needed to reach total mortality. Although the number of yachts encapsulated in this study was low, this experience has highlighted that the encapsulation duration suggested by laboratory results is too short. This demonstrates the need to test methodologies in the context in which they will be applied, as logistical constraints may only then become apparent.

Although longer than predicted by the laboratory study, the encapsulation period required to kill communities on pontoons and yachts in this study was notably shorter than the timeframe reported by Atalah et al. (2016). In their study, four-year-old communities with an average cover of 84% were encapsulated on experimental blocks and showed a decrease in percentage cover within 48 hours, but only achieved total mortality after 14 days. These differences could be a result of differences in the ratio of biomass to water in the two studies or the cooler water temperatures experienced in the Atalah et al. (2016) study as the communities were of similar age (three and four years old respectively) and dominated by similar taxa including colonial ascidians, bryozoans and mussels. The potential role played by temperature in regulating the time required to induce mortality in encapsulation systems is further highlighted by the fact that a study considering the solitary ascidian *Ciona savignyi* found that mortality was reached after 14 to 22 days at 7 °C, depending on the levels of dissolved oxygen (Pool et al. 2013). This suggests that the length of time required for effective encapsulation is likely to be longer in temperate regions and shorter in warmer more topical locations, and highlights how context should inform management approaches.

Based on the combined laboratory and field experiment results it is recommended that a national encapsulation program would be a valuable marine biosecurity tool. However, in recognition of the small sample size

of yachts used in this study and the fact that temperature (which varies notably among regions) is recognised to affect the duration of effective encapsulation, it is recommended that the findings of this study be used as a basis for region specific testing of the duration required for encapsulation in different contexts. For instance, in the South African context it is recommended that vessels be placed in encapsulation for at least five days, after which trained divers should assess mortality. Only once all biota are dead should the encapsulation device be removed.

There are a number logistical considerations that need to be accounted for when developing a national encapsulation program as a biosecurity tool to address fouling on newly arrived yachts.

1. Design of the encapsulation device

A berth similar in design to that proposed by Roche et al. (2015) is suggested to reduce the logistical challenges associated with encapsulating yachts. Such a design enables the vessel to be steered into the device, following which it can be sealed. This will minimise the challenge of drawing the device up around the vessel as was done in the present study. Such a design would also facilitate the potential use of additives (like acetic acid or chlorine) to speed up the encapsulation process as contaminated water can be removed and replaced with seawater before the vessel is released from the encapsulation device (Roche at el. (2015). When developing such a device it will be important to ensure that both the design and the materials used are strong enough to withstand multiple uses and potentially rough weather conditions that can have negative effects even in the sheltered confines of marinas. The design should also account for the need to remove as much water as possible when the yacht is first enclosed, as the higher the biomass to water ratio the faster mortality can be expected to occur. While such berths will require an initial capital outlay, once present in a port the device can be used repeatedly. The design of the encapsulation device will also need to account for the various dimensions of keeled yachts. For example a design that can fit a vessel with a length of 9 m and draft of 3.6 m was chosen for this study as it could accommodate 95% of the yachts that visit the region (Robinson *unpublished data*).

2. An adaptive management approach.

To facilitate the ongoing development of encapsulation as a biosecurity tool, it will be important that data be collected for every yacht that is encapsulated and that the methodology be routinely adapted in an evidence-based approach. To ensure the scientific rigor of such data, a standardised approach for data collection will need to be developed. For instance, standardised assessment of mortality will be vital. It is suggested that such a protocol include assessing response to a sharp stimulus (Floerl

et al. 2005), loss of physical integrity and colouration, and the ability to revive when placed in ambient conditions (Hopkins et al. 2016). It is also recommended that temperature within and outside of the berth be monitored, at least on a daily basis. In time this could give better insight into the relationship between temperature and the time required for effective encapsulation. This could be particularly important for regions that experience a range of coastal temperatures, like South Africa. Lastly, it is suggested that data on initial fouling cover also be collected. This is because encapsulation of very lightly fouled vessels may prove to be less effective than the use of divers to remove sparse fouling, especially when fouling is focused in niche areas. Despite the theoretical basis for this argument, data is needed to identify the point at which the benefit switches between these two approaches and support adaptive management that is responsive to findings from a growing dataset.

3. Stakeholder support

The primary stakeholder affected by a national encapsulation programme will be the state authority and this tool is unlikely to gain support if it adds significantly to the administrative burden already faced by biosecurity agencies. To minimise such a burden it is recommended that encapsulation be incorporated into current customs practices for foreign vessels arriving at ports. At least in the South African context, yachts are often docked at the customs jetty for up to seven days while awaiting customs clearance, providing the opportunity for encapsulation that would not further infringe on the stay of the yacht. Encapsulating foreign vessels during this time would thus centralize the administration of customs clearance and this aspect of biosecurity. Although the use of additives in encapsulation systems is allowed in some jurisdictions (e.g. New Zealand (Morrisey et al. 2016) and the United Kingdom (Roche et al. 2015)), this is not necessarily the case in all. For instance, the fact that acetic acid is not listed in the South African Water Guidelines has proved a challenge to its use in this country. However, it is hoped that cooperation between the relevant authorities may see this change in the future.

In conclusion, encapsulation holds promise as a management tool to minimise the introduction and spread of marine alien species by recreational vessels. Field studies have highlighted that laboratory studies are not necessarily directly transferable to the field. While context dependent requirements are acknowledged, in the South African context it appears that encapsulation for five days should result in mortality of all fouling biota. Nonetheless, it is recommended that the findings of this study be used as a basis for developing region specific protocols. An adaptive management approach should be used to optimise this encapsulation period to ensure effective encapsulation in different contexts.

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Supplementary material

The following supplementary material is available for this article:

Table S1. Species present in fouling assemblages considered during laboratory experiments.
Table S2. Water parameters (Mean (± SD)) at the start of the experiments and at the completion of the various treatments to which *Semimytilus algosus, Ciona robusta* and fouling communities were exposed.
Table S3. Main effect coefficient estimates and associated significance derived from the general mixed effects model considering the effect of treatment on dissolved oxygen, pH, ammonia and sulphide levels for Semimytilus algosus.
Table S4. Main effect coefficient estimates and associated significance derived from the general mixed effects model considering the effect of treatment on dissolved oxygen levels, pH, Ammonia and Sulphide for *Ciona robusta*.
Table S5. Main effect coefficient estimates and associated significance derived from the general mixed effects model considering the effect of treatment on dissolved oxygen, pH, ammonia and sulphide levels for fouling communities.
Table S6. Statistical results from Mann-Whitney U tests and t-tests comparing water measures inside and outside the encapsulation device on the first and final day of experiments for pontoons with a high fouling cover.
Table S7. Statistical results from Mann-Whitney U tests and t-tests comparing water measures inside and outside the encapsulation device on the first and final day of experiments for pontoons with a low fouling cover.
Table S8. Statistical results from Mann-Whitney U tests and t-tests comparing water measures (a) inside and (b) outside the encapsulation deviceon the first and final day of experiments for yachts.

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http://www.reabic.net/aquaticinvasions/2020/Supplements/AI_2020_Keanly_Robinson_SupplementaryTables.xlsx