

Kia tirotiro mangōpare, arā ko ngā tai e whā Look through the eyes of the mangōpare, observing in all directions

RESEARCH HIGHLIGHTS 2022



The Marine Biosecurity Toolbox is a 5-year (2019-24) research programme aimed at protecting New Zealand's marine environments from the impacts of non-indigenous species. The programme is jointly funded by the New Zealand Ministry of Business, Innovation and Employment (MBIE) and a unique group of science, Māori, regulatory and industry organisations. The programme's objective is to develop transformative 'tools' that empower regulators, industry, *Mana Whenua*, and the community to effectively manage risk pathways, prevent pest establishment, and detect and respond to new incursions.

The programme is built around four main research themes that target critical stages of the biological invasion process and support key elements of regional, domestic and international biosecurity management: PROTECT, DETECT, MANAGE & RESPOND and ECONOMICS & DECISION-SUPPORT.

Three years into the programme, we have made a great progress across all the workstreams. Here we present the key research highlights accomplished by 2022, along with the envisaged next steps towards the implementation of our science outputs.



Keeping marina pontoons fouling-free - one step closer!

Grant Hopkins, Nicholas Scott, Waikawa Marina, Port Marlborough, Bellingham Marine, Coastguard Nelson

Purpose of the study: Marina and port environments are widely known as hotspots for marine bioinvasions. Structures, like marina pontoons and wharf piles, are particularly prone to colonisation by non-native fouling organisms, including pest species that have become well-established in a region (e.g., Mediterranean fanworms, Pacific oysters). Once marine pests arrive and establish, they have proven to be very difficult to eradicate or control.

Earlier work undertaken during the Toolbox Programme has demonstrated that bubble streams can remove and prevent the settlement of early stages of biofouling. This project aims to develop and test prototype bubble stream systems that can be retrofitted to existing marina pontoon infrastructure and keep them fouling-free.

Brief description and main findings: We have developed a prototype that we believe will be highly effective in reducing biofouling accumulation on marina pontoons. The design consists of stainless-steel brackets that fix the diffusers to the marina pontoon, and 3D-printed clamps that allow the diffusers to be easily swapped out by divers (e.g., if the diffusers themselves become fouled) without the need for tools. This October we commenced a trial in Waikawa Marina, with the view to treat half a marina pontoon (and using the other half as an un-treated reference) during the spring/summer biofouling season. We then plan to treat marina structures at Westhaven Marina, as well as the drive-on pontoon structure used by the Coastguard vessel in Nelson.



Fig. 1. CAD drawing of diffusers retrofitted to a Bellingham Marine concrete pontoon.

Take-home messages: (1) Retrofitting systems to marina pontoons appears very feasible, (2) other structure types are amenable to treatment (e.g., boat docking systems), and (3) prototypes will be further refined and commercialised at the completion of the Toolbox Programme.

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Food for thought - can biocontrol work in temperate settings?

Grant Hopkins, Rebecca McMullin, Lauren Fletcher, Matt Miller

Purpose of the study: Our research has shown that native marine snails, including cat's eye (*kanohi* $p\bar{u}p\bar{u}$) and Cook's turban (*toitoi*), can be used to graze down and control populations of non-native biofouling on marina pontoons (= biological control, or biocontrol). The purpose of this study was to gain a better understanding of appropriate stocking densities for marina pontoons to ensure these native snails do not starve. Future field trials will also provide an opportunity to test and refine devices that aim to stop biocontrol agents escaping.

Brief description and main findings: This study provides the first attempt to match the dietary needs of a biological control agent to the food available in a biofouling control application. Using a NZ marina as a case study, we first aimed to establish a greater understanding of the nutritional composition of biofilm and biofouling communities on marina pontoons over timescales relevant to biocontrol applications, including periods of high (warmer months) and low (cooler) growth rates. This was accompanied by a review of the available information on the dietary requirements of cat's eye (and marine snails generally to identify any potential nutrient deficiencies.

Main findings: Experimental panels (375 x 500 mm) deployed in Nelson Marina during summer months had visible biofouling within two weeks (Fig. 1), developing into substantial fouling biomass after approx. two months (Fig. 2). Biofouling included the usual culprits: barnacles, hydroids, colonial ascidians, bryozoans, tubeworms, and algae. The contrast between summer and winter biofouling accumulation rates was large, with only a small smattering of macrofouling (e.g., barnacles, ascidians, tubeworms, and bryozoans) observed within the biofilm over the deployment period



Fig. 1. During summer months, food looks to be plentiful for control agents (top row), but during the cooler winter months, very little fouling growth was observed.



Fig. 2. Average (+1SE) biofouling dry weight following three deployment periods over summer and winter months. Summer deployments had near exponential growth, while winter fouling biomass remained low (<2 g dry weight).

Take-home messages: (1) there are strong seasonal differences in biofouling accumulation rates on artificial surfaces, (2) we expect that, based on our preliminary findings and the literature review, marina fouling should provide a nutritious food supply for control agents during the warmer months, however food supply is likely to be limiting in the cooler, winter months. This is likely to pose a considerable challenge around maintaining appropriate stocking densities on structures in temperate marinas. Control agent responses to periods of low food supply should be investigated, along with other potential strategies (e.g., supplementing food supply in leaner months, adjusting densities based on season).

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Nature-inspired antibiofouling surfaces

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Purpose of the study: Natural world species ranging from seabirds to aquatic spiders have evolved unique approaches for sustaining underwater entrapped air layers over long periods of time to facilitate diving into water. These properties have been replicated using a combination of surface functionalised textiles and solar-powered bubble streams.



Brief description: Feathers of the mallard duck (*Anas platyrhynchos*) consist of hierarchical fibrillar structures encapsulated with hydrophobic preen oil. These characteristics afford waterproofing through the entrapment of air pockets, enabling swimming and diving for such bird species. This liquid repellency mechanism for bird feathers has been mimicked by surface hydrophobisation of textiles to create large volumes of trapped air at the solid–liquid interface (plastron). To extend the trapped gas layer lifetimes, the transportation of air from the water surface to a submerged air bubble by the diving bell spider (*Argyroneta aquatica*) for respiration has been mimicked via solar-powered bursts of air bubbles.

Main findings: The trapped surface air layer formed during immersion of mallard bird feathers into water has been replicated using low cost and scalable plasmachemical functionalisation of textiles—mimicking a combination of fibrillar surface roughness and low surface energy. A strong correlation has been observed between liquid repellency, hydrostatic breakthrough pressure, and extent of gas layer entrapment (diameters of bubbles formed at the textile surface). Trapped gas layers last up to 4.8 days in static water immersion. Drawing bioinspiration from the diving bell spider, solar-powered pulsing of a gas bubble stream every 2 h further extends the trapped gas layer lifetime to 29 days. This use of gas bubbles to maintain the trapped air layers on functionalized textiles improves the antibiofouling performance in real-world outdoor conditions (pond water). The correlation between porosity and lifetime of trapped gas layer for the same surface chemistry confirms that there is gas cushion formation rather than a gas layer localised at just the substrate surface.



Take-home messages: Nature-inspired approaches hold significant potential for the development of eco-friendly antibiofouling systems without collateral harm to the environment. This example has combined the principles underpinning the buoyancy of mallard ducks and the respiration of diving bell spiders.

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Eco-engineered surfaces to enhance native species: model development and testing

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Purpose of the study:

We aim to design and build surfaces that increase the success of kutai (*Perna canaliculus*) on artificial structures under the working hypothesis that increasing the abundance of native species will reduce the success of invaders. This work is developing our understanding of how juvenile mussels interact with the surfaces they live on – in this case, mussel beds and their underlying rock – that vary in their complexity. We use 3D scanning and modelling to replicate the fine-scale topography of the mussel beds and rock and assess patterns of mussel dispersal in relation to the topographical features (Fig. 1A, B). Optimal features of the surfaces will be selected and tested in an iterative series of laboratory and field experiments that will guide the development of prototype models and engineered surfaces at larger scales.



Figure 1: Digital elevation model (A), juvenile kutai on a 3D model of a kutai bed (B) and complexity metrics for the 3D model where D = fractal dimension, R = rugosity, and H = overall variation in height (C).

Approach & progress:

The team has been assessing the relationship between juvenile kutai and the topography of mussel beds and rock surfaces in a series of laboratory experiments using 3D models of the reef at Waipu Cove. Preliminary data suggest that rock or flat control models generally retain fewer juvenile mussels than models of the mussel bed, indicating that juveniles might be more inclined to settle down among adults.

An important aspect of this work is defining and quantifying the complexity of the surfaces, so we can replicate them in next-generation models. We have developed code to analyse the digital-elevation models (DEMs) created by the scanning software that can be used to define complexity at different scales (Fig 1C). Complexity metrics (e.g., rugosity, fractal dimension) are now being related to the experimental data and we are describing the features that juvenile mussels have selected with the complexity metrics (and those that they have not). We are also manipulating the DEMs to crop, splice and manipulate (e.g., vary their density or size) optimal features to create mosaic models for further experimental work.

Some interesting patterns of dispersal have occurred in our experimental work. For example, some juvenile mussels climb to the apex of the adult-mussel shells, whereas others hunker down in the cracks and cervices among them. We hypothesise that the positioning of the juveniles will impact their short-term survival (juveniles on the apices will be eaten by voracious spotties) and we will test this in a field experiment using caged and uncaged models that have been pre-seeded with juveniles. To this end, we have designed, built, and piloted frames to deploy 3D models in the field (Fig. 2). This pilot work was a success with no frames or models being lost over a two-week period. We have also gathered an extensive data set on habitat affinities of juvenile mussels at

four sites on the South Island. There are strong differences among sites and habitats within sites in terms of the numbers and species of mussels using them and an indication that fine-scale topography produced by barnacles and small seaweeds might be important at some sites.



Figure 2: 3D models of mussel beds and rocky reef deployed on a frame at Fifeshire Rock, Nelson

Take home message:

The recruitment of native species like kutai is complicated and a detailed understanding of their habitat requirements is essential if bespoke engineered surfaces are to be produced. Our work is illuminating how kutai respond to variations in topography associated with their key habitats – an essential first step toward tailoring artificial habitat to benefit this iconic native species.

Next steps:

- A field experiment will be done in November and December to assess the effects of surface type (mussels, rock or control) and predators (caged or uncaged surfaces) on survival of juvenile kutai in the field.
- More statistical analyisis will be done to assess correlations between fine-scale (1 cm) complexity metrics and their selection by juvenile mussels in the laboratory experiments.
- New 3D models will be designed and printed based on data from our laboratory and field experiments, and we will test these with new experiments.
- We will also be broadening our geographic scope as Dayanitha heads to Sydney for the second half of her degree where she will continue to explore the role of habitat complexity in shaping marine assemblages.
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Material development to enhance native mussel settlement response

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Purpose of the study:

The settlement response of green-lipped mussels to substrates comprising replicated reef geometry was tested earlier in the project. We are now examining whether the addition of biogenic or geological additives to reef model construction materials can further enhance mussel recruitment and retention. This will inform the design of habitat models that have favourable surface characteristics, and that are made from a material that enhances mussel settlement.

Approach & progress:

A range of substrate materials are being produced using compounds (fillers) in the form of powder, added into a polymer matrix. The selection of fillers used in this research includes materials that are present naturally on intertidal reefs in our study area, and other that are thought to trigger settlement. Six fillers were identified from a literature review and have been selected for trials. So far, four of those fillers - ground mussel shells, bark tannin extract, ground rock, and coffee grinds - have been compounded to produce formulations at high and low filler concentrations (Figure 1A). We have now developed an initial batch of simple, flat tiles to test for mussel settlement preferences in relation to the presence fillers (Figure 1B).



Figure 1: (A): Fillers that have already been compounded. From left to right: Used coffee grinds; ground mussel shells; bark tannin extract; ground rock. The black pellets shown below are the PLA pellets (a material used for 3D printing). (B): Initial PLA test surfaces laced with finely ground rock from our study reef, produced via injection moulding and ready for experimental trials.

Next steps:

We are awaiting the remaining two fillers, Iron(III)-oxide and potassium sulfate, from the supplier. Once we have produced the remaining formulations, we will injection mould the flat tiles. All tiles will then be exposed to mussel spat during laboratory trials at Cawthron Institute.

Take-home message:

We have developed a technique for incorporating biogenic and geological materials into synthetic test surfaces. This is the first step towards developing substrates whose (1) structure and (2) material composition enhance settlement and retention of green-lipped mussels.

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Simplifying environmental DNA (eDNA) sampling and processing tools for in-field applications

Ulla von Ammon, Xavier Pochon, Gert-Jan Jeunen, Michelle Scriver, Jackson Treece, Jo Stanton, Neil Gemmell, Anastasija Zaiko

Invasive species, just as every living organism, shed eDNA into the environment. Filtering eDNA from the water column works as a great tracer for the early detection of invasive species and helps to direct efficient management. The DETECT team aims to simplify and streamline eDNA sampling and processing so that an easy-to-use toolkit can be handed over to kaitiaki, councils and schools to upscale biosecurity surveillance across the country. Three studies conducted over the last year are summarized here.

• Filter no more - the cruising speed net with a modified cod-end



Figure 1: The cruising speed net has been modified with a cod-end that contains an inbuilt 20 μ m filter collecting eDNA during the tow. Environmental DNA can be directly extracted from this filter.

• Passive sampling and in-field DNA extraction



Figure 2: Experimental design of an in-field vs mesocosm sampling site to analyze fish diversity from active filtration vs passive sampling and extracted via the standard Blood and Tissue Qiagen kit vs the novel in-field PdqEx extractor.

We tested different substrates to retain eDNA just by deploying them in the water. Their performance was compared to the 'gold standard' water filtration approach. To simplify processing step, eDNA from these filters was extracted with the novel PDQeX technology, allowing in-field extractions within 20 minutes. We could show that specific substrates such as nylon and sponges recover the same diversity as conventional filtration. We also received good DNA signals from high abundance taxa using the novel PdqEx technology. We will optimize this system for low abundant eDNA in a follow-up experiment focusing on the most promising passive sampler substrates.

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To circumvent our standard timeconsuming water filtration processes, we adjusted the codend of a plankton net to include a 20 µm membrane, so that waterborne material accumulates while the net is towed through the water at up to 5 knots. Our analysis showed that we discover the same eDNA richness and diversity as with conventional sample processing.



Specific tests to detect non-indigenous species from environmental samples

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The purpose of this study is to optimize and deploy molecular based tests which are specific for and sensitive to non-indigenous species of interest. Trustworthy assays that have been tailored for use in Aotearoa will give us the advantage necessary to detect and identify the presence of pest species, even before we can physically see them.



A conceptual scheme of the eDNA-based species-specific pest detection workflow – with qPCR or LAMP assay.

Approach

We are using established techniques and foundational knowledge to polish existing probe-based qPCR tests into shape for the New Zealand biosphere. Initially the focus is on six species of interest, and where applicable we are using previously published assays, while also designing new ones. Once these six species have robust, validated tests that can pick out our target from environmental samples, we can look into other species of interest and potentially the development of multi-target assays.

Ideally, we will be able to perform monitoring at locations of interest and after sampling from the sites and performing DNA extraction of the samples, we can test them to determine if our pest species are present or not.



DETECT focus is currently on the above non-indigenous species, selected in consultation with end-user partners (councils, Patuharakeke Te Iwi Trust, MPI, DOC). From left to right: *Undaria pinnifitida* (Wakeme, brown kelp), *Carcinus maenus* (European Green Crab), *Asterias amurensis* (North Pacific Seastar), *Caulerpa taxifolia* (Green feather kelp), *Charybdis japonica* (Asian paddle crab), *Sabella spallanzani* (Mediterranean fanworm).

Main Findings and interpretation

Initially, we built on the existing fully-validated qPCR/ddPCR assay for Mediterannean fanworm and designed a recombinase polymerase amplification (RPA) assay for this species. The RPA generates amplification within 20 minutes at 37-39°C, with a detection limit similar to that of the ddPCR. The results if the assay can be read-out with lateral flow strips (similarly to the COVID RAT tests). The assay applicability was verified with users without molecular laboratory experience and the overall positive written feedback on its usability for citizen science applications was obtained.

Next, we focused on *Undaria pinnatifida*, an invasive kelp species. So far, we have developed and validated an accurate test that is working well across multiple platforms. A probe-based qPCR assay is ready for use, with confirmed specificity (not picking other native species) and established sensitivity (level of detection). Currently we are adapting this for use with highly sensitive digital droplet PCR (ddPCR) as well as isothermic assay or single-temperature incubated tests, like LAMP (Loop-mediated amplification) that may be more easily deployed in field settings (e.g. by adopting LAMP testing platforms developed for rapid COVID diagnostics).

Take home message

These assays have the potential for early detection of known invasive species from environmental samples at low concentrations. For Undaria, our test needs less than a single cell's worth of the kelp's DNA for qPCR detection. The earlier we know about a NIS incursion, the sooner we can start responding to the threat. In areas of concern and open the response window for not only inhibiting the growth of pest species numbers, but also mitigating potential damage to at risk habitats and native species.

Future opportunities/Next Steps

- Develop multiplex assays using a complementary mix of molecular dyes, it is possible to create an assay that is capable of testing for multiple species in a single test.
- Move on to the larger list of non-indigenous species and continue to build a library of easy to run accurate assays, fully validate for New Zealand applications.
- Expand our library of single-temperature point-of-need tests for in-field use.
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A targeted eDNA approach to detect and quantify Undaria pinnatifida

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Environmental DNA (eDNA) bio-monitoring approaches offer solutions for the early detection of non-indigenous species (NIS), and has proven particularly powerful for detecting cryptic species in hard-to-reach environments. In a variety of scenarios, eDNA has detected and identified NIS faster and more cost-effectively than traditional methods, making routine surveillance and active preventative measures increasingly possible.

The purpose of this investigation was to see if eDNA methodology could be extended to proxy Biomass. We focused on *Undaria pinnatifida* (Figure 1), an invasive species present in New Zealand. Working alongside existing controlled removal work we used a species-specific qPCR assay on eDNA samples collected in parallel with biomass measurements to investigate whether quantification of a species based on eDNA sampling is feasible.



The field sampling was performed aboard the Polaris II (Figure 2) at 3 sites along the Otago coastline (Brinns Point, Butterfly Bay and Matainaka) marked off for U. pinnatifida removal. At each site, 5x 1L samples were taken from the surface and 5x1L samples – from the sub-canopy seaweed line at depth. The sampling was carried out on board using the vacuum filtration manifold (Figure 3). Then, the DNA was extracted from the filters and a speciesspecific qPCR assay was used to measure the DNA concentration and compare it with biomass measures of removed Undaria. We found broad correlation between eDNA and biomass measures, which was greater in water samples (Figure 4).

To improve resolution for eDNA-based species quantification, additional data will be collected within the East Otago Taiāpure along with Ngai Tāhu *Undaria* removal efforts.



Figure 4. *U.pinnatifida* DNA concentration (ng/uL) vs. *U. pinnatifida* Biomass (kg). Samples taken at depth indicated in Blue, samples taken at the surface indicated in Green.

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Understanding the dynamics of environmental DNA across tidal cycles in the Bay of Islands

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The aim of this study was to understand when to sample in a coastal environment to effectively detect marine pests from environmental DNA (eDNA). The focus was on how eDNA signals from species present in the area – including the marine non-indigenous species (NIS) - change across the tidal period.

A large study experiment was conducted in Opua, Bay of Island (NZ). Six sampling stations were set up in proximity to the marina (an assumed local hotspot of marine NIS accumulation) to capture eDNA signals over the tidal cycle (13-hour sampling window).



Samples were processed for eDNA metabarcoding analysis (simultaneous identification of many taxa/species within the sample based on their molecular fingerprints).



Main findings and interpretation:

There were slight differences between community composition (species detected) and structure (relative abundance of each species) between sampling stations and tide conditions: ebb (outgoing phase) & flow (incoming phase). However, the biodiversity detected was largely consistent across the tidal period. For the detected NIS communities, several of the species demonstrated similar patterns and their detectability was largely unchanged across tidal periods and locations.



Take-home message:

These overall consistencies across a tidal period are promising as they suggest that direct sampling for general community screening (which provides information on the species distribution within a given area) for marine biosecurity applications may not be affected by tidal changes; this information is vital for developing optimized sampling approaches for detecting eDNA.

Future opportunities/next steps:

- Run longer-term automated eDNA sampling in the area to understand the detectability of NIS over larger timescales and further optimize eDNA workflow for routine biosecurity surveillance.
- Employ oceanographic models to track and predict the movement of targeted marine NIS eDNA molecules to predict where the marine NIS source population is, which can help in control or direct management efforts.
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Advancing biosecurity surveillance through school & public outreach

Xavier Pochon, Ulla von Ammon, Jo Stanton, Martin Zirngibl, Michelle Scriver & Anastasija Zaiko

A critical step of the DETECT component is to develop and test user-friendly, portable and field-deployable tools for marine pest detection. Our ultimate vision is to provide all New Zealanders, a team of 5 million, with the ability to actively engage in marine biosecurity surveillance and to help us better protect our coastal environments. Over the last couple years, we've worked with teachers, school kids, community groups and tangata whenua to lay out the foundation of this vision.

From teachers to students

We started by a series of workshops and hands-on activities with partner science teachers from Nelson College and Nelson College for Girls to introduce key concepts in marine ecology, biodiversity, biosecurity and molecular methods for incorporation into the schools' curriculum (**Figure 1**).



Figure 1 Lead partner teachers: (a) Johnnie Fraser from Nelson College, (b) Natalie Tregidga from Nelson College for Girls, and (c) Gerd Banke from Nayland College.

Once activities were defined in agreement with the teachers, we actively engaged with a range of schools and year groups to showcase a variety of marine biosecurity concepts and detection tools, ranging from traditional methods to latest eDNA technologies (**Figure 2**).



Figure 2 Weaving biosecurity knowledge and eDNA tools into Society: (a) studying biofouling at Nelson College for Girls, (b) running DNA/RNA extractions, qPCR and Sequencing at Nelson College, (c) Seaweek at Nelson Intermediate School, (d) molecular lab at Nayland College, (e) collecting eDNA using aquabots with the Ministry Of Inspiration, (f) a rapid molecular assay for *Sabella spalanzanii* trialed by non-scientists published in *Frontiers in Marine Science* 2022, 9: 861657

This year we've hosted Gerd Banke, a science and biology teacher at Nayland College, who was awarded the prestigious Science Teaching Leadership grant to work with our team for six months. During this time, Gerd developed an engaging and well-resourced outreach programme that brings eDNA technologies into the classroom as a tool for students to learn about cutting edge technology, and at the same time to develop a strong sense of kaitiakitanga for their local environment.

North to South expeditions

In 2021, the DETECT team took part in research expeditions across two distant and ecologically different areas of Aotearoa. The first expedition onboard SV Manawanui launched from Waitematā Harbour in Tāmaki Makaurau Auckland, testing new sampling devices, collecting biosecurity data and exploring the wider marine environment of coastal Greater Auckland and Northland. The expedition team of 15 scientists, entrepreneurs, artists and students shared insights with the local communities, stakeholders and tamariki (**Figure 3**).



Figure 3 Public outreach during expedition 1 (June 2021): (a) School activities at the Leigh marine discovery centre and (b) on-board the SV Manawanui, (c) microplastics and eDNA hands-on workshop in Whangarei with participants from NRC, MPI, DOC, universities and Patuharakeke. The expedition resulted in multiple news articles, videos and blog posts.

The second expedition aboard the MV Strannik set off in October from Milford Sound to travel to George and Bligh Sounds for 6 days of sampling. We collected valuable samples for microplastics and biosecurity research, and conducted on-board laboratory analyses using simplified molecular detection approaches for high-profile non-indigenous species such as e.g. *Undaria* (**Figure 4**).



Figure 4 On-board eDNA-biosecurity lab during expedition 2 (October 2021): (a) Jo Stanton director of operations at the heart of (b) George Sound in Fjordland, and (c) the Strannik team with captain Rodney Russ (front centre).

Future opportunities/next steps

- Work closely with Gerd Banke to coordinate school outreach activities at Nayland College in 2023.
- Support the development of resources, activities and exchanges which have a Mātauranga Māori lens on marine biosecurity issues, but also the four big ideas about science: socio-scientific issues, science as an endeavour, communicating in science and investigations (collaboration with Patuharakeke Te Iwi Trust).
- Elaborate a national survey on citizen science and community engagement in New Zealand marine biosecurity systems (collaboration with the ECONOMICS & DECISION-SUPPORT team).
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Pest Alert Tool – a web-based application for flagging species of concern in metabarcoding datasets

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Advances in high-throughput sequencing (HTS) technologies and their increasing affordability have fueled environmental DNA (eDNA) metabarcoding data generation from freshwater, marine, and terrestrial ecosystems. Researchers and environmental authorities worldwide increasingly employ HTS methods for biodiversity assessments, new species discovery and ecological trends monitoring. Moreover, even non-scientists can now collect an eDNA sample, send it to a specialized laboratory for analysis and receive in-depth biodiversity record from a sampling site.

The large volume of data produced by metabarcoding also raises the possibility to detect the species of concern (e.g. marine non-indigenous species) across extensive temporal and spatial scales. To aid such detections, we developed the Pest Alert Tool – an online app for screening HTS datasets for marine species of concern in New Zealand (non-indigenous species, unwanted and notifiable organisms). The tool is currently accessible at: http://pest-alert-tool-prod.azurewebsites.net/



A screenshot of the Pest Alert Tool data screening results showing high-similarity (>99%) sequence matches with marine NIS (purple) and unwanted and notifiable organisms (red).

Future opportunities/next steps:

- Develop an extension app for online reporting quality assured detections and associated metadata
- Test the useability of the developed apps in automated eDNA collection study in Opua, Jan Feb 2023
- Adjust the functionality of the apps following end-users' (NRC and MPI) feedback
- Promote routine usage of these apps for metabarcoding datasets derived from Aotearoa's coastal waters
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Domestic pathway model: Overarching approach

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Purpose of the study:

To develop a process-based model of incursion risk to New Zealand ports, combining the influence of commercial, recreational and aquaculture vessels and equipment. The model will predict the changing risk of invasive species incursions to different locations over time, under business as usual and other management scenarios.

Approach & progress:

The dynamic model will take a process-based network approach. Network models consist of multiple 'nodes' connected by 'edges' of varying strength and directions. In this case, ports and other important locations will be considered 'nodes'. Vessel traffic between the nodes will form the foundation of the 'edges', or connection strengths between locations. The model will predict the relative risk of invasive species incursions at each location over time, which can then be linked to potential changes to market value.

The final model will allow for the application of different introduction and spread dynamics, species traits, and management scenarios, allowing comparison and selection of optimal management approaches based on risk reduction and market values.

Figure 1: Example of biosecurity model of New Zealand where green circles represent nodes and their size represent relative incursion risk, and orange lines represent edge strength, or connectivity, between nodes – directionality implied by following arcs in clockwise direction (Faubel et al in prep).



Main findings & next steps:

The modelling approach and conceptual model have been developed. The process of invasive species incursions will be captured as the strength of the edges, or connections, between the domestic nodes (ports, marinas, aquaculture sites, recreational destinations, marine protected areas, etc.). Edge strength will be calculated according to the conceptual model of invasion process described in Hilliam et al (in prep) Figure 2. For each edge, for each time-step the following process will be used to calculate the strength of movement:

- Total transmissible population available (or, potential propagule pressure, PPP)
- Population entrained on vessels at the source node,
- Proportion of entrained population surviving transport,
- Proportion of transported population introduced to the destination node
- Proportion of introduced population successfully established at destination port.



Figure 2: Process of calculating edge strength in each timestep

The conversion of the conceptual model into a quantitative model is now in its early stages. Full drafts of the commercial and recreational input data have been developed, and the aquaculture data is in progress (see other MANAGE & RESPOND research updates for more detail).

The immediate next steps will involve finalising the model structure code, selecting or developing the equations for each process step and identifying any missing parameters or data. The longer-term next steps are to incorporate the spread and management scenarios into the code (see the Biosecurity Management Response Scenarios research update for more detail). The project team would welcome the opportunity to discuss the nuances of the modelling approach with anyone interested.

Take-home message

The dynamic risk model will provide a comprehensive understanding of incursion risk from major sources, and allow for systematic spatial prioritisation and selection of management approaches to secure New Zealand's maritime resources.

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Domestic pathway model: Commercial vessels

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Purpose of the study:

To develop and test a flexible and highly transferable framework for quantifying the marine biosecurity incursion and spread risks for potential non-indigenous species (NIS). We used risk proxies derived from geographic data and network dynamics representing the movement of all commercial marine vessels across New Zealand, and their associated hull-based and ballast-based characteristics.

Approach & progress:

Two complementary proxies were designed to quantify a) the potential NIS movement via a ship's wetted hull surface area, and b) the potential transport though internal ballast water. The commercial vessel movement network was developed from port-call data from all movements into and around New Zealand for the period from January 2016 to December 2019. Our analysis quantified the relative international and domestic incursion risk, as well as the risk of spread of NIS introductions throughout the entire New Zealand seascape.

All raw commercial vessel traffic has been summarised (420,000 movements by 2,388 vessels from 165 international sources) for all years of data and movements classified into 5 key patters: 1) Vessel sitting at port/mooring for service and/or on/off loading, 2) domestic-only movement between ports within NZ, 3) local (domestic) excursion to sea without visiting another port/site, 4) International departures from New Zealand, 5) International arrival into New Zealand.

The figure illustrates all commercial vessels moving into New Zealand (light grey arcs), as well as all vessel movements between domestic ports (blue arcs). Ports are sized relative to the total hull entering a port.



Main findings & next steps:

Across New Zealand, sites with the highest relative incursion risk are ports or Auckland, Tauranga, Lyttelton, Wellington and Napier. We also identified two ports that have a high relative incursion risk but are currently not considered for national biosecurity management: Timaru and Whanganui. Many domestic ports were identified as contributing to the domestic spread risk as either stepping-stone ports and/or local super-spreaders. Our network-based framework also identified suites of ports that are at significantly elevated incursion risk due to their accumulated secondary exposure risk profiles (e.g., Tauranga) – a risk not currently considered in marine biosecurity approaches.



Figure 1 Example of combining network metrics and incursion and spread risk proxies for NIS moving through hull (left) and ballast (right) pathways (Faubel et al. in prep).

Take-home message:

The potential incursion risk from ballast water and biofouling on commercial vessels may impact areas across New Zealand, with strong geographic patterns (north, west, & south).

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Domestic pathway model: Recreational Vessels

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Purpose of the study:

This study aims to determine where, when, and how long recreational vessels travel and the potential biosecurity risk they pose to their destinations. This is important as recreational vessels can be a key pathway for spreading invasive marine species. But as recreational vessels aren't tracked, there is no readily available information about their movements.

Approach & progress:

Information on recreational vessels' movements was gathered through an online map-based survey. This survey allowed participants to specify where they travelled and record valuable trip information. Yachting New Zealand, the New Zealand Marina Operators Association and regional harbourmasters throughout the country helped us distribute the survey. There were over 1,800 respondents to the survey providing over 12,000 visits to domestic destinations. This sample of the recreational boating population was then scaled up to represent the annual movements for the entire country.



Integration of the survey data with information on mooring facilities and destinations around the entire NZ coastline (gathered from the Ministry for the Environment, harbourmasters, Land Information New Zealand, New Zealand Marina Operator's Association and the aquaculture industry) identified approximately 600 locations that recreational

vessels can visit around New Zealand (shown on above). These locations will be used across all pathways and the overall transport model.

Main findings & next steps:

We identified several marinas and moorings areas throughout New Zealand that play a key role in the spread of marine pests throughout the network shown below, as they possess over 150 connections to other sites. These are located in Waitematā Harbour, Gulf Harbour, Great Barrier Island, Tauranga Harbour, the Bay of Islands and Nelson. We also identified which destinations are visited most by recreational vessels from marinas and moorings. These are islands, which are extremely popular, followed by coastal hydro systems (estuaries/harbours) and beaches.

The next steps in this pathway analysis will focus on which destinations are most at risk from the introduction of invasive species by recreational vessels. This will be done using quantitative information on cumulative vessel length arriving and time spent at each destination. This relative risk will highlight particular places or types of sites, that are most at risk of invasion through recreational vessels and should receive extra attention regarding monitoring and management actions. Simulated incursions and management actions will also be run in the future within the recreational vessel pathway and overall transport model.



Figure 1 Movement of recreational vessels. Gray arcs represent documented movements and points represent unique destinations (size and colour related to the amount of visits).

Take-home message:

Relatively few key marinas and mooring areas contribute to the vast majority of spread within the recreational vessel network, largely located in the northern North Island.

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Domestic pathway model: Aquaculture network

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Purpose of the study:

This project is building a network of movements among New Zealand's marine aquaculture sites to understand their exposure to, and ability to spread, marine invasive species. The network model is being built via direct discussions with aquaculture companies to create node lists (places) and edge lists (movements between nodes by industry vessels, equipment, and stock). The resulting network will inform risk analyses for pest spread and risk management opportunities that can prevent significant losses to aquaculture and other values in New Zealand.

Approach & progress:

Marine farmers in the salmon, greenshell mussel, and oyster industries of New Zealand took part in interviews focused on movements of vessels, stock, and equipment among marine farms, home ports, processing facilities, and other company sites. The information gathered directly from farmers is being converted to node and edge list data that represent sites and movements per company. The movements include smolt and spat transfers, stock transfers, harvesting, maintenance activities and daily staffing routines. The network includes marine sites only and land-based sites connected directly to a marine site (e.g., hatcheries and processing factories). Movements among nodes that occur by sea or overland are included. Data have been collated for all three sectors (salmon, mussels, and oysters) and the node list and node maps have been created. The edge list is under construction and a final round of proofing or addressing data gaps will occur before analyses in 2023.

Findings & Next Steps:

The aquaculture network is made up of 26 nodes for salmon, 1198 for mussels, and 268 for oysters. There are notable geographic footprints within and among sectors; for example, the salmon industry occurs exclusively on the South Island and each company has a quite distinct area of operation with very little overlap. Mussel and oyster farming is more distributed around the country and long-distance movements of stock (usually by land) interact with more regional movements of equipment and vessels.

After the aquaculture network model is constructed, analyses will focus on network characteristics that generate risk of pest spread and the relative importance of nodes or edges for introduction risk. The industry applies biosecurity management practices to sites and transfer mechanisms, and scenario modeling will account for management actions already underway and scenarios that would increase or reduce risk if practices change.



Take-home message:

The aquaculture industry is sensitive to direct impacts of pests and disease on food production and profitability. This aquaculture pathway model will provide additional understanding and actionable management strategies to protect, manage, and respond to pest and disease incursions for an important primary industry.

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Domestic pathway model: Great Barrier Island case study

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Purpose of the study:

Recently, an established population of a non-indigenous tunicate, *Clavelina oblonga*, was discovered near Port Fitzroy, Aotea Great Barrier Island (GBI), New Zealand. GBI is not a designated international arrival port and mostly known as a popular destination for domestic recreational vessels. This discovery was the first record of *C. oblonga* for New Zealand and unusual in that GBI is not a site where one would expect a non-indigenous species (NIS) to become first established. There is considerable effort mounting to manage this incursion and limit the potential further spread around GBI and further.

The objective of this work is to support management of *C. oblonga* through the identification and prioritisation of surveillance sites by (i) quantifying the role of GBI in New Zealand's domestic vessel transport network, (ii) identifying 'upstream' locations that may be potential origins of the GBI incursion, and (iii) identifying likely destinations that may be at risk of receiving infections from GBI through secondary spread pathways.

Approach & progress:

We use network and geospatial modelling approaches to quantify and understand the patterns of potential infection and spread using three types of data: (i) domestic port-call records derived from a global vessel movement database; (ii) high-resolution vessel GPS tracks extracted through Automatic Identification System (AIS) signals; and (iii) an existing dataset on domestic recreational vessel movements.

Two proxy measures for the potential propagule pressure (PPP) of NIS moving through the seascape were used: the hull total wetted surface area (TWSA) proxy was calculated by combining a vessel's wetted surface area and vessel-specific hull complexities (niches areas such as propellers, thrusters, keels, etc.). Ballast water discharge (BWD) is a proxy based on the vessel type, a vessel's maximum ballast water capacity, and ballast water exchange frequencies characteristic for the vessel types. These proxies were applied across all vessel types and data sources.

Findings & Next Steps:

GBI is an important node in New Zealand's domestic vessel network and can act both as a source and a recipient of NIS. Tryphena, Smokehouse Bay, and Port Fitzroy are GBI sites of recent NIS outbreaks - and were identified by our analysis as the sites receiving the greatest PPP from mainland New Zealand. Interestingly, our AIS analyses uncovered a number of recreational vessels that arrived at GBI from international origins – something that is unlawful unless express advance permission from government was obtained. We suggest biosecurity efforts should focus on eradication and containment to ensure future incursions are minimised. Current surveillance *for C. oblonga* within GBI suggests that local managers are looking in the right areas for additional outbreaks. However, there are sites with significant relative risk where marine biosecurity surveillance is currently not undertaken, including Rangiahua Island, Bradshaw Cove and Katherine Bay (see Figure).

Sites at risk of further incursion span the Auckland region and bays to the north, but also include locations throughout the North Island (see Figure). Surveillance may need to be stratified across bays around GBI, and include sites around Auckland and seascapes further north and south to maximise the likelihood of detecting early signs of spread.



Figure 1: Potential spread of C. oblonga through the total wetted surface area pathway (TWSA). Connections (moving in a clockwise direction) depict potential spreading patterns from two locations with existing populations (pink circles). The left panel displays the total potential spread throughout New Zealand, the right panel displays a high-resolution figure of potential spread within GBI. oblonga influx are displayed in the table.

Take-home message:

Real-time management can be efficiently informed through the analysis of vessel movement data to identify the potential downstream spread risk and optimise surveillance for early detection. We are presently conducting similar analyses to examine the potential spread of the alga Caulerpa brachypus that was also (and more recently) discovered at GBI.

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Domestic pathway model: Biosecurity risk in future climates

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Purpose of the study:

Marine non-indigenous species (NIS) are being introduced beyond their native ranges at increasing rates. As waters warm under climate change, the range of suitable habitat is expected to change for existing and prospective NIS, and move toward the poles. In this study, the risk of NIS invasion to ports in New Zealand was estimated by combining the likelihood of introduction and the potential of establishment. The likelihood of introduction was derived from a transport model developed using marine vessel movements from Automatic Identification Systems, the New Zealand Department of Conservation, and ferry and water taxi timetables. The potential of establishment was predicted using environmental suitability (or the environmental match) of locations using species distribution modelling (SDM) approaches.

Our objective was the development of a risk-based tool to identify domestic locations at a high relative risk of (i) the spread of NIS already established in some parts of New Zealand, and (ii) new potential NIS should these make it through the border. The potential of establishment for eight model NIS were explored, with two illustrated here (the north-Pacific sea star, *Asterias amurensis*, the Mediterranean fanworm, *Sabella spallanzanii*), for contemporary ocean climatologies and those predicted for future climate change scenario in 2050 and 2100.

Approach & progress:

We defined the relative risk of target species incursions as a combination of: (i) the likelihood of introductions, and (ii) the suitability of locations for establishment by the target NIS. To examine differences in the introduction potential of target species across domestic locations, we calculated the 'vessel inflow' to each port, defined as the sum of the lengths (metres) of all vessels that entered each port during one year. The suitability of coastal locations within the study domain was estimated using two complementary species distribution modelling methods: MaxEnt and boosted regression trees (BRTs), both machine learning methods to quantify the species' niche.



Figure 1. Preliminary results for A. amurensis habitat suitability under a 'high emissions' climate scenario (RCP 8.5). (A) Under current conditions for full study domain (B) under current conditions in top of North Island (C) in 2050, full study domain (D) in 2050, in top of North Island (E) in 2100, full study domain (F) in 2100, in top of North Island (Phipps et al. in prep).

Main findings & next steps:

Our work to date has shown that ports in the New Zealand seascape are located in areas of suitable habitat for a broad suite of NIS, and domestic vessel movements between these ports provide ample opportunities for spread. Auckland is one of the first ports of call for many international vessels and has a high environmental suitability for many of the species studied. The risk of invasion is predicted to rise at many ports across the country as climate

change causes warming ocean temperatures. Areas of suitable habitat will increase in size and move into areas that are currently unsuitable under contemporary climate conditions.



We are currently refining the analysis and outcomes and preparing the research for publication.

Figure 2 Example for Predicted risk for A. amurensis in ports under future climate conditions (RCP 8.5, 2100). Bubbles increase in size as level of risk increases. The change in colour represents the proportions of vessel metres from international vessels. (Phipps et al. in prep)

Take-home message:

By modelling the risk of invasion for contemporary and future climate conditions, managers will have access to species-specific and local-scale information required to act now and plan for the future

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Domestic pathway model: Incursion and response scenario modelling

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Purpose of the study:

The domestic maritime network model developed in MANAGE & RESPOND will incorporate three major pathways for the transport of marine non-indigenous species: commercial shipping, recreational boating and marine aquaculture (see separate research updates). Once finalised, the model will be used to simulate and co-design (with industry, government, Māori and community end-users) a range of potential marine pest incursion and response scenarios relevant to New Zealand's regions and industries. These simulations will be combined with bioeconomic cost-benefit models and used to derive optimised intervention strategies encompassing both proactive and reactive approaches and extending over relevant management time scales. The aim of this work is to co-design (with industry, government, Māori and community end-users) the context, structure, and approach for appropriately capturing realistic incursion and spread dynamics and meaningful response scenarios.

Approach & progress:

The development of meaningful pest incursion and response scenarios, and the incorporation of modelling results into regional operations and policies, requires an interactive and multidisciplinary team. We have established a collaborative Working Group (WG) that includes representatives from regional councils, regional biosecurity partnerships, central government, Māori, aquaculture industry, and programme scientists. Presently, WG members are in the process of gathering key information for this project: (i) costs, constraints, regulatory frameworks and level of success of current marine biosecurity management practices; and (ii) priority and real-world response or management options for scenario development (Figure 1).

Model parameterization with species' life-history attributes, and a deliberate 'starting distribution', will provide us with valuable information such as: spread of the species (via shipping, boating or aquaculture) over realistic management timeframes (e.g., 1-10 years), contribution of key 'hubs' in the network, or the relative role of different transport pathways. Incorporating simulations corresponding to the use of existing, improved or novel interventions (e.g. infrastructure sanitation, vector



Figure 1: Which species, incursions and intervention options should be simulated for best assisting marine biosecurity management in New Zealand?

hygiene, surveillance, population control, quarantine), at a range of intensities, will allow us to quantify their relative effectiveness relative to 'do nothing', or 'status quo' scenarios.

Next steps:

Once data and information gathering by the WG has been completed (November 2022), all materials will be summarised to form the basis of a workshop to begin the decision and prioritisation process regarding incursion and response scenario modelling.

Take-home message:

A process is underway to identify priority incursion and response scenarios that will be modelled to determine the relative cost-effectiveness of different intervention strategies. The project team and Working Group welcome input from other programme members and end-users that assists us in this process.

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ECONOMICS & DECISION-SUPPORT

To clean or not to clean: views and preferences of recreational boat owners on keeping hulls free from biofouling organisms

Richard Yao, Melissa Welsh, Alaric McCarthy, Mark Newton, Oliver Floerl and MBT end users (central government agencies, councils and industries)

Purpose of the study: Recreational boatowners play a crucial role in controlling the spread of biofouling organisms. However, there is no study to date that has evaluated boatowners' views and aspirations around keeping their hulls free from biofouling organisms. This study aims to evaluate the preferences, values and motivations of New Zealand boatowners, using a survey-based approach that combines environmental economics (choice experiment) and psychological (theory of planned behaviour) approaches.

Brief description and findings: The study focuses on owners of recreational boats that are permanently in the marine environment, as they are the most prone to colonisation by biofouling organisms (Figure 1). The team has drafted a national survey instrument based on discussions with end users of the Marine Biosecurity Toolbox (e.g., regional councils, central government agencies) and a focus group. Questions on the costs of and time involved in keeping hulls clean were tested at a focus group in Nelson in October 2021. We found, from a sample of eight boatowners, that an average boatowner spent approximately \$3,500 per year on general repairs and maintenance plus an additional \$2,000 for antifouling paint and removal of biofouling (Figure 2). This indicates that biofouling is a major cost item, increasing boat maintenance cost by 57%.



Figure 1. Examples of recreational boats in Nelson that are permanently in the marine environment.



Figure 2. Average cost of a boat's general maintenance and additional costs from keeping hulls free from biofouling organisms (n=8).

Given the increase in a boat's maintenance cost, it is important to assess and demonstrate the benefits of these additional expenses. To achieve this, the team is implementing a framework to assess the broader benefits of measures to keep hulls clear of biofouling, as well as identify the factors that would underpin the boatowner's values and behaviour in this space. For example, concern for the marine environment, views on the long-term benefits and willingness to spend time in training on the latest innovations and technologies for keeping hulls clean. The key factors that the team identified so far include improvement in marine ecosystem health, reduction in boat maintenance and fuel costs, increasing abundance of iconic seafood species, and improved consistency of policies across different regions. These factors are compiled in a choice situation that enables each boatowner to evaluate and exercise trade-offs across competing outcomes (Figure 3). Analysing the data collected from this type of survey will allow the estimation of willingness to pay or to spend additional time for the improvement of these environmental factors. These values which are usually not accounted for in market transactions can be indirectly elicited from respondents. Elicited data on these preferences will enable the estimation of values that can be incorporated in policy decision making.

Attribute	Status quo	Option A	Option B
Marine ecosystem health	Declining limiting enjoyment of environments	Improving increasing enjoyment of environments	Stable constant enjoyment of environments
Boat maintenance & fuel cost	Increasing cost of maintenance and fuel use	Stable cost of maintenance and fuel use	Decreasing cost of maintenance and fuel use
Ioonic seafood species abundance	Declining abundance of seafood species	Improving abundance of seafood species	Stable abundance of seafood species
NZ hull cleanliness policies	Different policies across NZ regions	One set of policies across NZ regions	Improved coordination between regions policies vary based on risk
Additional annual payment in hull cleaning action (S/year)	\$0	\$100 per year for 5 years?	\$200 per year for 5 years?
Willingness to spend time on training for hull cleanliness	0 hours	32 hours per year for 5 years	16 hours per year for 5 years
I would choose	0	0	0

Figure 3. An example of the developing choice task for the choice experiment.

Take-home message: Keeping boats free from biofouling results in a significant increase in maintenance cost to boatowners but can realise significant economic and environmental benefits in the longer term. We envision that results from this study will inform the development of policies to support and encourage recreational boat owners to keep their hulls clean.

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