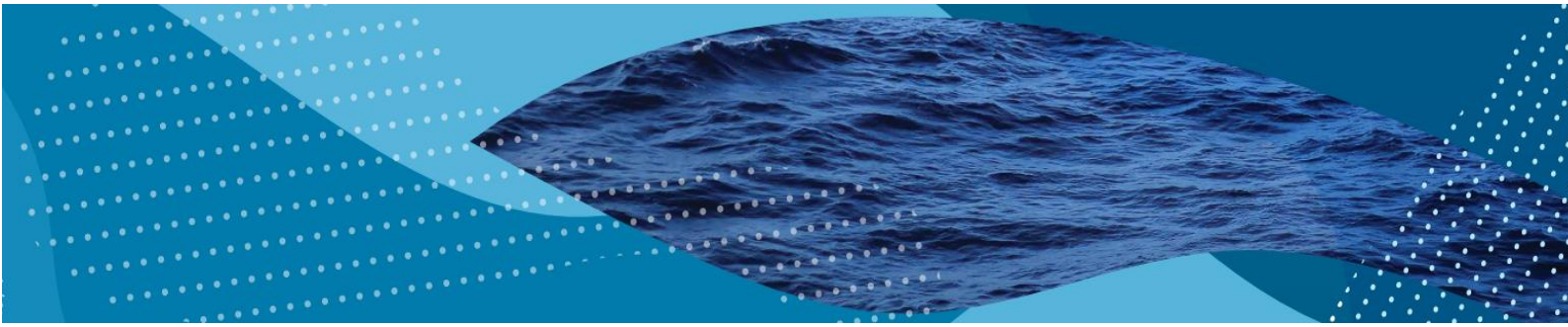

Queensland Seaports eDNA Surveillance (Q-SEAS) marine pest pilot program 2019-2020

Port of Gladstone

Winter/spring (event 1) and summer (event 2) report

March 2021

FINAL

Queensland Government

This publication has been compiled by Carolyn Trewin of Biosecurity Queensland, Department of Agriculture and Fisheries.

Executive Summary

Gladstone Ports Corporation (GPC) was one of five port authorities to partner with Biosecurity Queensland (Department of Agriculture and Fisheries), to implement an innovative state-wide marine pest surveillance pilot program across Queensland. The Queensland Seaports environmental DNA (eDNA) Surveillance (Q-SEAS) pilot program used molecular technologies to analyse environmental samples collected from potentially high-risk areas within port berthing areas, to test for the presence of invasive marine species.

Q-SEAS is the first government-industry collaborative state-wide marine biosecurity surveillance program in Queensland. Some ports had previously undertaken independent marine pest monitoring using traditional techniques however Q-SEAS established a consistent methodology and coordinated surveillance network for the early detection and proactive management of marine biosecurity threats at Queensland's seaports in Brisbane, Gladstone, Mackay, Townsville and Cairns. Q-SEAS was developed as part of the Queensland Marine Pest Prevention and Preparedness Project (under the Queensland Biosecurity Capability Implementation Program) and aligns with the Australian Government's *MarinePestPlan 2018-2023* and the *National Marine Pest Surveillance Strategy (2019)*. The program was designed to be risk-based, adaptable and scalable to the level of identified risk at each port, transformative, cost-effective and simple for ports to implement. The pilot program provided significant advancements in detection capability through the use of molecular diagnostic techniques, increased spatial coverage with the participation of five ports and cost efficiencies due to centralised, coordinated administration. Q-SEAS was managed by Biosecurity Queensland and delivered in partnership with participating port authorities, who provided significant financial and in-kind contributions to support its delivery.

The purpose of the program was to establish early detection capability, at locations identified as high risk for marine pest introductions, in collaboration with industry. The earlier a marine pest is detected, the better the chances are for eradication and control and to minimise impacts on the marine environment and industries.

A target list of marine pest species was defined and a combination of risk assessment and pathway analysis determined biannual surveys during winter/spring (2019) and summer (2019-2020) seasons would best target the lifecycles of these species. Methods incorporated collection of DNA and eDNA using dual-substrates, through deployment of settlement arrays to capture fouling organisms and collection of plankton samples to assess biota in the water column. Environmental samples were analysed using DNA metabarcoding through next generation sequencing (NGS) technologies, by eDNA Frontiers laboratory at Curtin University. DNA metabarcoding allowed for testing of an unlimited number of target taxa, which was important for assessing a taxonomically broad list of marine pests. The application of cytochrome *c* oxidase subunit 1 (CO1) and 18S ribosomal RNA gene (18S) metabarcoding assays increased the breadth and specificity of taxa detected, enabling further classification to species level. Imagery of fouled settlement plates was used as another line of evidence in the assessment process, for cross-examination against DNA results.

This report describes the activities and findings of the Q-SEAS pilot program for the Port of Gladstone. The primary objective was to identify the presence of invasive marine pests, as listed in the *Biosecurity Act 2014* or otherwise identified by Biosecurity Queensland. There was little overlap between taxa identified in either substrate type (plates and plankton) or metabarcoding assay (CO1 and 18S), with the CO1 assay providing resolution of taxa to genus and species level. This allowed further interrogation to help rule out or confirm suspected marine pest detections. Stringent quality control measures were undertaken both in the field and in the laboratory to avoid cross-contamination and to preserve sample integrity. Final DNA sequences were screened against an in-house genetic sequence reference library, with outputs further investigated using a conservative stepwise criteria assessment process.

The Q-SEAS pilot was successfully completed at the Port of Gladstone between September and November 2019 (event 1) and November to January 2020 (event 2). There were no detections of

invasive marine pests. A total of 151 families were identified across all substrates in event 1, including taxonomic classification of 62 genera and 54 species. Only 6 families were identified in all substrates by both assays. In event 2, a total of 106 families were identified from all substrates, including taxonomic resolution of 39 genera and 18 species, with only 4 families identified in all substrates by both assays. Results demonstrated the importance of using multiple substrates and assays across different seasons to increase the likelihood of detecting target taxa.

Barnacles, worms, ascidians, mussels, oysters and seaweed belonging to target marine pest 'family' classifications were identified in both events, with diatoms from target marine pest families also identified in event 2. Results ruled out the presence of the invasive marine pest targets, except for the brown macroalga (seaweed) from the *Sargassum* genus. *Sargassum* seaweed was identified on plates from Barney Point in event 1, and in plankton for both events. The analyses could not resolve this detection to species level and rule out the presence of the invasive *Sargassum muticum*. Seaweed belonging the *Sargassum* genus is one of the most diverse genera of brown marine algae known to commonly occur in shallow tropical and subtropical waters. *Sargassum* seaweed is very prevalent and speciose across Australia, and the lack of available reference sequences for native *Sargassum* species meant that this result was classified as 'inconclusive'. No further testing was undertaken. The introduced feather-duster worm *Branchiomma bairdi* was detected on the settlement arrays at Barney Point. This worm is introduced but not recognised as an invasive marine pest in Australia and is not a target for management, however future surveillance could monitor for the ongoing presence and distribution of this species.

Q-SEAS successfully established a robust and widespread marine biosecurity screening network, which significantly improved the ability to detect small and cryptic taxa using methods that are faster, cheaper and safer than traditional marine pest surveillance. The multifaceted, holistic approach and high level of scientific rigour used for Q-SEAS resulted in identification of a wide diversity of taxa with high taxonomic resolution, providing confidence in its application as an early warning surveillance tool for marine pests.

Critical baseline data has been collected to inform the current status of marine pests in Gladstone, with results providing a comprehensive snapshot of marine biodiversity in some areas of the port environment. Outcomes have enabled evidence-based decisions to inform marine pest surveillance efficacy, practices, management and policies, with results already used to guide future surveillance efforts. Q-SEAS provides the best chance of early detection and early intervention, particularly with continued surveillance over varying temporal and spatial scales. Continuity will improve knowledge on the status of marine pests in Queensland, with biobanking of DNA samples allowing for retrospective analyses as technologies improve, or for understanding changes in biological communities.

The pilot year of Q-SEAS at the Port of Gladstone provided informative data on the status of marine pests, though it is important to note that results do not cover the port in its entirety with sampling focused at key locations based on identified marine pest introduction risk and suitability of the location for the surveillance apparatus. In the event that a pest is detected in the program results, further investigations will be undertaken to confirm the presence of the species and determine a suitable course of action to respond to the detection.

The program provides important and valuable data, to add to previous surveillance undertake at the Port of Gladstone, and from which future surveillance activities can build upon. The accumulation of spatial and temporal data over future years will facilitate the early detection of new marine pests, help to better understand the distribution of known pests, and provide an ever-increasing level of confidence in the absence of target marine pests of concern.

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Acronyms

COC	Chain of Custody form
CO1	Cytochrome *c* oxidase subunit 1
DAF	Queensland Department of Agriculture and Fisheries
DNA	Deoxyribonucleic acid
DPIRD	Western Australian Department of Primary Industries and Regional Development
eDNA	Environmental DNA
GBO	General Biosecurity Obligation
GPC	Gladstone Ports Corporation
NATA	National Association of Testing Authorities
NCBI	National Centre for Biotechnology Information
NGS	Next Generation Sequencing
PCR	Polymerase Chain Reaction
qPCR	Quantitative Polymerase Chain Reaction
Q-SEAS	Queensland Seaports eDNA Surveillance
SOP	Standard Operating Procedures
SWASP	State-Wide Array Surveillance Program
ZOTU	Zero-radius Operational Taxonomic Units

Glossary

Assay	In the context of eDNA metabarcoding, assay refers to a PCR test that selectively targets a subset of biota from an eDNA sample. The use of multiple assays will always detect a wider diversity of taxa than a single assay, and the type of assay should be selected based on the objectives of a study.
Biobanking	Storage of biological samples for future interrogation.
CO1 gene region	The gene region that is used as the standard barcode for most animal groups.
Ct	In a qPCR assay, a positive reaction is detected by accumulation of a fluorescent signal and the cycle threshold (Ct). The cycle Ct is defined as the number of PCR cycles required for the DNA signal to exceed background level.
DNA	Deoxyribonucleic Acid which is the hereditary material that contains the genetic information of an organism
eDNA	Environmental DNA is DNA that is collected from an environmental sample such as water, soil, air or snow. Sources of eDNA include fragments of living and dead biota, faeces, mucus, gametes or scales.
DNA metabarcoding	Genetic technique for simultaneously amplifying and sequencing barcode regions (eg 18S, CO1) of an unlimited number of species.
18S rRNA	A conserved gene region of nuclear DNA, which codes for a subunit of the ribosome and is found in all eukaryotes, making it a good candidate for DNA barcoding.
Marine pest	Marine animal and plant species introduced to waters outside their natural range. If these introduced species establish and spread, they may become invasive and cause irreversible harm to the environment and economy. Marine pests can impact upon industry, the environment and the community, resulting in significant economic burden.
Marine biosecurity	Activities to prevent the introduction and spread of marine pests of concern, responding to marine pest detections, and managing marine pest incursions.
PCR	A molecular laboratory technique used to detect specific DNA, by rapidly amplifying millions of copies of target DNA in a sample using specially designed primers and probes.
Settlement array	Specially designed frames which float just below the water's surface with small PVC plates attached to provide a surface for marine 'fouling' organisms to settle and grow. These devices are deployed for a period of several weeks before plates are removed and tested for the presence of marine pests.
Settlement plate	Small PVC square 'plates' which are attached to the 'settlement array' frame, to provide a surface for settlement and growth of marine organisms (which are often planktonic during their larval (juvenile) stages, before seeking hard surfaces on which to complete the next phase of their lifecycle).
Plankton	Microscopic plants (phytoplankton) and animals (zooplankton) including the juvenile stages of larger marine invertebrates and fish, which live in the water column and are usually carried by tides and currents.
qPCR	A molecular laboratory technique which amplifies targeted DNA molecules during the PCR testing process in 'real time'. Also known as real-time PCR.

Acknowledgements

Biosecurity Queensland wishes to acknowledge the financial and in-kind support provided by Gladstone Ports Corporation, which enabled successful implementation of the Q-SEAS program in Gladstone. We appreciate the valuable advice and recommendations provided by the Western Australian Department of Primary Industries and Regional Development (DPIRD) during the development of this program. Laboratory analyses were conducted by eDNA Frontiers at Curtin University, with efforts to tailor analyses for the Q-SEAS program and assistance with further diagnostic investigations greatly appreciated by Biosecurity Queensland.

1 Introduction

As an island nation, Australia is reliant on shipping to service trade and support the economy, with over 95% of trade by volume carried by ships (DAWR, 2018). Ports are therefore a fundamental component of Australia's trade and support maritime industries and local communities. The preservation and protection of maritime port infrastructure and activities is essential for trade to continue efficiently into the future (IA, 2011). Queensland has 21 ports (16 of these are trading ports) along its 6,973 km of mainland coastline, which are a major component of the State's supply chain and economy (DTMR, 2019; GA, 2019). These ports facilitate trade, service the shipping industry, support regional industries, sustain coastal communities and tourism, and support Australia's national defence and security (DTMR, 2019).

1.1 Marine pests

Marine pests are animals and plants introduced to waters outside their natural range. If these introduced species establish and spread, they have the potential to become invasive and cause irreversible harm to our environment and economy. Marine pests can impact upon industry, the environment and the community, resulting in significant economic burden. Marine pests can be translocated from interstate or international waters as biofouling on vessels, or in seawater systems such as ballast water, water intake pipes or bilge water.

Queensland's coastline supports numerous ports, many of which are the first port of call for international shipping vessels on the east coast of Australia. Risks of marine pest introduction have intensified due to increases in international and domestic vessel traffic, as well as a rising need for marine infrastructure along the Queensland coastline. These factors increase the risk for the introduction of marine pests not yet established in Queensland. Changes in the dynamics of vessel movements and the environment are shifting the potential for marine pests to be introduced to Australian waters (DAWR, 2018). Establishment of an invasive marine pest could result in:

- Restrictions to vessel movements and access to waterways, ports and marinas
- Increased costs for vessel inspections and cleaning of vessels or infrastructure
- Damage to vessels and marine infrastructure resulting in decreased efficiency, increased maintenance and safety risks (i.e. clogging of seawater pipes/filters, fouling of navigation buoys)
- Changes, limitations and economic loss to commercial/recreational fishing and aquaculture
- Introduction of disease that can affect human health and fisheries resources
- Changes to marine ecosystems (e.g. outcompeting native species for food and habitat)
- Risks to the heritage-listed Great Barrier Reef Marine Park or other ecologically significant areas
- Reduced public amenity, tourism and recreational opportunities.

Protecting Australia's marine environment and maritime industries from the introduction, establishment and spread of marine pests is crucial. Some marine pests have already established in Australia, resulting in significant impacts to industry and the environment. Appendix 7.1 provides some examples of the impacts that introduced aquatic pests and disease can have on the economy and the environment. While many marine species have been introduced to Australia, those which are recognised as 'invasive' marine pests pose the biggest threat to the environment and economy.

1.2 Marine biosecurity

The role of marine biosecurity is to prevent the introduction and spread of pests of concern, respond to detections, and manage incursions. Eradicating an established marine pest is extremely difficult, if not impossible, therefore prevention is the primary strategy, followed by early detection and early intervention. Surveillance is an essential component of any effective marine biosecurity program. Marine biosecurity is a shared responsibility for governments, industry and the community, particularly those who operate in the marine environment.

All Queenslanders have a 'general biosecurity obligation (GBO) under Queensland's *Biosecurity Act 2014*. Under the GBO, individuals and organisations whose activities pose a biosecurity risk must take all reasonable and practical steps to prevent or minimise each biosecurity risk; minimise the likelihood of causing a 'biosecurity event' and limit the consequences if such an event is caused; prevent or minimise the harmful effects a risk could have, and not do anything that might make any harmful effects worse. Participation in the Q-SEAS program by port authorities demonstrates that they are taking their GBO seriously and taking practical and innovative steps to minimise and manage biosecurity risks in their areas of operation. The voluntary participation and support provided by port participants also demonstrates an acknowledgement that the benefits of early detection and early intervention significantly outweigh the risks associated with establishment of marine pests.

1.3 Marine biosecurity risk pathways

The primary risk-pathways for the introduction of marine pests are ship biofouling or ballast water, or through naturally occurring environmental pathways, such as ocean currents and cyclones. Increased coastal development and port expansion also has the potential to change the suitability of habitats for introduced marine species, with invasive species often favouring disturbed habitats and artificial structures. While advancements have been made in ballast water management globally, biofouling regulations are only just starting to be developed and implemented, and other risk pathways in the marine environment remain difficult or impossible to control, so improvements in preparedness and surveillance capabilities that facilitate early detection and reduce the chances of marine pests establishing will continue being important.

The biosecurity risk and impact profile for Queensland ports varies and is dependent on:

- Type of maritime operations and infrastructure
- Type and number of visiting vessels, their origins and time spent at sea and at berth (eg. international vessels originating from countries likely to have established marine pest species recognised as key threats to Queensland)
- The environment in which they operate (eg river, harbour, degree of disturbance, hydrodynamics, connectivity with suitable habitat to support establishment or spread of marine pests)
- Proximity to sensitive or protected areas (eg Great Barrier Reef, aquaculture, tourism)
- Marine biosecurity preparedness, planning and risk-management.

Shifting environmental conditions such as sea surface temperatures, salinity, ocean heat content and ocean surface currents (CMS, 2019) have the potential to alter the risk profiles and pathways for introduction of marine pest in Queensland. Climate change is already causing shifts in ecosystem diversity and in the future, habitats previously unlikely to support the establishment of marine pests might become at risk of incursion (CSIRO, 2014).

1.4 Why Queensland is unique and marine biosecurity is important

Queensland supports the highest levels of shipping trade and operates the busiest ports in Australia (Figure 1 and

Figure 2). With shipping activity forecast to increase ~250% in the next 20 years at ports within the Great Barrier Reef region (PGM Environment, 2012), and a predicted doubling of bulk commodity exports and container imports each decade, Queensland has recognised the risks and seized the opportunity to be proactive and protect its infrastructure, industries, tourism, trade and unique marine environment from the risk of invasive marine pests. The connectivity between high-risk vector pathways, key maritime industries and habitats of international significance, coupled with

Queensland's significant marine tourism industry, provide the incentive for collaborative action in a way that fosters shared responsibility for marine biosecurity.

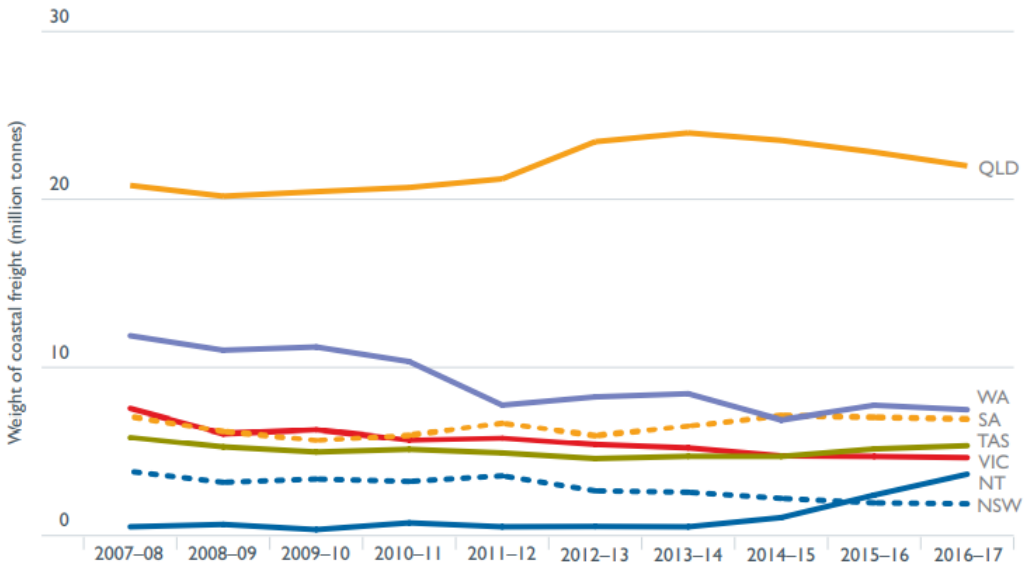

Figure 1: Coastal sea freight loaded in Australian states/territories 2007-2017 (BITRE, 2019)

Figure 2: Ships entering Australian ports by State/Territory 2005-17 (adapted from BITRE, 2019)

1.5 Marine pest surveillance approaches

1.5.1 Traditional methods

Traditional methods of marine pest surveillance are relatively expensive, time consuming and limited to identification of species using morphology and taxonomy. They inherently rely on highly specialised taxonomists, and surveillance typically requires labour-intensive techniques such as visual diver surveys and collection of biological samples (e.g. benthic sleds, trawls, pylon scrapes, traps, plankton, sediment cores) from areas that can be difficult to access. Surveys generally occur infrequently, due

to high costs and site-specific logistical challenges, and limitations to this approach include the inability to detect small and cryptic life stages of target species. Traditional techniques rely on the collection and preservation of viable specimens, which must be intact, of a suitable size, and mature enough for visual taxonomic identification to be effective.

Prior to the Q-SEAS pilot, GPC engaged consultants to undertake independent marine ecology surveys at the port of Gladstone using traditional surveillance methods where identification of potential pests relied on visual identification of potential pest species. Results and reports were not routinely shared with DAF.

1.5.2 Molecular methods

DNA technologies are rapidly becoming a more accessible, informative and cost-effective tool for evidence-based environmental monitoring, including for the early detection of marine pests. DNA is relatively stable and can be readily isolated from fresh, frozen or chemically preserved specimens, and molecular analysis of composite environmental samples (eg. fouling, sediments, water, plankton) can be used to audit environmental DNA (eDNA) for species composition and biodiversity. Sources of eDNA include, but are not limited to, living biota, fragments of living and dead biota, faeces, mucus or scales. The eDNA from even small environmental samples can reveal an incredible amount of information about biological diversity, including native and invasive pest species.

Multi-species detection of environmental eDNA using next generation sequencing (NGS) is a fast, efficient and cost-effective way to characterise the presence of an unlimited number of species from an environmental sample. This approach also provides data on local biodiversity and can be used to retrospectively analyse changes in biological communities. NGS approaches allow for samples to be collected in a non-destructive, cost-effective and timely manner, using sampling techniques that are relatively safer and easier than traditional methods. This technique, if designed and tailored to suitably address the study objectives, can increase the chance of detecting an invasive species at the early phase of an incursion, while a population is confined to a small area and at low density, before it has a chance to establish and spread. This provides opportunity for proactive intervention and management and allows for detection of cryptic species that would be impossible to identify visually.

Metabarcoding characterises DNA ‘barcodes’ (genetic sequences) from all organisms in a sample, providing a rapid biodiversity assessment and the simultaneous amplification of evolutionarily conserved genes from multiple taxonomic groups. Outputs are subsequently characterised through bioinformatic comparison with reference databases. This provides a vast amount of taxonomic data to inform biodiversity studies, or for detecting presence of introduced species. DNA metabarcoding is able to characterise samples through sequencing of molecular markers (genes) that amplify a short, standardised DNA fragment specific for a species (Hajibabaei *et al.* 2007). Different markers are used depending on the taxa group of interest, which is why the analyses used must be carefully designed to address the objectives of individual studies. The most commonly used marker for metabarcoding of animals is the cytochrome *c* oxidase subunit 1 (CO1), because it can be amplified from an enormous range of organisms, it has high resolution (allows classification of taxa to species level) and can be found across taxonomically verified databases (Deagle *et al.* 2018). The 18S ribosomal RNA gene is another commonly used marker for phylogenetic and biodiversity studies, because it is highly conserved and can detect a diverse range of taxa.

The choice of metabarcoding marker can greatly affect detection capabilities and a combination of markers can help to overcome the limitations of individual markers, therefore improving the taxonomic breadth and resolution capabilities of the analysis and reducing the rates of false negatives and false positives (Zhang *et al.* 2018). A reliable genetic reference library developed from taxonomically verified voucher specimens and associated DNA extracts is another important component of the metabarcoding approach (Deagle *et al.* 2018). While DNA metabarcoding cannot provide quantitative data (the number/population of a species present in the environment) nor determine whether taxa was living at the time of collection, it does effectively indicate ‘presence’ (or risk) and provides evidence-

based data to inform how and where further attention (investigations, surveillance) should focus. The sampling effort and surveillance strategy must be carefully designed if molecular methods are to be effective and should aim to achieve detection from different pathways and in varying habitats, with sufficient spatial and temporal coverage (Zaiko *et al.* 2018).

Metabarcoding uses a broad taxonomic approach, which seeks to identify as many organisms in a sample as possible. In the event of a suspected marine pest detection, follow up testing using a targeted molecular approach may be required, if the metabarcoding outcomes cannot be resolved with a high level of confidence (due to a lack of reference sequences for closely related species).

2 The Q-SEAS marine biosecurity program

Gladstone Ports Corporation (GPC) is one of five port authorities to partner with Biosecurity Queensland (DAF), to implement an innovative state-wide marine pest surveillance pilot program across Queensland. The Queensland Seaports eDNA Surveillance (Q-SEAS) program was developed as part of the Queensland Marine Pest Prevention and Preparedness Project (under the Queensland Biosecurity Capability Implementation Program) and used molecular technologies to analyse environmental samples collected from potentially high-risk areas within port berth and harbour areas, to test for the presence of invasive marine species. The eDNA approach is an advancement on previously used traditional surveillance methods and is particularly valuable because using molecular diagnostics:

- improves our ability to detect small and cryptic organisms that would otherwise not be discernable in a visual inspection
- is rapid and cost-effective
- facilitates regular surveillance
- reduces safety concerns associated with divers in the water
- requires fewer resources than traditional marine pest monitoring methods.

This, along with the increased education and awareness and the strong partnership relationships built with ports, represents a significant enhancement of marine pest surveillance capabilities at the participating ports and for Queensland.

2.1 Development of the state-wide model

Q-SEAS is the first state-wide marine biosecurity surveillance program in Queensland and was designed to be risk-based, adaptable, transformative, cost-effective, and in alignment with the Australian Government's *MarinePestPlan 2018-2023* (DAWR 2018). The model for a Queensland-wide marine pest surveillance program was initially discussed between Biosecurity Queensland and the Western Australian Department of Primary Industries and Regional Development (DPIRD), who collaborate with Port Authorities in Western Australia (WA) to deliver the WA State-Wide Array Surveillance Program, or SWASP. Ideas were further explored between Biosecurity Queensland, DPIRD and Queensland's port authorities after the Queensland Biosecurity Capability Review (2015) recommended marine biosecurity as a key focus area for improvement in Queensland. The Q-SEAS program was subsequently developed in early 2019 after a high level of interest from the ports.

The Q-SEAS program design was informed through consultation with participating ports with regards to operational delivery, a review of high-risk vector pathways and invasive marine species biology, assessment of local environmental attributes and with reference to the scientific literature. Support, information and advice was provided to Biosecurity Queensland by DPIRD and other academic researchers throughout the creation of the Q-SEAS program, including learnings from the SWASP. Opportunities for improvement or enhancement of previous studies were thoroughly investigated in order to comprehensively inform future surveillance options, such as trialing of additional sampling substrates and genetic markers.

2.2 Strategic alignments

The Q-SEAS program implemented coordinated and collaborative activities that are in alignment with key national and state level strategic programs, which are summarised in Table 1. Importantly, resources were targeted towards agreed national priorities to provide lasting benefits and demonstrated how marine biosecurity can become a shared responsibility between the Queensland Government and industry, with support from research organisations. The Q-SEAS model engages with stakeholders to improve marine pest biosecurity management and works to achieve Australia's 'national vision' of *maintaining Australia's healthy and resilient marine environment that is protected from the threat of marine pests, which supports our economy and social amenity* (*MarinePestPlan* 2018-23).

Table 1: Strategic alignments of the Q-SEAS program

Key Strategic Plans	Strategic Alignment
Australian Government *MarinePestPlan* 2018-23 (DAWR 2018)	Implementing coordinated and collaborative activities, to ensure resources are targeted towards agreed national priorities to provide lasting benefits. Demonstrates how marine biosecurity can become a shared responsibility between government, industry and research organisations; enhances preparedness and response capability; engages stakeholders to better manage marine pest biosecurity; focuses on prevention/early detection and strengthens the surveillance system.
Reef 2050 Long-Term Sustainability Plan (Commonwealth of Australia 2018)	Improving capability and capacity in the marine biosecurity system through education and awareness, building relationships with stakeholders/partners and developing surveillance; supporting goals of protecting the future of the Great Barrier Reef World Heritage Area (GBRWHA) through early detection and proactive management of invasive marine pests.
Australia's Biosecurity Future (CSIRO 2014)	Adopting a future-focused approach to understand Queensland's marine biosecurity status and seeking to strengthen biosecurity, using technology and innovation for surveillance.
DAF Strategic Plan 2019-23 (DAF 2019)	Working with researchers to drive innovation; building capacity to meet sector challenges; establishing collaborative solutions to address critical challenges and opportunities; and promoting responsible biosecurity practices to protect productivity and environmental sustainability.
Priority Ports Master Planning Guideline (State of Queensland 2020)	Contributing to managing balance between environmental protection of the GBRWHA and economic growth at priority port development areas.
Queensland Government Strategic Plan 2018-22 (State of Queensland 2018)	Working with public and private sectors to support Queensland's future, using new ideas; systems and processes; with integrity, transparency and accountability.
North-East Shipping Management Plan (AMSA 2014)	Improves confidence in stakeholder perceptions of marine biosecurity in Queensland, for minimising risks to the GBRWHA and other areas of value.
Biosecurity Queensland Strategy 2018-2023 (DAF 2018)	Transformative; uses emerging technologies; focuses on early detection and prevention; helping to protect ecosystems and way of life.

2.3 The Q-SEAS approach

Experimental design for marine pest surveillance is more effective if it incorporates multiple substrates (eg plates, plankton, water, sediments; Koziol *et al.* 2018). The Q-SEAS method incorporated collection of DNA and eDNA using a multi-substrate approach, through deployment of settlement arrays to capture fouling organisms, and collection of plankton samples to assess biota in the water column. Samples were analysed using CO1 and 18S DNA metabarcoding assays by eDNA Frontiers laboratory at Curtin University. The metabarcoding approach allowed for testing of an unlimited number of target taxa (and reduced the risk of missing target taxa), which was an important consideration when assessing a taxonomically broad list of marine pests as defined in the *Queensland Biosecurity Act 2014* and other species identified as a potential threat for introduction to Queensland (section 7.2 and section 7.3), across a diverse geographic area. Photographs of fouled settlement plates were taken upon retrieval, and subsequently used for cross-examination of plates with DNA results, thereby providing an additional line of evidence in the assessment process. DNA samples collected during the trial phase have been "biobanked" in secure long-term storage, to facilitate future analysis if the need arises. These samples could also be used to optimise laboratory protocols for improving future Q-SEAS sample analysis techniques.

The Q-SEAS pilot program aimed to identify the benefits and limitations to application of innovative molecular methods for marine pest surveillance, with monitoring designed to be flexible and evolve as technologies and sampling methodologies improve, and as Q-SEAS outcomes are able to inform refinement of the method. Q-SEAS was managed by Biosecurity Queensland and delivered in

partnership with participating port authorities, who provided significant financial and in-kind contributions to support its delivery.

The central point of coordination and independent oversight by Biosecurity Queensland enabled development and delivery of Q-SEAS with support from suitably qualified and experienced marine biosecurity personnel, enabling a high level of scientific rigour for all aspects of the program's delivery and significant cost-efficiencies through bulk purchasing and supplier chain setups. Key functions of this role included program design, development and delivery of ready-to-deploy equipment 'kits' to each participating port, site-based support and training for equipment deployment and sample retrieval, coordination of sample preservation, transport and analysis, interpretation and reporting of results, managing potential detections and follow-up investigations, and stakeholder liaison and contractual engagements. Biosecurity Queensland was also able to provide Q-SEAS partner ports with unique insight into new marine biosecurity threats.

2.4 Key achievements of Q-SEAS

Q-SEAS established a robust, consistent and coordinated surveillance network for the early detection and proactive management of marine biosecurity threats at Queensland's ports in Brisbane, Gladstone, Mackay, Townsville and Cairns. Q-SEAS significantly improved the ability to detect small and cryptic taxa using methods that are faster, cheaper and safer than traditional marine pest surveillance methods, without reliance on morphology and visual taxonomic identification. Outcomes have enabled evidence-based decisions to inform marine pest surveillance efficacy, practices, management and policies, with results already used to inform and guide future surveillance efforts.

Q-SEAS significantly improved Queensland's marine biosecurity capacity, providing improved early detection, early intervention and preparedness, helping to protect valuable port operations, maritime industries and the environment. Continued surveillance over varying temporal and spatial scales will improve knowledge on the status of marine pests in Queensland, with biobanking of DNA samples allowing for retrospective analyses as technologies improve, or for understanding changes in biological communities in response to key events, including future marine pest incursions. Opportunities exist for cross-purposing data for other biodiversity or research-based priorities, and future surveillance should continue to explore options for knowledge-sharing and mutually beneficial collaborations.

Alliances developed between participating port authorities and Biosecurity Queensland demonstrated how marine biosecurity responsibilities can be shared between government and industry. Q-SEAS was coordinated, collaborative and targeted towards agreed national priorities, using a risk-based approach that focused on achievable outcomes. Biosecurity Queensland's role as the centralised point for coordination was critical for its success, providing the conduit between the science, research, industry and government, and supported the platform for delivery of a mutually beneficial partnership program, in a cohesive and consistent manner yet that provided flexibility to be tailored to each port's needs.

The pilot program resulted in improved understanding of marine pest diagnostics and the application of DNA analysis for surveillance, with returns to industry and government evident through the effectiveness of the early warning surveillance network.

2.5 Standard Operating Procedures

Standard Operating Procedures (SOP) for Q-SEAS (DAF, 2020) were developed as a standalone document, and aim to support the ongoing delivery of surveillance, as well as to assist with expansion of Q-SEAS to new locations. The SOP provides detailed protocols for all site-based activities and was provided as a guide for all participating ports. The SOP will continue to be reviewed and updated to incorporate learnings from pilot program implementation.

3 Methods

3.1 Scope

Marine pests of interest are listed in Queensland's *Biosecurity Act 2014* and other key marine pest target lists, for example, the Australian Priority Marine Pest List (ABARES 2019) (sections 7.2 and 7.3), and refers to those recognised as potentially invasive. Q-SEAS scope excluded diseases in the marine environment and species native to Australia, including those undergoing range expansions such as the crown of thorns starfish.

3.2 Schedule

Surveillance events were scheduled on a biannual basis, to capture differences in seasonality and associated changing environmental conditions. Q-SEAS event 1 targeted the 2019 winter/spring period (August/September), and event 2 targeted the 2019-2020 summer period (December/January). The first deployment of settlement arrays at the Port of Gladstone was completed on 16 September 2019, with sample retrievals completed on 14 November 2019. Event 2 settlement arrays were deployed on 14 November 2019 and retrieved on 14 January 2020. Plankton samples were collected on 17 September 2019 and 15 January 2020.

3.3 Notification flyers

Prior to deployment of equipment and collection of samples, a 'notification flyer' was distributed to notify port users of the upcoming marine pest surveillance activities (Appendix 7.4).

3.4 Site Selection

Factors considered for the selection of placement sites for the settlement arrays included the proximity to high-risk vectors, site conditions, ease of access, safety, the biology of target marine pest species and port operations (Table 2).

Table 2: Criteria for selection of settlement array placement sites and plankton tows

Key criteria	Factors for consideration
Proximity to high-risk vectors	• Commercial vessels may transport marine pest species from international waters through hull fouling or in ballast water. Ports and harbours are the first point of entry for international vessels and port environments/infrastructure often provide sheltered areas with artificial and natural substrates that might be suitable or preferable for the settlement of marine pests. • Recreational vessels such as yachts that undertake international or interstate voyages and spend time at marina facilities
Site conditions	• Hydrodynamics - tides, water currents, water circulation, salinity and freshwater incursion, proximity to rivers or protected harbours • Disturbance – protection from waves, swell, ship thrusters, frictional wear or human interference
Accessibility and safety	• Safety and ease of entry/exit with equipment • Security, restricted areas, permits, inductions and approvals for entry to site • Public access (risk of tampering or theft)
Marine pest biology/ecology	• Preferred habitats, environmental tolerances, dispersal pathways of marine pests
Port operations	• Identify vessel berthing areas where international vessels originate from countries likely to have established marine pest species recognised as key threats to Queensland

3.5 Settlement arrays

Settlement arrays were deployed at four locations (Figure 3) for a period of two months during each surveillance event. Grey square PVC plates (120 mm x 120 mm x 4.5 mm) were attached to the arms of each settlement array frame using cable ties. Each plate was scuffed (80-120 grit sandpaper) prior to deployment to roughen the surface, aiming to provide a more suitable surface to facilitate settlement and growth of marine fouling organisms. Eight plates were attached to the settlement array for surveillance event 1 (four vertically and four horizontally; Figure 4). For surveillance event 2, additional plates were attached to make plate 'sandwiches', where two plates were horizontally aligned parallel to one another (four plates vertically and six plates horizontally; Figure 5). The purpose of this approach was to provide more protected areas for growth of marine organisms, away from predators and in areas less exposed to sunlight and water movement.

Upon retrieval, settlement arrays were photographed and visually inspected for unusual or heavy fouling. Photographs were taken of both sides of each plate as they were removed from the array, prior to being placed in thick resealable plastic bags and immediately preserved (frozen) on dry ice pellets. Six plates were carefully selected for analysis based on types and quantities of fouling observed, and the remaining plates were retained in the dedicated marine pest freezer on site at the port as a contingency. Fouling on the settlement array frames and floats was also visually inspected for any unusual or excessive growth. Frozen plate samples were subsequently sent via a specialist courier to the analysing laboratory for DNA testing. All plate samples remained frozen from the point of retrieval until being processed in the laboratory. Images of settlement array activities at the Port of Gladstone are provided in Figure 6.

Figure 3: Settlement array monitoring locations at the Port of Gladstone

Figure 4: Settlement arrays at the Port of Gladstone

Figure 5: Settlement array setup for surveillance event 2, showing plate 'sandwich'

Figure 6: Settlement array deployments and retrievals at the Port of Gladstone

3.6 Plankton sampling

Plankton sampling was undertaken in addition to deployment of settlement arrays to improve the program's detection capabilities, by capturing planktonic life stages or DNA that may otherwise go undetected on settlement plates.

Plankton samples were collected on 17 September 2019 (event 1) and 15 January 2020 (event 2), from fifteen locations illustrated in Figure 8. Methods for collection of plankton consisted of fifteen 100 m horizontal tows, with sampling sites concentrated at a distance of approximately 500 m apart. Plankton collection sites were located around RG Tanna Wharf (Figure 8) due to this area being visited by vessels from international waters of concern and therefore considered at risk of potential translocation or introduction of marine pests. The distribution of sites was based on the premise of seeking to detect something that is either present in very low abundance, or not known to occur at all. The replication of samples and frequency of sampling aimed to be scientifically robust but practicable - fitting within the stringent time and budget limitations of the program. The distribution of sampling sites gave consideration to relevant environmental influences and confines.

GPC also requested the addition of plankton sample collection from East Banks Spoil Disposal Site (EBSDS) (Figure 9) as part of the pilot program and undertook this sampling on 18 September 2019 (event 1) and 15 January 2020 (event 2) for the purpose of monitoring and reporting on potential marine pest introductions related to port dredging activities.

The plankton kit was comprised of a 50 cm x 150 cm x 50 µm plankton net and bridle with an 11 cm diameter lead-weighted PVC cod end with detachable lower section, lined with 50 µm mesh (Figure 7). The plankton net was secured to the vessel by rope and a flow meter was attached to the mouth of the plankton net to measure the approximate volume of water sampled. Vessel-based plankton tows were completed at slow speeds (~1 to 1.5 knots depending on tidal currents and tow direction), with the net submerged just below the water surface for the duration of each transect. The flow meter was reset to zero prior to commencement of each transect and flow meter readings were recorded upon each net retrieval. Transect lengths were measured using a handheld GPS device.

Plankton samples were concentrated in the cod end, with detritus removed using a small aquarium net and beaker, before being transferred to labelled 50 mL sampling vials for preservation and submission to the analysing laboratory. Around 10 mL of headspace was left in each vial to allow for expansion of samples when frozen. Vials were immediately placed in dry ice for preservation and stored in the dedicated marine pest sample freezer prior to being transported to the laboratory. Plankton samples remained frozen from the point of retrieval until being processed in the laboratory. Plankton sampling activities at the Port of Gladstone are illustrated in Figure 10.

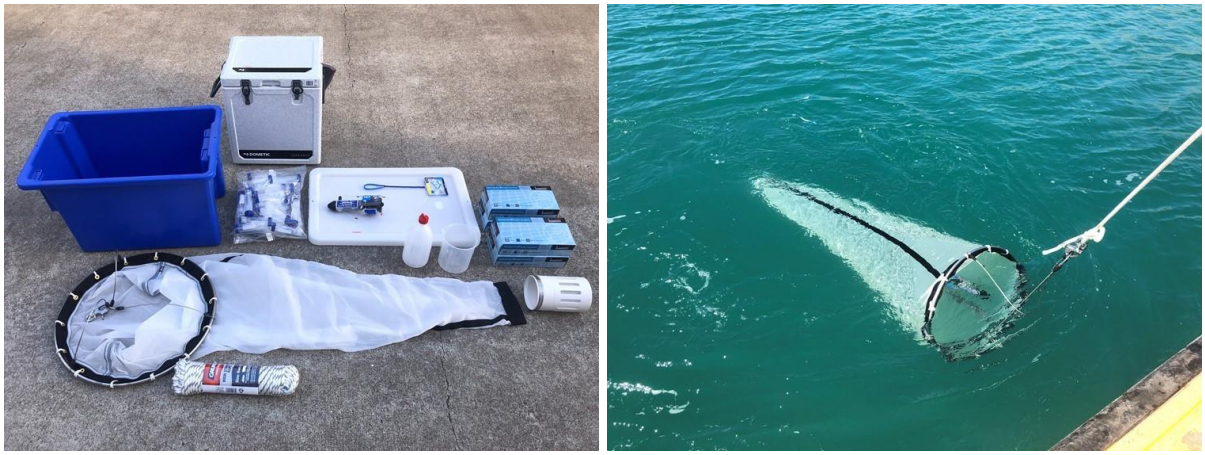

Figure 7: Plankton sampling equipment kit

Figure 8: Plankton sampling locations at the Port of Gladstone

Figure 9: Location of East Banks Spoil Disposal Site (EBSDS)

Figure 10: Q-SEAS plankton sampling at the Port of Gladstone

3.7 Quality Control Measures

Strict quality control measures are essential for sample collection during all environmental monitoring programs, with specific protocols enacted when genetic analysis is applied to the study. Full measures are outlined in the Q-SEAS SOP (DAF 2020), however a summary of procedures applied in the field and in the laboratory are outlined below.

3.7.1 Field

Settlement arrays

Laboratory grade nitrile gloves were worn at all times during retrieval of settlement arrays and for handling of settlement plates, with gloves changed between each array. Clean plastic sheeting was placed underneath each settlement array prior to retrieval, to avoid cross-contamination with the underlying surface. All implements used for sampling processes (eg scissors, knives used to remove settlement plates) were decontaminated between sites using a diluted bleach solution, and rinsed well prior to use. Each settlement plate sample was double bagged using high quality Sandvik 80 µm press-seal bags, with waterproof labels inside each bag to identify the samples. Samples were immediately preserved on dry ice upon collection. A clean cooler box was purchased solely to preserve and transport Q-SEAS settlement plate samples, and a dedicated marine pest freezer was purchased for use as a clean and secure temporary repository for all Q-SEAS samples. Photographs were taken of all sites, arrays during deployment and retrieval, and each settlement plate was carefully photographed (both sides) upon retrieval. Consumption of food, beverages or smoking was not permitted during sample handling. Settlement plate samples remained frozen at all times after collection, including while being transported to the analysing laboratory.

Plankton sampling

Laboratory grade nitrile gloves were worn at all times during retrieval of plankton samples, with gloves changed between each sample. Each plankton sample was carefully labelled with a unique identifier in accordance with the standard naming convention (3.7.5), and each screw-top vial lid was securely sealed using parafilm. Samples were immediately preserved on dry ice upon collection. A clean cooler box was purchased solely to preserve and transport Q-SEAS plankton samples, and a dedicated marine pest freezer was purchased for use as a clean and secure temporary repository for all Q-SEAS samples. All equipment was thoroughly decontaminated after use using a diluted bleach solution and rinsed well in clean fresh water prior to being placed in storage. Plankton samples remained frozen at all times after collection, including while being transported to the analysing laboratory.

3.7.2 Sample preservation

Samples were immediately frozen upon collection, using dry ice pellets which were stored in dedicated marine pest settlement plate and plankton cooler boxes. Samples remained frozen at all times after collection, including while being transported to the analysing laboratory.

3.7.3 Sample transport

Nitrile gloves were worn at all times when packing or handling samples for transport to the laboratory. Chain of custody (COC) documentation was sent with all samples, with information including the date sampled, preservation methods and unique sample identifiers. The COC was signed upon dispatch by the sender and by the recipient, with sample receipt and acknowledgement of sample integrity confirmed by the receiving laboratory. A specialist courier was used to transport frozen plate samples via air freight to the analysing laboratory for DNA testing, to ensure rapid transport and preserve DNA integrity (by keeping samples frozen at all times).

3.7.4 Laboratory

The eDNA Frontiers laboratories are dedicated areas for demand-driven contract research and routine sample processing established and under a Quality Management System with standard operating protocols to meet commercial needs. The sample preparation laboratory is an isolated and dedicated space for processing environmental samples for DNA extraction. This laboratory contains dual robotic DNA extraction systems, with capacity for a throughput of 150 samples per standard day. Dedicated sequencing and analysis laboratories are used for NGS technologies and quantitative PCR analyses. The laboratories are accredited and operate within a Class 5.1.1 Biosecurity Containment Level 1 (BC1) Microbiological facility, which includes an Advanced Clean Environment (ACE) 434 m^2 pressurised, class 100 (ISO 5) clean air space housing five class 10 (ISO 4) ultra-clean laboratory. The facility contains cutting edge technology and liquid handling robots to eliminate contamination and increase precision and accuracy in pre-PCR set-up. This facility is a major contributor to eDNA Frontiers being able to provide confident data detection, at levels which are filtered out in other testing facilities. It is a unique facility in the southern hemisphere (eDNA Frontiers, 2020). The laboratory is currently undergoing National Association of Testing Authorities (NATA) accreditation.

The “Pawsey Supercomputing Centre” is one of two, Tier-1, High Performance Computing facilities in Australia, with expertise in supercomputing, data, cloud services and visualisation. The Centre is used for completing bioinformatics analysis of sequencing outputs. All analytical procedures undertaken by eDNA Frontiers as part of the Q-SEAS program were done so in accordance with strict operating procedures for eliminating cross contamination risks and providing reliable, robust outcomes that can be used with confidence.

3.7.5 Sample naming conventions

Unique identifiers were assigned to all samples and named in accordance with the following protocol:

- Three letters to represent the Port location (GLD = Gladstone)
- Two numbers to represent the Event (01 or 02)
- One letter to represent the method (A = array, P = Plankton)
- Two or three digits (combination of letters, numbers or both) to represent the site (eg BP for Barney Point, BSL for Boyne Smelters Limited)
- Two numbers to represent the sample (plate) number (01, 02, 03… etc)

Examples of naming applied: settlement plates GLD01ABP01; plankton GLD01P01.

3.7.6 Documentation and record keeping

Q-SEAS-specific datasheets were developed for recording information relating to equipment deployment and sample retrieval for both settlement arrays and plankton sampling. Copies of the datasheet templates are provided in the Q-SEAS SOP.

3.8 Molecular analysis

Frozen settlement plate and plankton samples were transported via a specialist courier from the port to the eDNA Frontiers laboratory at Curtin University. The biofouling was scraped from the six plates and material from two plates was pooled together (to make 3 replicates), homogenised and subsampled for DNA extraction. Plankton samples were thawed, homogenised and subsampled for DNA extraction. Biomass DNA was extracted from samples using a commercial tissue genomic DNA extraction kit following the eDNA Frontiers standard operating procedure. Extracted DNA was used for metabarcoding analysis and remaining samples were stored at -80°C.

Settlement plate samples were analysed using the 18S (Pochon *et la.* 2013) and CO1 (Leray *et al.* 2013) metabarcoding assays, and plankton samples were amplified with the 18S assay only. The application of two metabarcoding assays increased the diversity and resolution of taxa detected from each surveillance sample (see Appendix 2 at section 7.2). NGS was completed using an Illumina MiSeq© platform, with negative controls included in the DNA extraction phase and PCR stages of the workflow to detect potential contamination and maintain quality control procedures.

Bioinformatics assessment was completed by eDNA Frontiers by transforming unique sequences into zero radius operational taxonomic units (ZOTUs) to provide sensitive taxonomic resolution. The ZOTUs were queried against the nucleotide database NCBI (Genbank) for taxonomic assignment. Sequences were converted to the lowest possible taxon based on similarities and differences to the National Centre for Biotechnology Information (NCBI) Genbank sequence database. The final DNA sequences were then screened against the eDNA Frontiers in-house database, which contains the genetic sequence reference library compiled by DPIRD for target taxa, which contains the same marine pest taxa required for the Q-SEAS screening. Final outputs were then conservatively assessed against the stepwise criteria for the marine pest assignments (Figure 11).

Using the metabarcoding approach, a possible marine pest detection occurs when comparison of results (DNA sequences) through the bioinformatics assessment (checking results against available genetic databases to determine what species they belong to) is found to match that of a known marine pest target species. When DNA sequences for closely related locally occurring species are known, and target pest sequences have been generated and verified using vouchered specimens, the metabarcoding results (if resolved to species level) can be cross-checked against these and confidently assigned, therefore confirming or ruling out the possible detection. If verified DNA sequences for closely related locally occurring species are unavailable, then the ability to confidently distinguish a marine pest species from a local species is difficult. Conservative taxonomic assignments must then be made and can result in the outcomes being described as ‘inconclusive’.

If the metabarcoding approach identifies a suspected marine pest from surveillance samples, follow-up investigations using species-specific targeted qPCR technologies are an additional line of analysis that, provided validated assays (primers and probes) are available, are incorporated into the Q-SEAS assessment process.

Developments in genetic technologies, including optimisation of assays and availability of more comprehensive genetic databases will continue to improve the sensitivity and specificity of molecular tools, reducing the limitations and improving the outcomes of their application. When a holistic approach is adopted for analysing surveillance samples, including the use of multiple metabarcoding assays from multiple substrates, with species-specific qPCR for follow-up investigations, these techniques provide effective methods for early detection of invasive marine pests.

Criteria				Confidence
1	Is a sequence >97%* similar to an AIS reference sequence	Yes Go to 2	No	
2	Can the assay differentiate the AIS from other species	Yes Go to 3	No	Inconclusive
3	Does the sequence match to an AIS at 99%*	Yes Go to 4	No	Inconclusive
4	Are there available reference sequences for closely related local taxa	Yes Go to 5	No	Possible
5	Has the AIS been reported in the area before	Yes Go to 6	No	Probable
6				Highly Probable

Figure 11: The eDNA Frontiers stepwise criteria for assessing sequences against the marine pest reference library

3.9 Key considerations and limitations

Q-SEAS experimental design was selected based on reviews of available scientific literature, with the most scientifically robust method that fit within the time and budget limitations of the pilot program adopted. Future reviews and evaluations will be used for understanding where opportunities for refinement or enhancement can be achieved. Key factors for determining the fit-for-purpose sampling design and optimised surveillance strategy are outlined in Table 3.

Table 3: Key considerations for Q-SEAS experimental design and surveillance strategy

Key considerations	Comments
Species tolerances	• Habitat preferences, lifecycles, spawning conditions etc. (see section 7.5).
Seasonality	• Changes in environmental conditions influences species diversity, population abundance and spawning cycles, through changes in water temperature and salinity.
Sampling environment	• Majority of eDNA work in aquatic environments is for freshwater species or for surveillance of a single known target (eg endangered species), where optimised sampling and analysis protocols are available. The marine environment is vastly different and research on achievable detection rates and optimal sampling methods for introduced species is limited (including genetic research on locally occurring species). Surveillance in dynamic marine waters must consider size of sampling area (eg. river, enclosed harbour, open water), and levels of influence from tidal regimes, freshwater influx and water mixing and contaminants. Results for some ports may represent an assessment of a particular high-risk area within port waters, rather than an assessment of the port in its entirety.
Sampling substrates	• Types of substrates (eg. plates, water, sediments, plankton) were thoroughly researched for determining most viable options for Q-SEAS. Settlement plate sampling and plankton sampling were selected as practicable options. Plankton sampling is able to concentrate samples of microscopic plankton (≥50 µm) from large water volumes using simple vessel-based collection. This method can capture living biota or fragments of DNA in the water column, including early life stages that are unlikely to be found on settlement arrays, provided effective preservation and analytical methods are adopted. Sampling sensitivity, optimal number of tows and most suitable diagnostic methods can be refined over consecutive years, based on outcomes from each round of surveillance. • Water sampling was determined to not yet be an appropriate technique for routine marine pest surveillance, due to inadequate knowledge of detection sensitivities and optimal number of samples required (replication). Water sampling may be applicable for testing DNA in closed water bodies (eg. rainwater tanks, dams, creeks, ponds). However, in dynamic marine environments there are more challenging variables to overcome (eg hydrodynamics), and further trials and testing of water sampling for the purposes of eDNA detection of invasive species in marine waters is required for development of optimum, reliable and standardised sampling protocols, before this approach can be applied to routine marine pest surveillance. • Sediment sampling was also considered however the capabilities of laboratories to receive prepare and analyse samples using eDNA techniques were not sufficiently proven to support this method as part of the pilot program.
Laboratories	• Accessibility to commercially operating laboratories with adequate capacity and capabilities for provision of services that are suitable for analysis of marine samples, preferably with experience in marine biosecurity (eg. access to in-house DNA libraries for target marine pest species). • Affordability and analytical turnaround times critical - Q-SEAS could not fund lengthy and expensive experimental trials, testing and validation of new laboratory assays or protocols. • Diagnostic screening needed to be able to test for an unlimited number of target pest species from bulk environmental samples, with levels of sensitivity and specificity high enough to detect something that may be present in very low numbers. • Access to DNA reference libraries for marine pest target species of concern for Queensland.
Practicality	• Logistical constraints, availability of staff, equipment, site accessibility and safety. • Site-based activities were designed to be as simple as possible, to minimise risk of cross-contamination and limit handling of samples by site operators (eg plate scraping and processing not done on site, no filtering of samples, no dilution of chemicals, and provision of high-quality equipment that was easy to use). • Sample preservation techniques (eg freezing or use of chemicals) that were appropriate, safe, affordable and practicable (particularly for transportation) to ensure viability of DNA.
DNA integrity	• Sample collection and preservation techniques must maintain DNA integrity, and laboratory analysis must be effective in amplifying target DNA with high levels of quality control. Results must be interpreted with consideration for DNA degradation rates, which are variable and depend on target species, life stage, lifestyle, morphology, shedding rates and environmental influences including sunlight and water chemistry (water temperature, pH, salinity, contaminants).

4 Results and Discussion

Application of the dual substrates (plates and plankton) and two metabarcoding assays (18S and CO1) increased the diversity of taxa identified, with many taxa classified to genus and species level. This helped to rule out or confirm potential marine pest detections, with results being conservatively interpreted using a stepwise approach. Results provided critical baseline data for understanding the current status of marine pests at the Port of Gladstone, and pilot program demonstrated that the method does significantly improve our ability to detect small and cryptic taxa using methods that are faster, cheaper and safer. While the approach was not reliant on morphology and visually-based taxonomic assessment, imagery of fouled settlement plates provided an important additional level of evidence in the assessment process, for supporting the interpretation of results. Examples of settlement plate fouling from surveillance event 1 and 2 is illustrated in Figure 12, Appendix 7.5 and Appendix 7.6.

Figure 12: Representative settlement array fouling at the Port of Gladstone (event 1)

4.1 Event 1

Q-SEAS event 1 was successfully completed at the Port of Gladstone between September and November 2019. Two metabarcoding assays (18S and CO1) were applied to settlement plate samples, and the 18S metabarcoding assay was applied to plankton. A total of 151 families from 22 phyla were identified across all substrates, with only 6 families identified by both assays in both substrates (Balanidae barnacles, Ascidiidae and Styelidae ascidians, Bougainvilliidae hydroids and Ceramiaceae red algae). This demonstrates the importance of using multiple substrates and assays to increase the likelihood of detecting target taxa.

The diverse range of taxa identified using the 18S assay included:

- 47 families in plankton (15 phyla; Figure 13 and Figure 14)
- 90 families on settlement plates (16 phyla; Figure 13 and Figure 15).

The diverse range of taxa identified using the CO1 assay included:

- 57 families on settlement plates (12 phyla) including resolution of 62 genera and 54 species (Figure 13 and Figure 16).

There was little overlap between taxa identified in either substrate type or metabarcoding assay, with 19 families identified by the 18S assay in both plankton and plate samples; and 23 families identified on plates by the two different assays. Only 6 families were identified on both plates and in plankton (Figure 13).

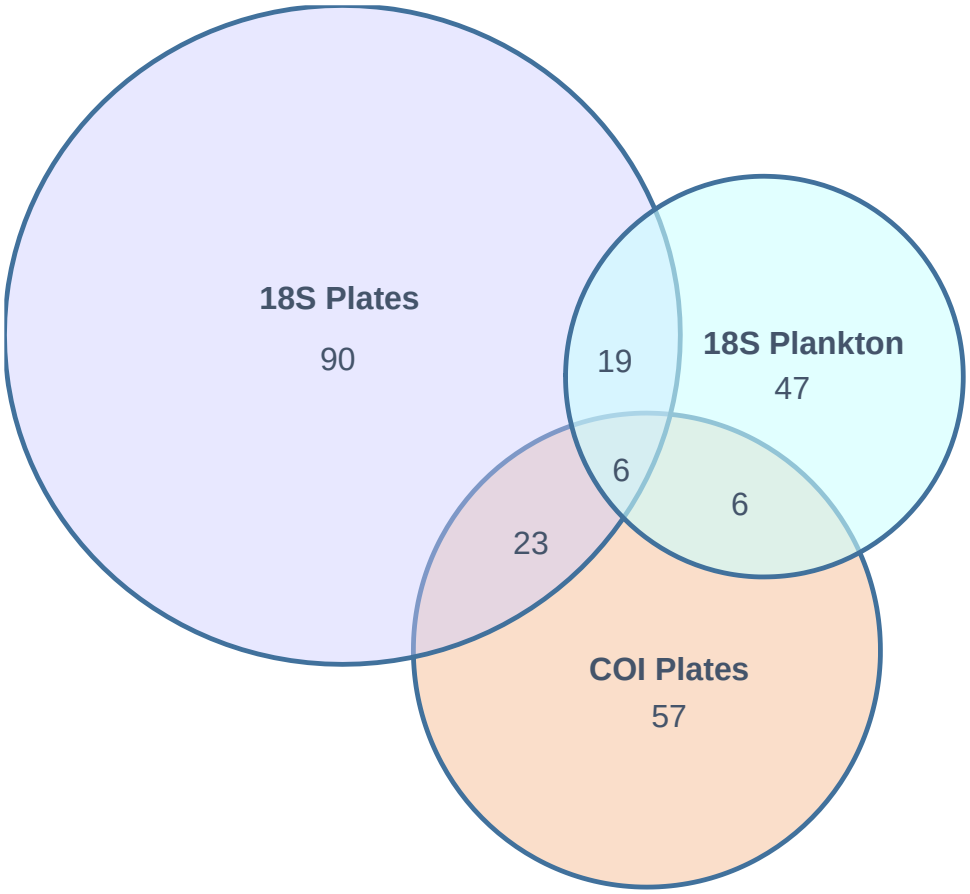

Figure 13: Venn diagram showing number of families identified in event 1, using the 18S and CO1 assays on plates and in plankton. There was little overlap between taxa identified in either substrate type or metabarcoding assay. Note: total numbers for each parameter include the overlap.

No target marine pests (as listed in section 7.2 and 7.3) were detected from samples collected in the winter/spring sampling event. Worms, ascidians, mussels, barnacles, oysters and seaweed belonging to target marine pest 'family' classifications were identified (Table 4). The 18S and CO1 assays provided information to help rule out the presence of the invasive worms *Sabella spallanzannii* and *Marenzelleria spp.,* ascidians *Didemnum sp*., mussels *Arcuatula senhousia and Perna sp.*, barnacles *Amphibalanus improvisus* and Crassostrea oysters. Taxonomic resolution allowed identification of worms as *Bispira crassicornis, Scolelepis sp.* and *Branchiomma bairdi*, ascidians as *Diplosoma listerianum*, mussels as *Modiolus sp., Brachidontes sp.* and *Trichomya sp.,* barnacles as *Amphibalanus amphitrite*, oysters as *Dendostrea sp.* and *Saccostrea sp.* These are known to occur locally except for *B. bairdi*, which is a feather-duster (tube-dwelling) worm native to central Florida to Atlantic Panama and the Caribbean Islands and has been introduced to the Pacific Coast of Mexico and Panama, the Galapagos Islands, Hawaii, the Mediterranean, the island of Madeira and Queensland, Australia (Fofonoff *et al.* 2018). The first known occurrences of *B. bairdi* in Queensland were at Lizard Island (Capa and Murray, 2015; Capa *et al.* 2013). This species is introduced but not recognised as an invasive marine pest in Australia, and future surveillance could monitor for the ongoing presence and distribution of this species.

Seaweed belonging to the *Sargassum* genus (brown macroalga) were also identified in plankton and on plates at Barney Point (Table 4), but the analyses could not resolve these detections to species level and rule out the presence of the invasive species *Sargassum muticum*. Seaweed belonging the *Sargassum* genus is one of the most diverse genera of brown marine algae known to commonly occur in shallow tropical and subtropical waters. *Sargassum* seaweed is very prevalent and speciose across Australia, with at least 373 species of known to occur in Australia (Diaz-Pulido and McCook, 2008), and up to 537 species represented globally (Guiry, 2020). The widespread occurrence of this diverse algae and the lack of available reference DNA sequences for native *Sargassum* species meant that this result was classified as 'inconclusive', and no further testing was undertaken.

Table 4: Gladstone metabarcoding bioinformatics results from both substrates and assays (event 1)

Target marine pest species	Identification (Family level)	Identification (Genus level)	Results
Results from 18S assay			
Fanworm – *Sabella spallanzanii*	Sabellidae	Sabella	Sequences identified as *Bispira crassicornis*
Burrowing worm – *Marenzelleria spp.*	Spionidae	*Marenzelleria*	Sequences identified as *Scolelepis sp.*
	Detected at GLD01ABSL and plankton	Not detected	
Barnacle – *Amphibalanus improvisus*	Balanidae	Amphibalanus	Sequence ID unable to be confirmed
Ascidian – *Didemnum spp.*	Didemnidae	*Didemnum*	Sequences identified as *Diplosoma sp.*
	Detected at GLD01AGM, GLD01AFL	Not detected	
Mussels – *Arcuatula senhousia, Perna spp.*	Mytilidae	Arcuatula, Perna	Sequences identified as *Brachidontes sp*, *Modiolus sp.* and *Trichomya sp.*
Seaweed – *Sargassum muticum*	Sargassaceae	Sargassum	Sargassum sp. – sequence ID unable to be confirmed
Results from COI assay			
Fanworm – *Sabella spallanzanii*	Sabellidae	*Sabella*	Sequences identified as *Branchiomma bairdi*
	Detected at GLD01ABP, GLD01AGM	Not detected	
Barnacle – *Amphiblanus improvisus*	Balanidae	*Amphibalanus*	Sequences identified as *Amphibalanus amphitrite*
	Detected at GLD01ABP, GLD01AGM, GLD01AFL	Not detected	
Ascidian – *Didemnum spp.*	Didemnidae	*Didemnum*	Sequences identified as *Diplosoma listerianum*
	Detected at GLD01AGM, GLD01AFL	Not detected	
Oyster – *Crassostrea spp.*	Ostereida	*Crassostrea*	Sequences identified as *Dendostrea sp.* and *Saccostrea sp.*
	Detected at GLD01ABP, GLD01AGM, GLD01ASBL	Not detected	

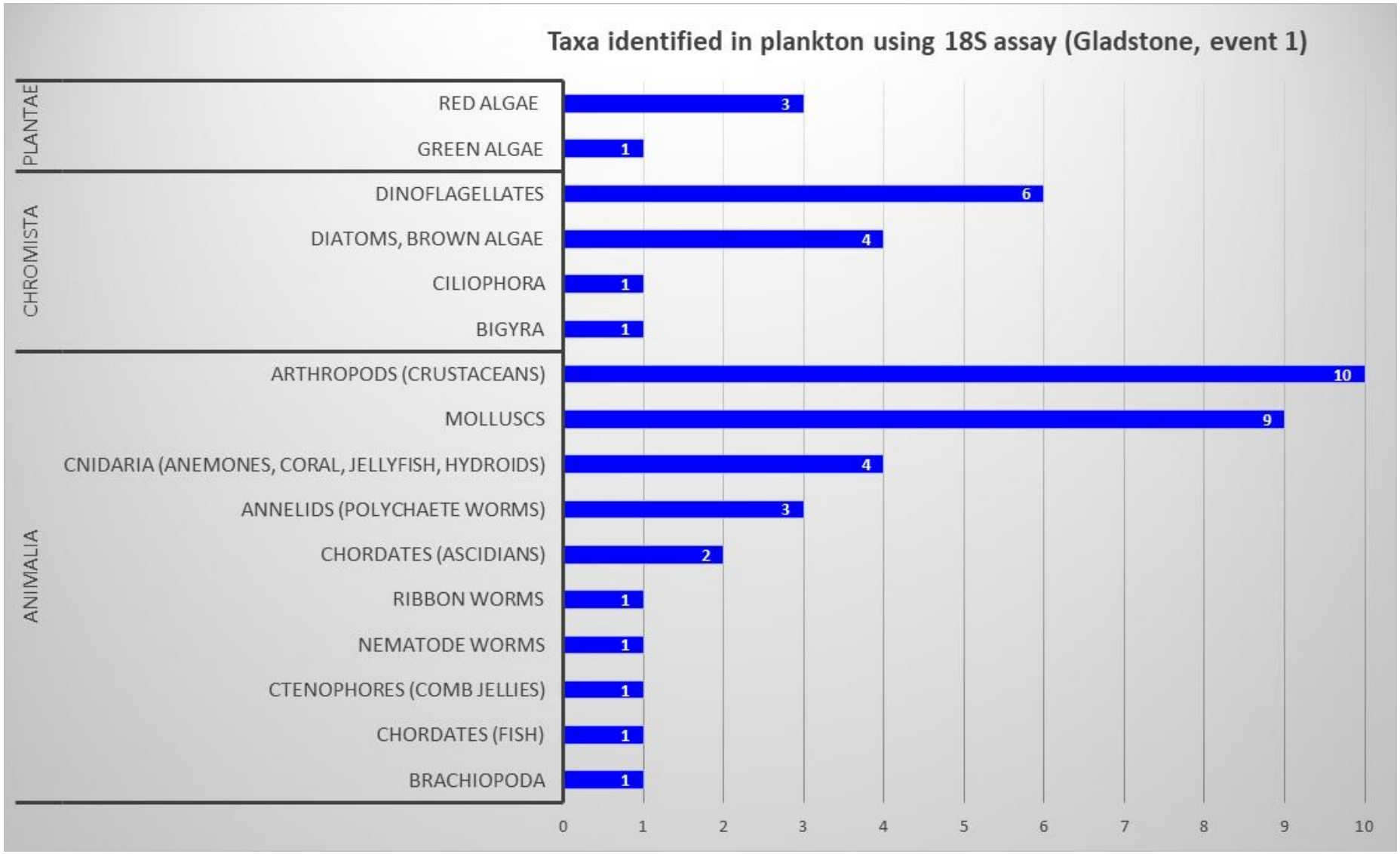

Figure 14: Number of taxa identified to family level (grouped by phyla) in plankton using the 18S assay for event 1. Note: 49 taxa identified with 47 identified to family level.

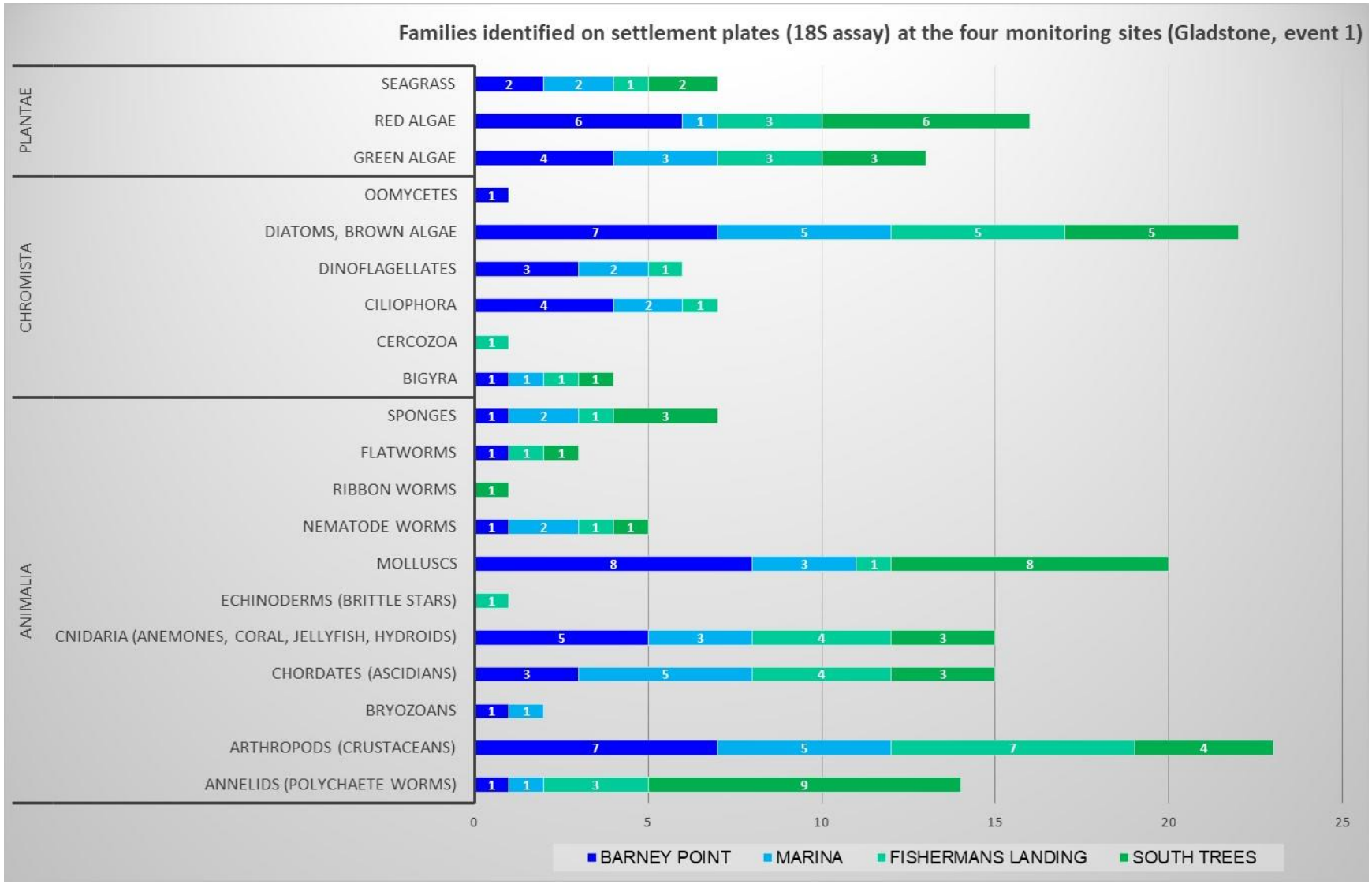

Figure 15: Number of taxa identified to family (grouped by phyla) on settlement plates using the 18S assay at all monitoring sites, for event 1. Note: 97 taxa identified with classification of 90 families.

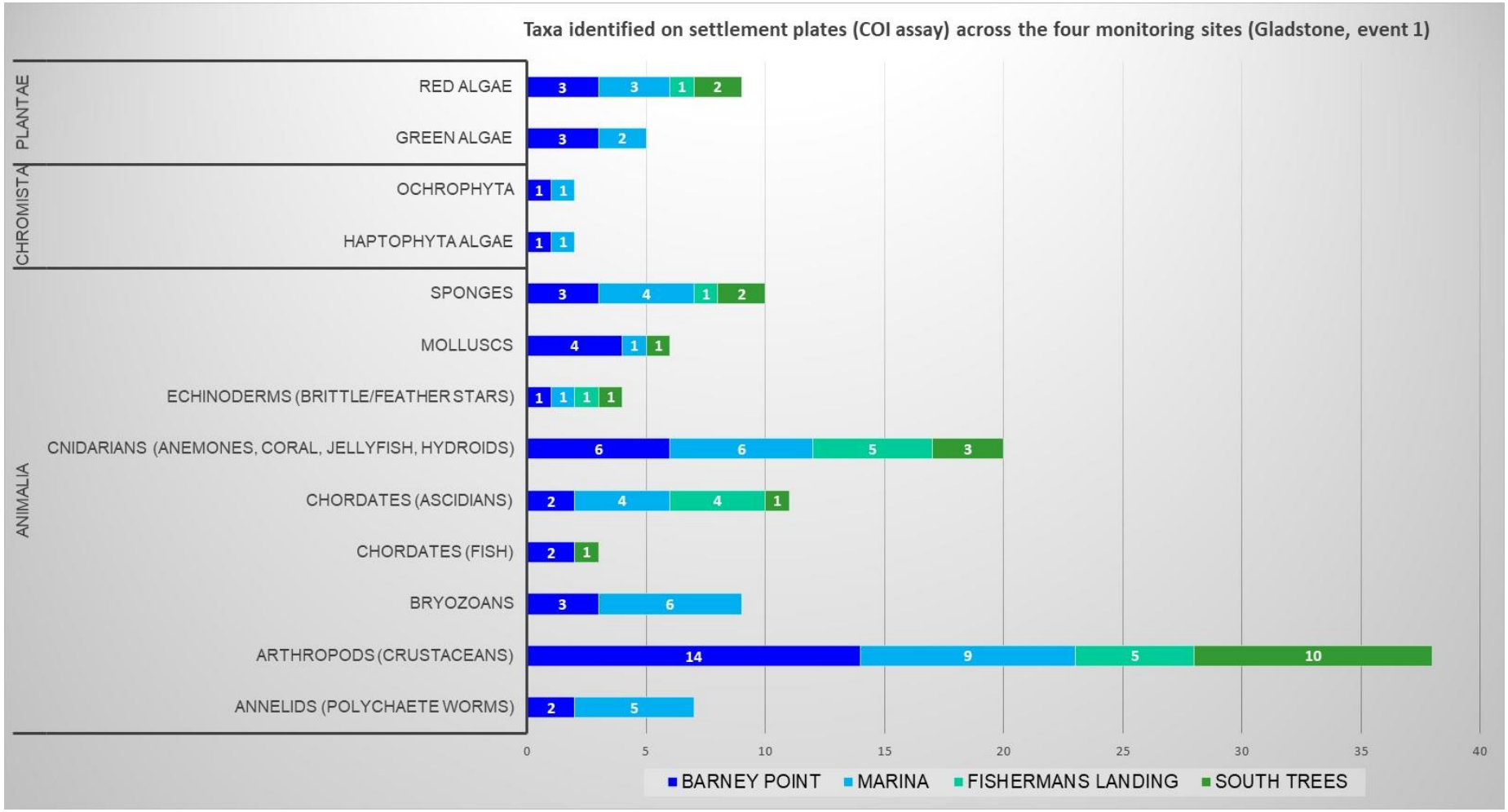

Figure 16: Number of taxa identified to genus and species (grouped by phyla) on settlement plates using the CO1 assay at all monitoring sites, for event 1. Note: 73 taxa identified including idientificaton of 57 families, 62 genera and 54 species.

4.1.1 Additional plankton samples at East Banks Spoil Disposal Site (Event 1)

Plankton samples collected from EBSDS were analysed using the 18S metabarcoding assay. A wide range of taxa from 58 families and 16 phyla were detected.

No target marine pests (as listed in section 7.2 and 7.3) were detected from samples collected in the September 2019 sampling event.

Worms and mussels belonging to target marine pest 'family' classifications were identified (Table 4). The 18S assay provided information to help rule out the presence of the invasive worm *Marenzelleria spp.* and mussels *Arcuatula senhousia and Perna sp.* Taxonomic resolution allowed identification of worms as *Aurospio dibranchiata*. This species has not previously been recorded in Australia (Atlas of Living Australia) but are not considered to be an invasive marine pest and future surveillance could monitor for the ongoing presence and distribution of this species. The mussels detected from the family Mytilidae were determined to be *Modiolus sp.* Species from this genus are known to occur locally.

Table 5: Gladstone metabarcoding bioinformatics results from EBSDS plankton (event 1)

Common name - Target species	Family	Genus	Comments
Results from 18S assay			
Burrowing worm – *Marenzelleria spp.*	Spionidae	*Marenzelleria*	Sequences identified as *Aurospio dibranchiata*
	Detected in 2 samples	Not detected	
Mussels – *Arcuatula senhousia, Perna spp.*	Mytilidae	*Arcuatula, Perna*	Sequences identified as *Modiolus sp.*
	Detected in 8 samples	Not detected	

4.2 Event 2

Q-SEAS event 2 was successfully completed at the Port of Gladstone between November 2019 and January 2020. Two metabarcoding assays (18S and CO1) were applied to settlement plate samples, and the 18S metabarcoding assay was applied to plankton. A total of 106 families from 18 phyla were identified across all substrates, with only 4 families identified by both assays in both substrates (Lumbrineridae worms, Ascidiidae ascidians, Styelidae ascidians and Ostreidae oysters). This demonstrates the importance of using multiple substrates and assays to increase the likelihood of detecting target taxa.

There was little overlap between taxa identified in either substrate type or metabarcoding assay, with 19 families identified by the 18S assay in both plankton and plate samples; and 23 families identified on plates by the two different assays. Only 6 families were identified on both plates and in plankton (Figure 13).

The diverse range of taxa identified using the 18S assay included:

- 58 families in plankton (41 orders, 14 phyla; Figure 13 and Figure 14)
- 40 families on settlement plates (12 phyla, 29 orders; Figure 13 and Figure 15).

The diverse range of taxa identified using the CO1 assay included:

- 39 families on settlement plates (9 phyla, 28 orders) including resolution of 39 genera and 18 species (Figure 13 and Figure 16).

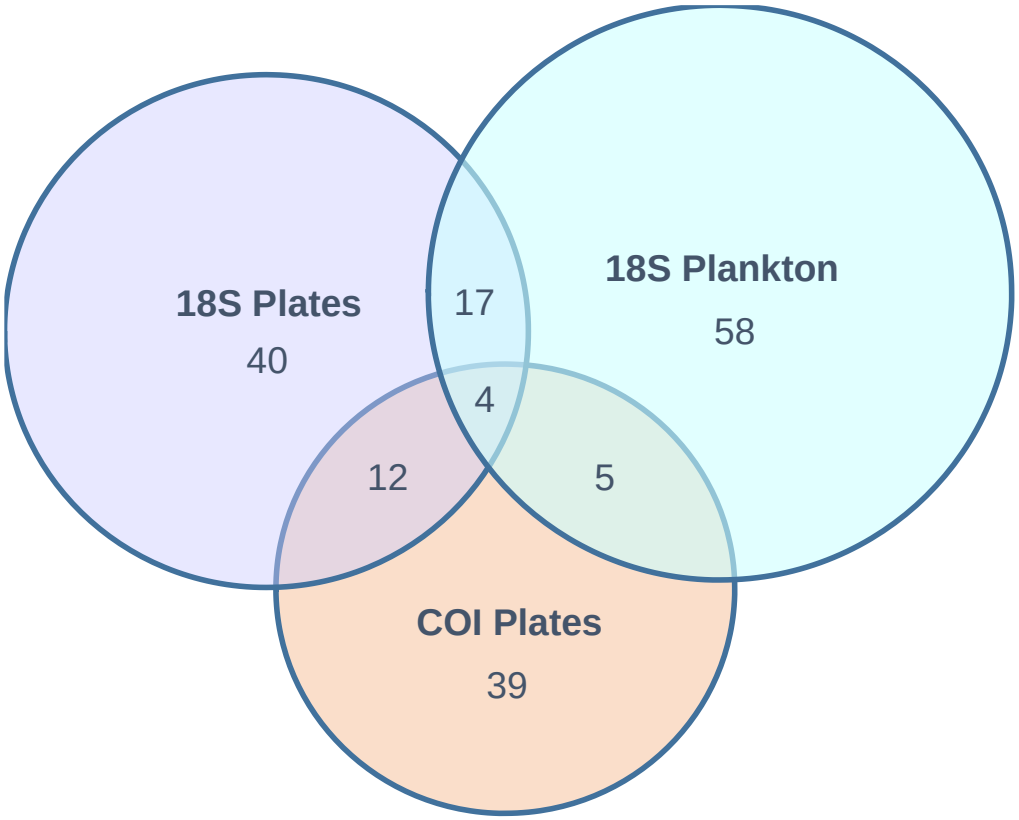

Figure 17: Venn diagram showing number of families identified in event 2, in the 18S and CO1 assays on plates and in plankton. There was little overlap between taxa identified in either substrate type or metabarcoding assay. Note: total numbers for each parameter include the overlap.

No target marine pests (as listed in section 7.2 and 7.3) were detected from samples collected in the summer sampling event. Barnacle, worms, ascidians, mussels, seaweed, oysters and diatoms belonging to target marine pest 'family' classifications were identified (Table 6). The 18S and CO1 assays ruled out the presence of the invasive barnacles *Amphibalanus improvisus,* worms *Sabella spallanzannii* and *Marenzelleria spp.,* ascidians *Didemnum spp.*, mussels *Arcuatula senhousia and Perna sp.*, oyster *Crassostrea spp.* and diatom *Pseudo-nitzschia seriata*. Taxonomic resolution identified these as locally occurring species, as outlined in Table 6.

Branchiomma bairdi was detected on settlement plates from the Gladstone Marina. As described in section 4.1, *B. bairdi* is a feather-duster worm which is introduced but not recognised as an invasive marine pest in Australia, and future surveillance could monitor for the ongoing presence and distribution of this species. Seaweed belonging to the *Sargassum* genus (brown macroalga) were detected in plankton, but the analyses could not resolve these detections to species level to rule out the presence of the invasive *Sargassum muticum*. As described in section 4.1, seaweed from this genus is very diverse and the lack of available reference DNA sequences for native *Sargassum* species meant that this detection was classified as 'inconclusive'. No further testing was undertaken.

Table 6: Port of Gladstone metabarcoding bioinformatics results from both substrates and assays (event 2)

Target marine pest species	Identification (Family level)	Identification (Genus level)	Results
Results from 18S assay			
Burrowing worm – *Marenzelleria spp.*	Spionidae	*Marenzelleria*	Sequences identified as *Aurospio sp* , *Scolelepis sp.*
	Detected in plankton	Not detected	
Barnacle – *Amphibalanus improvisus*	Balanidae	*Amphibalanus*	Sequence ID unable to be confirmed
	Detected at GLD02ABP, GLD02AFL	Not detected	
Ascidian – *Didemnum spp.*	Didemnidae	*Didemnum*	Sequences identified as *Diplosoma sp.*
	Detected at GLD02ABP, GLD02AGM, GLD02AFL	Not detected	
Mussels – *Arcuatula senhousia, Perna spp.*	Mytilidae	*Arcuatula, Perna*	Sequences identified as *Modiolus sp.*
	Detected at GLD02ABP and plankton	Not detected	
Oysters – *Crassostrea sp.*	Ostreidae	*Crassostrea*	Sequences identified as *Saccostrea sp.*
	Detected at GLD02ABP and plankton	Not detected	
Seaweed – *Sargassum muticum*	Sargassaceae	*Sargassum*	*Sargassum sp.* – sequence ID unable to be confirmed
	Detected in plankton	Detected in plankton	
Diatom – *Pseudo-nitzschia seriata*	Bacillariaceae	*Pseudo-nitzschia*	Sequences identified as *Psammodictyon sp.*
	Detected at GLD02ABP, GLD02AFL, GLD02ABSL	Not detected	
Results from COI assay			
Fanworm – *Sabella spallanzanii*	Sabellidae	*Sabella*	Sequences identified as *Branchiomma bairdi and Parasabella sp*
	Detected at GLD02AGM	Not detected	
Barnacle – *Amphiblanus improvisus*	Balanidae	*Amphibalanus*	Sequences identified as *Amphibalanus amphitrite and Amphibalanus reticulatus*
	Detected at GLD02ABP, GLD02AFL, GLD02ABSL	Detected at GLD02ABP, GLD02AFL, GLD02ABSL	
Ascidian – *Didemnum spp.*	Didemnidae	*Didemnum*	Sequences identified as *Diplosoma listerianum*
	Detected at GLD02AFL	Not detected	
Oyster – Crassostrea spp.	Ostreidae	*Crassostrea*	Sequences identified as *Dendostrea sp.* and *Saccostrea sp.*
	Detected at GLD02ABP, GLD02AGM, GLD02ASBL	Not detected	

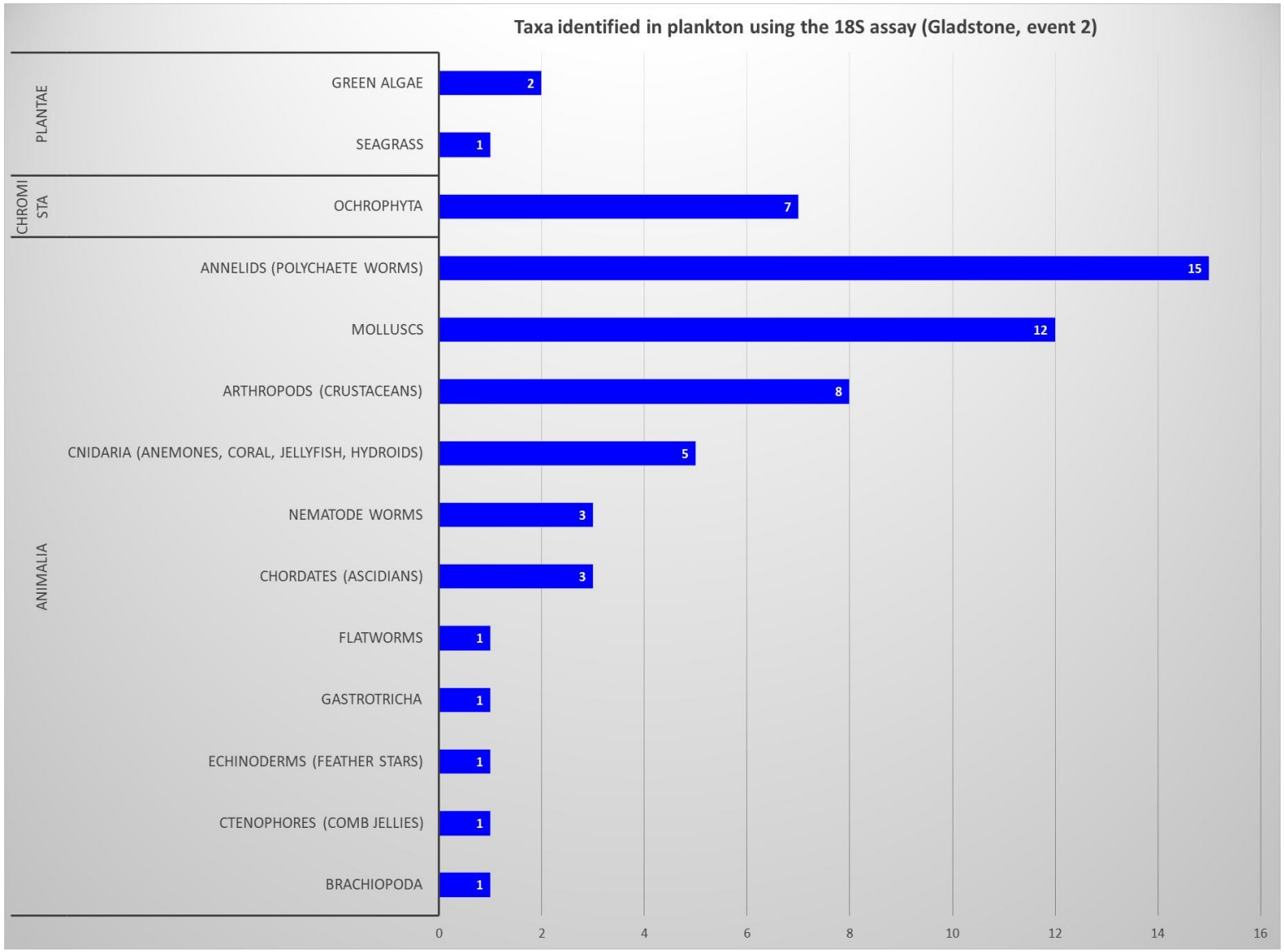

Figure 18: Taxa identified in plankton (grouped by phyla) using the 18S assay, for event 2. Note: 61 taxa detected, with classification of 58 families.

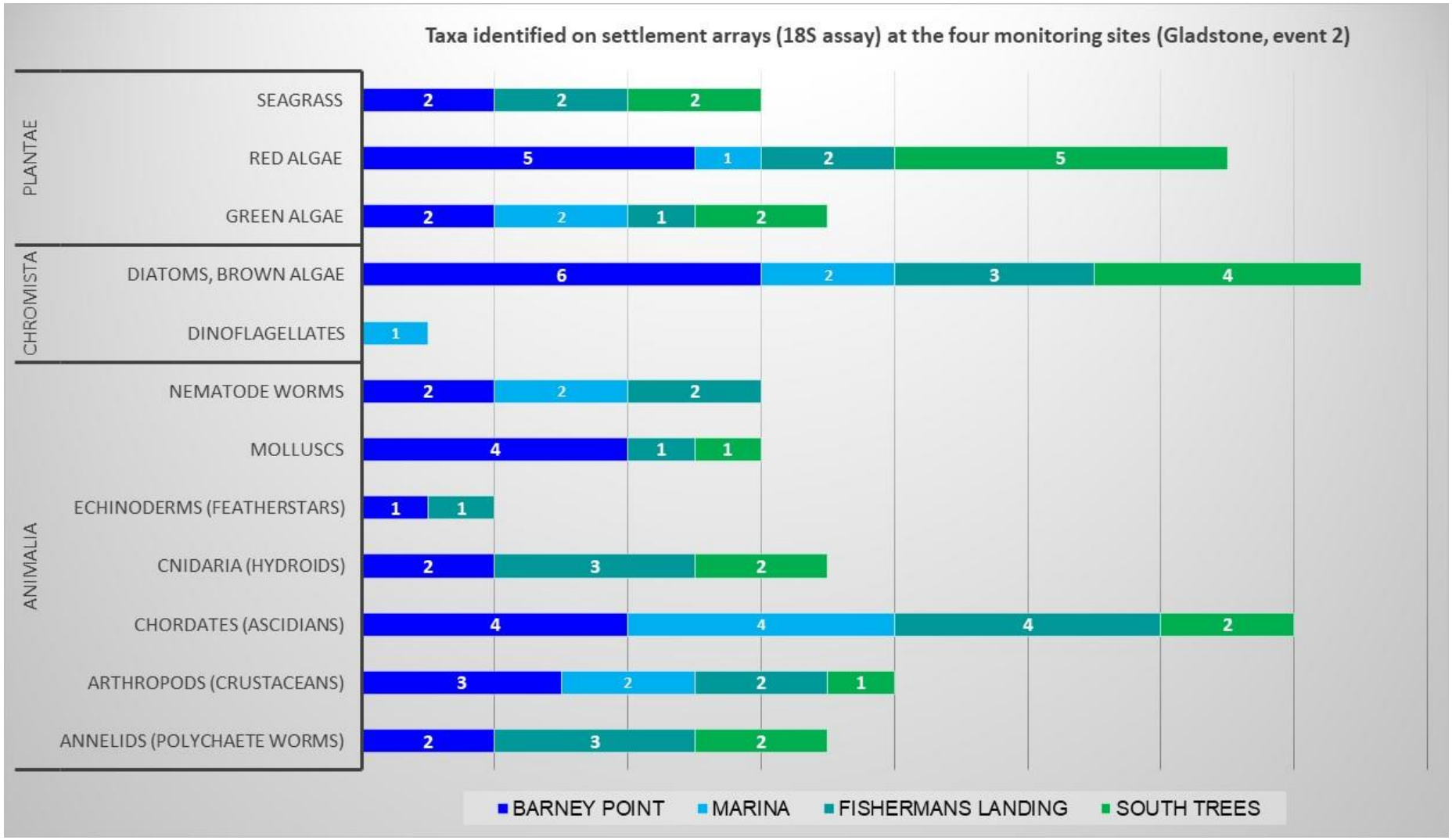

Figure 19: Taxa identified on settlement plates (grouped by phyla) using the 18S assay at all monitoring locations, for event 2. Note: 42 taxa identified, with classification of 40 families.

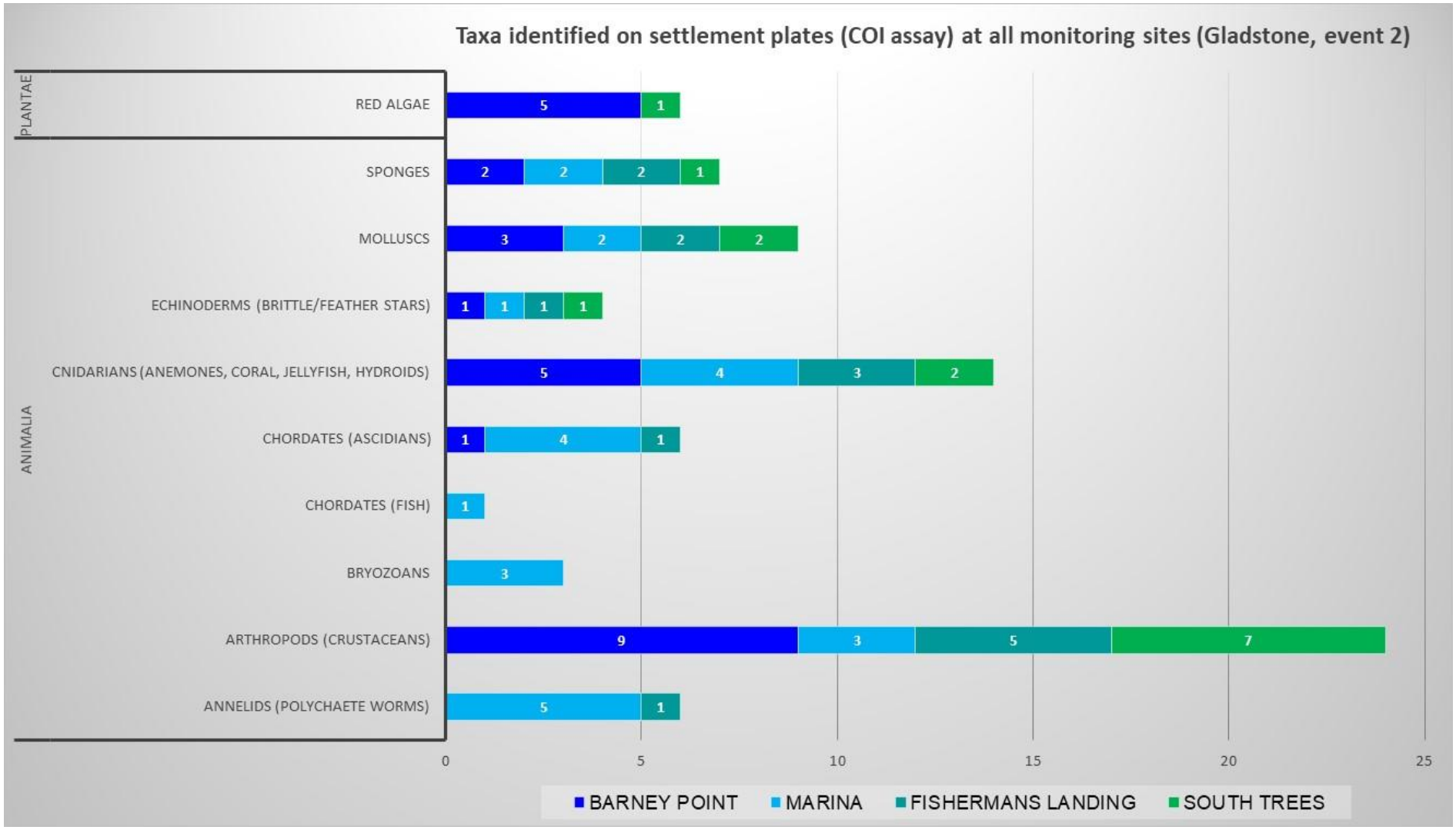

Figure 20: Number of taxa (grouped by phyla) identified on settlement plates at all sites using the CO1 assay, for event 2. Note: 48 taxa identified, with classification of 39 genera and 18 species.

4.2.1 Additional plankton samples at East Banks Spoil Disposal Site (Event 2)

Plankton samples collected from EBSDS were analysed using the 18S metabarcoding assay. A wide range of taxa from 38 families and 14 phyla were detected.

No target marine pests (as listed in section 7.2 and 7.3) were detected from samples collected in the January 2020 sampling event.

Seaweed and mussels belonging to target marine pest 'family' classifications were identified (Table 7). The 18S assay provided information to help rule out the presence of the invasive mussels *Arcuatula senhousia and Perna sp.* The mussels detected from the family Mytilidae were determined to be *Modiolus sp.* Species from this genus are known to occur locally.

Seaweed belonging to the *Sargassum* genus (brown macroalga) were detected in plankton, but the analyses could not resolve these detections to species level to rule out the presence of the invasive *Sargassum muticum*. As described in section 4.1, seaweed from this genus is very diverse and the lack of available reference DNA sequences for native *Sargassum* species meant that this detection was classified as 'inconclusive'. No further testing was undertaken.

Table 7: Gladstone metabarcoding bioinformatics results from EBSDS plankton (event 2)

Common name - Target species	Family	Genus	Comments
Results from 18S assay			
Mussels – *Arcuatula senhousia, Perna spp.*	Mytilidae	*Arcuatula, Perna*	Sequences identified as *Modiolus sp.*
	Detected in 6 samples	Not detected	
Seaweed – *Sargassum muticum*	Sargassaceae	*Sargssum*	*Sargassum sp.* - Sequence ID unable to be confirmed
	Detected in 6 samples	Detected in 6 samples	

5 Conclusions

Q-SEAS is Queensland's first state-wide marine biosecurity surveillance program, which provides a coordinated surveillance network for the early detection and proactive management of marine pest threats at Queensland's ports. The program is risk-based, adaptable, transformative, cost-effective and in alignment with the Australian Government's *MarinePestPlan 2018-2023*. The multifaceted, holistic approach and high level of scientific rigour adopted by Q-SEAS resulted in identification of a wide diversity of taxa with high taxonomic resolution. This proof of principle has provided confidence in its feasibility as an early warning surveillance tool for marine pests.

Q-SEAS was successfully implemented at the Port of Gladstone from June 2019 to July 2020. Methods included collection of eDNA from settlement plates and from plankton, in potentially high-risk areas. Complimentary surveillance techniques used to strengthen the experimental design included capturing imagery of fouled settlement plates for retrospective comparison to metabarcoding results. DNA metabarcoding was used to analyse samples for the presence of a broad taxonomic list of invasive marine species identified as a potential threat for introduction to Queensland. This genetic technique enabled surveillance for an unlimited number of target taxa, and the application of the two diagnostic assays (CO1 and 18S) greatly increased the breadth and specificity of taxa detected, with little overlap between taxa detected on either substrate type (plates/plankton) or diagnostic assay. The CO1 metabarcoding assay was able to provide resolution to genus and species level for many of the taxa detected, which allowed further interrogation to help rule out or confirm suspected marine

pest detections. Final DNA sequences were screened against an in-house genetic reference library, which ensured a robust detection capability, with outputs further investigated using a conservative stepwise criteria assessment process.

There were no detections of invasive marine pests at the Port of Gladstone. The 18S and CO1 metabarcoding assays identified a wide range of taxa that included 151 families in event 1, and 106 families in event 2. The CO1 assay allowed resolution of many taxa to genus and species level, providing important information to help rule out or confirm potential marine pest detections. There was little overlap between taxa identified in either substrates or assay type, which demonstrates the importance of using multiple substrates and assays to increase the likelihood of detecting target taxa. Barnacles, worms, ascidians, mussels, oysters and seaweed belonging to target marine pest 'family' classifications were identified in both events, with diatoms from target marine pest families also identified in event 2.

The 18S metabarcoding assays undertaken on the additional plankton samples collected from EBSDS identified 58 families in event 1, and 38 families in event 2. Worms, mussels and seaweed belonging to target marine pest 'family' classifications were identified.

Results ruled out the presence of the invasive marine pest targets, except for the brown macroalga (seaweed) from the *Sargassum* genus. *Sargassum* seaweed was identified on plates from Barney Point in event 1, in plankton from RG Tanna wharf for both events and from EBSDS in event 2. Seaweed belonging the *Sargassum* genus is one of the most diverse genera of brown marine algae known to commonly occur in shallow tropical and subtropical waters. *Sargassum* seaweed is very prevalent and speciose across Australia, and the lack of available reference sequences for native *Sargassum* species meant that this result was classified as 'inconclusive'. No further testing was undertaken. The introduced feather-duster worm *Branchiomma bairdi* was detected on the settlement arrays at Barney Point and the Gladstone Marina. This worm is introduced but not recognised as an invasive marine pest in Australia, and future surveillance could monitor for the ongoing presence and distribution of this species.

Results have demonstrated that Q-SEAS successfully established a robust and widespread marine biosecurity screening network, which significantly improved the ability to detect small and cryptic taxa using methods that are faster, cheaper and safer than traditional marine pest surveys. While this approach does not rely on expensive and laborious morphology and visual taxonomic identification of settlement plates, involvement from suitably qualified marine biosecurity personnel for inspection of sample imagery can provide another important line of evidence to support this new approach for marine pest surveillance in Queensland. Molecular data has been collected to inform the current status of marine pests in key areas of the Gladstone port environment, with results providing a comprehensive snapshot of marine biodiversity. Outcomes have enabled an evidence-based decision-making process, to inform marine pest surveillance efficacy, practices, management and policies, with results already used to guide future surveillance efforts.

Biosecurity Queensland's role as the centralised point for coordination of the Q-SEAS program provided the conduit between the science, industry and government organisations, to facilitate a platform for delivery of a mutually beneficial partnership program in a cohesive and consistent manner. This was a key factor for the success of the Q-SEAS program. Q-SEAS maintained the integrity of scientific outputs and facilitated coordination of surveillance across a wide geographical area with multiple stakeholders. This approach also ensured independent review of molecular data and achieved significant cost-efficiencies through bulk purchasing and the supply chain. The Q-SEAS SOP (DAF, 2020) was developed to ensure state-wide surveillance is conducted consistently, and to allow for continued refinement and delivery of a robust science-based program into the future. This consistent approach allows for ongoing comparison of data both within and between study areas.

The Q-SEAS pilot program aimed to identify the benefits and limitations to application of innovative molecular methods for marine pest surveillance, with future monitoring designed to be flexible and evolve as technologies and sampling methodologies improve. Key outcomes and recommendations of the Q-SEAS program (from a state-wide perspective) will be documented in a separate report upon completion of the Q-SEAS pilot program evaluation. While the pilot year of Q-SEAS provided informative, evidence-based data on the status of marine pests at the Port of Gladstone, it is important to note that results do not cover the port in its entirety. Rather, the program establishes important and valuable molecular data, from which future surveillance will build upon. The accumulation of spatial and temporal data over future years will facilitate the early detection of new marine pests, help to better understand the distribution of known pests, and provide an ever-increasing level of confidence in the absence of target marine pests of concern.

Strengthening of the public-private partnerships between participating port authorities and Biosecurity Queensland demonstrate how marine biosecurity responsibilities can be shared between government and industry. Q-SEAS was coordinated, collaborative and targeted towards agreed national priorities, using a risk-based approach that focused on achievable outcomes. Q-SEAS provides Queensland with a model for preparedness, by providing a program for the early detection and early intervention of marine pests, particularly with continued ‘routine’ surveillance over varying temporal and spatial scales. Continuity will improve knowledge on the status of marine pests in Queensland, with biobanking of DNA samples allowing for retrospective analyses as technologies improve, or for understanding changes in biological communities in response to future marine pest incursions.

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7 Appendices

7.1 Appendix 1 - Examples of impacts from introduced aquatic pests/pathogens

Pest/pathogen	Location	Date	Cost	Pathway	Impact
Whitespot disease	Australia (Queensland)	2016	~$AU49.5 million for prawn farmers ~$AU20.5 wild fisheries GVP at risk	Unconfirmed	Direct financial and economic impact on prawn farms, wild prawn/crab fisheries. Indirect impacts to community and recreational fisheries through job losses, constrained access to infected zones and movement restrictions (costs unknown).
Black-striped mussel *Mytilopsis sallei*	Australia (Northern Territory)	1998-99	AU$2.2 million (excluding personnel)	Biofouling	Incursion contained to Darwin marina; eradication costs considered minor in comparison to potential costs to fisheries/aquaculture industries/environment.
Northern pacific seastar *Asterias amurensis*	Australia (Tasmania/Victoria)	1986 / 1995	Unknown	Ballast water	Major predator of commercial and non-commercial shellfish. Loss of commercial and recreational fisheries harvests declines in aquaculture productivity, disruptions to natural marine ecology, smothering of benthic habitats and impacts to amenity for local beaches. Connected to decline of endangered handfish.
European fanworm *Sabella spallanzanii*	Australia (Western Australia, South Australia, Victoria, New South Wales)	1965 / 1996	Unknown	Shipping or aquaculture	Forms dense colonies and consumes vast amounts of food to the detriment of native species. Fouls infrastructure increases operating costs for industry. No known predators, considered a major threat to benthic assemblages due to impacts on nutrient cycling.
Zebra mussel *Dreissena polymorpha* Quagga mussel *Dreissena bugensis*	North America	1993-99	>US$5 billion	Ballast water	Ongoing impacts to industries, businesses and communities as a result of cleaning and control measures/clogged pipes, power industry shutdowns costing over US$5,000 per hour.
Shipworm *Teredo navalis*	USA	1920s	US$205 million per year (1992 dollars)	Ballast water or rafting	Major cause of damage and destruction to submarine timber structures.
European green crab *Carcinus maenas*	New England, Novia Scotia	1996	~$44 million annually	Ballast water	Destroyed commercial fishing habitats and soft-shell clam fisheries. Reduced viability of other commercially important bivalves.

Source: *ALA, 2019; Arthur et al., 2015; Bax et al. 2002; Bott et al., 2012; Cohen and Carlton, 1995; DAWR, 2015; DPI, 2019; IANST, 2009; Lafferty and Kuris, 1996; Nelson, 2019; RP, 2017; USDS, 2009*.

7.2 Appendix 2 – Marine pest target species list showing the taxonomic resolution of 18S and CO1 metabarcoding assays for species as listed in the *Biosecurity Act 2014*

Target	Common name	Scientific name	Taxonomic resolution	
			18S assay	CO1 assay
1	American slipper limpet	*Crepidula fornicata*	Family	Species
2	Asian bag/date mussel	*Arcuatula senhousia*	Species	Species
3	Asian clam	*Corbula (Potamocorbula) amurensis*	Species	Unknown
4	Asian green mussel	*Perna viridis*	Species	Species
5	Asian seaweed	*Sargassum muticum*	Genus	Unknown
6	Black striped mussel	*Mytilopsis sallei*	Genus	Genus
7	Brown mussel	*Perna perna*	Genus	Species
8	Centric diatoms	*Chaetoceros concavicorne, C. convolutes*	Unknown	Unknown
9	Chinese mitten crab	*Eriocheir spp.*	Family	Genus
10	Colonial sea squirt	*Didemnum spp. – exotic invasive strains*	Not available	Species
11	Comb jelly	*Mnemiopsis leidyi*	Family	Unknown
12	European barnacle	*Amphilbalanus improvisus*	Species	Unknown
13	European clam	*Varicorbula gibba*	Species	Species
14	European fan worm	*Sabella spallanzanii*	Species	Genus
15	European green crab	*Carcinus maenas*	Species	Species
16	Green macroalga	*Caulerpa taxifolia – exotic strains*	Species	Genus
17	Green macroalga	*Codium fragile ssp. tomentosoides*	Species	Unknown
18	Jack-knife clam	*Ensis leei (formerly E. directus)*	Genus	Unknown
19	Japanese/Asian shore crab	*Hemigrapsus sanguineus*	Family	Unknown
20	Japanese seaweed	*Undaria pinnatifida*	Not available	Species
21	Lady crab/Asian paddle crab	*Charybdis japonica*	Species	Species
22	Marbled spinefoot/rabbitfish	*Siganus rivalatus*	Species	Species
23	New Zealand screwshell	*Maoricolpus roseus*	Species	Unknown
24	Northern Pacific seastar	*Asterias amurensis*	Genus	Genus
25	Pacific crab	*Hemigrapsus takonoi/penicillatus*	Family	Species
26	Pacific oyster	*Crassostrea gigas*	Genus	Unknown
27	Pennate diatom	*Pseudo-mitzschia seriata*	Family	Unknown
28	Rapa whelk	*Rapana venosa (syn Rapana thomasiana)*	Family	Species
29	Red gilled mudworm	*Marenzelleria spp.*	Genus	Species
30	Red macroalga	*Grateloupia turuturu (syn Grateloupia doryphora)*	Species	Unknown
31	Round goby	*Neogobius melanostomus*	Species	Species
32	Soft shell clam	*Mya arenaria*	Species	Unknown
33	Toxic dinoflagellates	*Dinophysis norvegica, Alexandrium monilatum, Pfiesteria piseicida*	Unknown	Unknown

7.3 Appendix 3 – Marine pest target species able to be detected by the 18S metabarcoding assay by eDNA Frontiers (based on genetic sequences from vouchered specimens by DPIRD)

Group	Species	Common name
Annelid	*Sabella spallanzanii*	Mediterranean fan worm
Annelid	*Marenzelleria arctia*	Red-gilled mud worm
Annelid	*Hydroides dianthus*	Tube worm
Cirripedia	*Amphibalanus improvisus*	Bay barnacle
Cirripedia	*Balanus glandula*	Acorn barnacle
Cirripedia	*Hesperibalanus fallax*	Epibiotic barnacle
Cirripedia	*Chthamalus proteus*	Atlantic barnacle
Clam	*Corbicula fluminea*	Freshwater clam
Clam	*Ensis directus*	Jack-knife clam
Clam	*Varicorbula gibba*	European clam
Clam	*Potamocorbula amurensis*	Asian clam
Clam	*Mya arenaria*	Soft-shell clam
Clam	*Anadara transversa*	Transverse ark clam
Cnideria	*Mnemiopsis leidyi*	Comb jelly
Cnideria	*Blackfordia virginica*	Black Sea jelly
Decapoda	*Pseudodiaptomus marinus*	Asian copepod
Copepoda	*Tortanus dextrilobatus*	Asian copepod
Decapoda	*Charybdis japonica*	Asian paddle crab
Decapoda	*Carcinus maenas*	European shore crab
Decapoda	*Callinectes sapidus*	Blue crab
Decapoda	*Carcinoscorpius rotundicauda*	Mangrove horseshoe crab
Decapoda	*Dikerogammarus villosus*	Killer shrimp
Decapoda	*Eriocheir sinensis*	Mitten crab
Decapoda	*Hemigrapsus sanguineus*	Japanese shore crab
Decapoda	*Hemigrapsus takanoi*	Pacific crab
Decapoda	*Pachygrapsus fakaravensis*	Polynesian grapsid crab
Decapoda	*Rhithropanopeus harrisii*	Harris' mud crab
Fish	*Tridentiger barbatus*	Shimofuri goby
Fish	*Tridentiger bifasciatus*	Shokohazi goby
Fish	*Tridentiger trigonocephalus*	Chameleon goby
Fish	*Acanthogobius flavimanus*	Goby
Fish	*Neogobius melanostomus*	Round goby
Fish	*Siganus luridus*	Dusky spinefoot
Fish	*Siganus rivulatus*	Marbled spinefoot
Gastropod	*Rapana venosa*	Veined rapa whelk
Gastropod	*Crepidula fornicata*	American slipper limpet
Macroalgae	*Codium fragile*	Green macro algae
Macroalgae	*Caulerpa taxifolia*	Green macro algae

Group	Species	Common name
Macroalgae	*Caulerpa racemosa*	Green macro algae
Macroalgae	*Sargassum muticum*	Asian seaweed
Macroalgae	*Grateloupia turuturu*	Red macro algae
Macroalgae	*Bonnemaisonia hamifera*	Red macro algae
Macroalgae	*Womersleyella setacea*	Red macro algae
Macroalgae	*Fucus evanescens*	Brown macro algae
Mussel	*Perna viridis*	Asian green mussel
Mussel	*Perna perna*	South African brown mussel
Mussel	*Perna canaliculus*	New Zealand mussel
Mussel	*Arcuatula senhousia*	Asian bag mussel
Mussel	*Limnoperna fortunei*	Golden mussel
Mussel	*Geukensia demissa*	Ribbed mussel
Mussel	*Mytella charruana*	Charru mussel
Mussel	*Mytilopsis sallei*	Black-striped mussel
Mussel	*Mytilopsis leucophaeata*	Dark false mussel
Mussel	*Dreissena bugensis*	Quagga freshwater mussel
Mussel	*Dreissena polymorpha*	Zebra freshwater mussel
Oyster	*Crassostrea gigas*	Pacific oyster
Oyster	*Crassostrea virginica*	American oyster
Oyster	*Crassostrea ariakensis*	Suminoe oyster
Oyster	*Anomia nobilis*	Saddle oyster
Sea star	*Asterias amurensis*	Northern pacific seastar

7.4 Appendix 4 – Notification flyer

Gladstone Ports Corporation
Growth. Prosperity. Community.

Queensland Government
Department of Agriculture and Fisheries

MARINE PEST SURVEILLANCE

COMING TO QUEENSLAND PORTS SOON

WHAT IS A MARINE PEST?

Marine pests are animals and plants introduced to waters outside their natural range. They can travel vast distances attached to vessels as biofouling or living in internal seawater systems such as bilge water, ballast water or water intake pipes.

WHAT WE ARE DOING

Gladstone Ports Corporation (GPC) and the Department of Agriculture and Fisheries (DAF) have established a marine biosecurity surveillance pilot program, which will be implemented from September 2019 to June 2020. The surveillance program focuses on the early detection of marine pests that are not known to occur in Queensland, and that we are actively trying to keep out.

WHY?

There is high potential for translocation of marine pests via shipping activity. If these pests were to arrive in Queensland, they could threaten seaport operations, fisheries resources, the economy, the environment, and our ability to use and enjoy local marine and coastal areas.

WHEN WILL THIS HAPPEN?

We will be conducting surveillance activities in the Port of Gladstone between September 2019 and March 2020, to maximise the chance of detection over a variety of seasons and environmental conditions.

ADDITIONAL INFORMATION

To find out more about GPC go to: www.gpcl.com.au
To find out more about DAF go to: www.daf.qld.gov.au
To learn more about marine pests go to:
https://www.qld.gov.au/environment/coasts-waterways/marine-pests

WHAT TO EXPECT

We will deploy metal frames from four berths in the harbour, which will be tethered to infrastructure with a rope (see example below). Small PVC plates attached to each frame will provide a surface for marine organisms to settle and grow. The DNA of these 'fouling' organisms will be analysed for marine pests. Each frame will float just below the water surface and be anchored to the ground to maintain its position for two months. A sign will be erected during each deployment as illustrated below.

Plankton will also be collected. This will involve vessel-based tows using a small net (pictured below) in the vicinity of ship berthing areas.

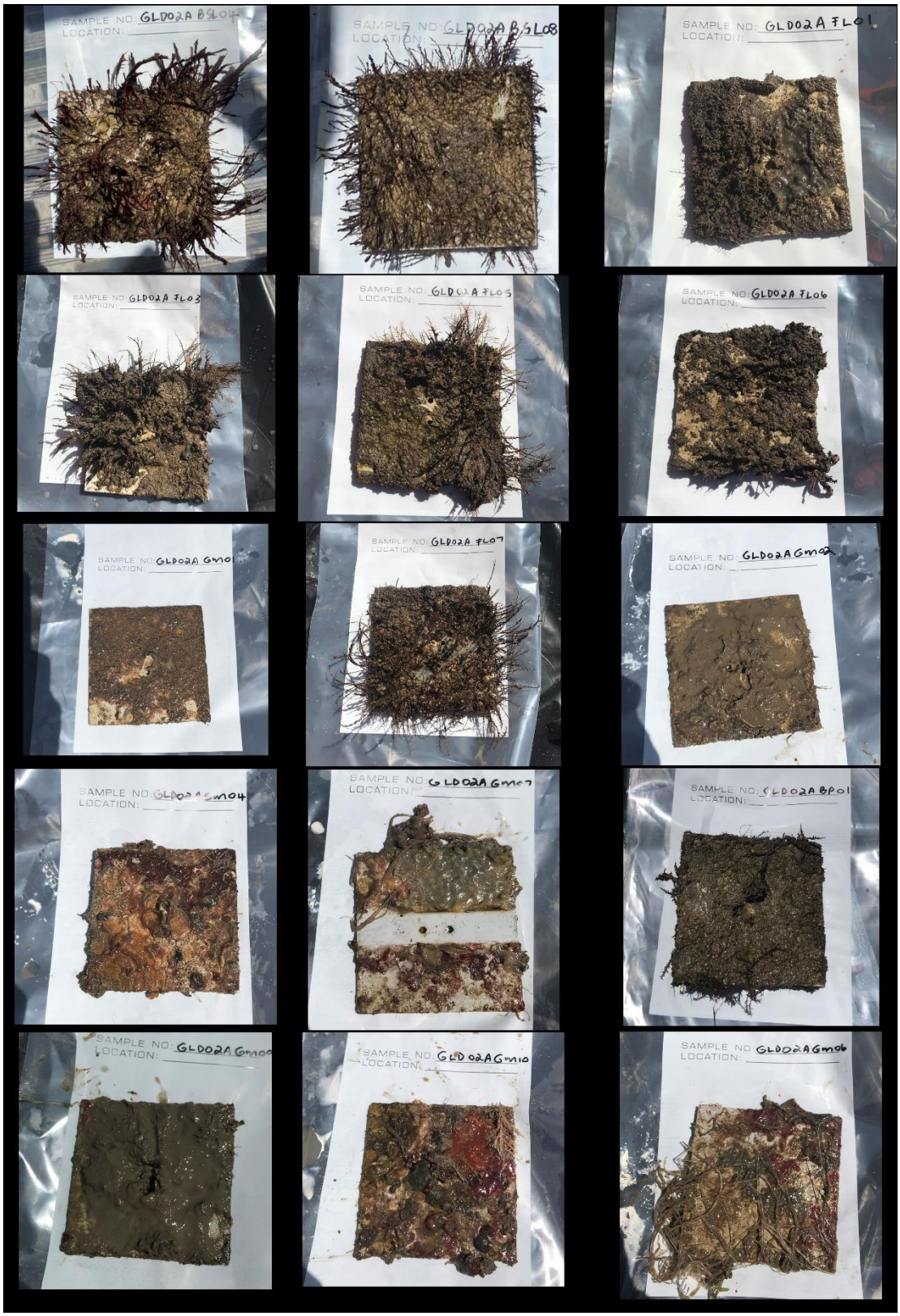
WHO TO CONTACT

If you have any concerns or questions about these activities please contact GPC on 1800 243 472 or Carolyn Trewin (DAF) on 0436 935 125.

Marine biosecurity
Look. Report. Protect.
13 25 23
biosecurity.qld.gov.au

7.5 Appendix 5 – Representative settlement array plate fouling (event 1)

7.6 Appendix 6 – Representative settlement array plate fouling (event 2)



7.7 Appendix 7 – Examples of marine pest species habitats, environmental tolerances and reproductive cycles

	Species	Habitat	Colonising substrate	Temp	Salinity	Spawn	Planktonic larval stage
	Asian green mussel *Perna viridis*	Mid-intertidal to subtidal	Any artificial surfaces	11-32°C	18-33 ppt	Annually	14-21 d (2-3 w)
	Asian bag mussel *Arcuatula senhousia*	Intertidal to 20 m	Soft sediments, hard substrates	11-27°C	17-33 ppt	Autumn	45-55 d (6-7 w)
	Black-striped mussel *Mytilopsis sallei*	Shallow & intertidal	Vertical surfaces	5-40°C	0-55 ppt	Autumn/ winter	Few days only
	Brown mussel *Perna perna*	Subtidal & low shoreline	Hard rocky substrates	8-30°C	15-50 ppt	Winter	10-12 d (1-2 w)
	Chinese mitten crab *Eriocheir sinensis*	River banks & shallow coast	Muddy sediments	7-31°C	5-25 ppt	Autumn/ winter. Eggs hatch in summer	Zoea 2-8 weeks
	Harris' mud crab *Rhithropanopeus harrisii*	Subtidal estuaries	Sheltered structures, sandy/muddy	0-30°C	0-40 ppt	Summer	16 d (2 w)
	Japanese seaweed *Undaria pinnatifidia*	Tidal zone to 15 m	Any hard surface	10-25°C	12-30 ppt	Spring/ Summer	11-43 d (2-6 w)

7.8 Appendix 8 – eDNA Frontiers laboratory report (Event 1)

7.9 Appendix 9 – eDNA Frontiers laboratory report (Event 2)

7.10 Appendix 10 – eDNA Frontiers laboratory report – plankton samples from East Banks Spoil Disposal Site (Event 1)

7.11 Appendix 11 – eDNA Frontiers laboratory report – plankton samples from East Banks Spoil Disposal Site (Event 2)