ENVIRONMENTAL RISK MANAGEMENT AUTHORITY
NGĀ KAIWHAKATŪPATO WHAKARARU TAIAO

FORM NO2N

Application for approval to

IMPORT INTO CONTAINMENT ANY NEW ORGANISM THAT IS NOT GENETICALLY MODIFIED

under section 40 of the Hazardous Substances and New Organisms Act 1996

Application Title: Import into containment marine organisms (both prokaryotic and eukaryotic) for scientific research.

Applicant Organisation: National Institute of Water and Atmospheric Research Ltd.

ERMA Office use only

Application Code: ☐☐☐☐☐☐☐☐ Formally received:____/____/____

ERMA NZ Contact: _____ Initial Fee Paid: $

Application Status:

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Application for approval to import into containment any new organism that is not genetically modified, under Section 40 of the Hazardous Substances and New Organisms Act 1996

IMPORTANT

1. An associated User Guide is available for this form. You should read the User Guide before completing this form. If you need further guidance in completing this form please contact ERMA New Zealand.
2. This application form covers importation into containment of any new organism that is not genetically modified, under section 40 of the Act.
3. If you are making an application to import into containment a **genetically modified organism** you should complete **Form NO2G,** instead of this form (Form NO2N).
4. This form, together with form NO2G, replaces all previous versions of Form 2. Older versions should not now be used. You should periodically check with ERMA New Zealand or on the ERMA New Zealand web site for new versions of this form.
5. You can talk to an Applications Advisor at ERMA New Zealand who can help you scope and prepare your application. We need all relevant information early on in the application process. Quality information up front will speed up the process and help reduce costs.
6. This application form may be used to seek approvals for importing more than one new (non-genetically modified) organism into containment where the organisms are of a similar nature.
7. Any extra material that does not fit in the application form must be clearly labelled, cross-referenced, and included as appendices to the application form.
8. Commercially sensitive information must be collated in a separate appendix. You need to justify why you consider the material commercially sensitive, and make sure it is clearly labelled as such.
9. Applicants must sign the form and enclose the correct application fee (plus GST). The initial application fee can be found in our published Schedule of Fees and Charges. Please check with ERMA New Zealand staff or the ERMA New Zealand website for the latest schedule of fees. We are unable to process applications that do not contain the correct initial application fee.
10. Unless otherwise indicated, all sections of this form must be completed for the application to be progressed.
11. Please provide an electronic version of the completed application form, as well as sending a signed hard copy.

You can get more information by contacting us. One of our staff members will be able to help you.

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Application for approval to import into containment any new organism that is not genetically modified, under Section 40 of the Hazardous Substances and New Organisms Act 1996

Section One – Applicant Details

1.1 Name and postal address in New Zealand of the organisation or individual making the application:

Name > National Institute of Water and Atmospheric Research Ltd

Postal Address > Private Bag 14-901
Kilbirnie
Wellington

Physical Address > 301 Evans Bay Parade
Greta Point
Wellington

Phone > 04-386-0300

Fax > 04-386-0574

E-mail >

1.2 If application is made by an organisation, provide name and contact details of a key contact person at that organisation

This person should have sufficient knowledge to respond to queries and have the authority to make decisions that relate to processing of the application.

Name > Elizabeth Maas

Position > Scientist

Address > Private Bag 14-901
Kilbirnie
Wellington

Phone > 04-386-0539

Fax > 04-386-0574

E-mail > e.maas@niwa.co.nz

1.3 If the applicant is an organisation or individual situated overseas, provide name and contact details of the agent authorised to transact the applicant's affairs in relation to the application

This person should have sufficient knowledge to respond to queries and have the authority to make decisions that relate to processing of the application.

Name >

Position >

Address >

Phone >

Fax >

E-mail >

Application for approval to import into containment any new organism that is not genetically modified, under Section 40 of the Hazardous Substances and New Organisms Act 1996

Section Two – Purpose of the Application

This form is to be used for an application to import into containment any new organism that is not genetically modified. For an application to import into containment **a genetically modified organism**, use **Form NO2G**.

2.1 Give a short summary statement of the purpose of this application to be used on ERMA New Zealand's public register. (Maximum of 255 characters).

Briefly describe the organism(s) to be imported into containment, and the purpose(s) for which you wish to import the organism(s).

> To import into containment microorganisms and other small (<30mm) marine organisms from marine water and sediment samples; and marine invertebrates (<120mm) from the NZ EEZ, Southern Ocean and Antarctica waters for scientific research.

2.2 Provide a short description of the background and aims of the project suitable for lay readers.

Describe the purpose of the importation and rationale for the overall project these organisms are to be used in so that people not directly connected with the research can understand why these organisms are required.

> Although marine bacteria form an integral part of the food web in the oceans, and are involved in the cycling of all the major elements, there is currently very little information about the diversity and functioning of marine bacteria in New Zealand's ocean, the Southern Ocean and the Ross Sea, Antarctica. NIWA scientists would like to better understand the oceanic food webs in these oceans at a microscopic level. In order to do this, we wish to collect water and sediment samples from these locations, especially for the isolation of marine microscopic organisms (bacteria, fungi, micro-algae etc) so that we can better understand their functioning and diversity. In addition little information is available on the likely response of key marine invertebrate species (e.g., amphipods, bivalves and echinoderms) to environmental changes, such as ocean acidification and increase in ocean temperatures associated with climate change. We wish to collect these marine organisms so that their responses can be assessed, and the implications of these responses to the wider ecosystem determined.

Section Three – Information on the Organism(s) to be imported

If the application is for importation of more than one organism, this section must be completed separately for each organism. If there are commercial reasons for not providing full information here, alternative approaches must be discussed with and agreed by ERMA New Zealand.

3.1 Give the unequivocal identification of the organism(s) to be imported

These names will be on the public register and should clearly identify the organisms. Please provide details of the following:

Latin binomial, including full taxonomic authority:

>

Common name(s), if any:

>

Type of organism (eg bacterium, virus, fungus, plant, animal, animal cell):

>All microscopic (<30mm) marine organisms and marine invertebrates (<120mm) from the New Zealand EEZ, Southern Ocean, and Antarctica waters.
Including:
Eukaryotes (fungi, yeasts, thraustochytrids, micro-algae, protists and invertebrates)
Archaeabacteria
Bacteria

Taxonomic class, order and family:

>N/A

Strain(s) if relevant:

>N/A

Other information, including presence of any inseparable or associated organisms:

> The shells/tests of the molluscs and echinoderms will be carefully examined for the presence of any associated organisms (e.g., encrusting fauna), and any found will be removed prior to importation to NZ.

3.2 Characteristics of the organism(s) to be imported

Provide information on the biology, ecology and the main features or essential characteristics of each organism(s) to be imported. For example, note production of spores/seeds/pollen, conditions for growth and reproduction. Also provide information on affinities of the organism(s) with other organism(s) in New Zealand. This information should be relevant to the identification of the risks of the organism (section 5).

> The organisms that we plan to collect as part of this application are all from the marine environment. The temperature range of the oceanic water from which they will be collected will be varied, ranging from polar temperatures of -1.9°C to sub-tropical temperatures of 25°C. The habitats that we plan to collect from include oceanic water (down the water column to the seafloor) and sediments (ranging from shelf (100m-600m) to abyssal plain (>4000m) depths). We expect to see some sediment-specific organisms, but in general due to the mixing of the water and ocean currents around New Zealand and Antarctica (Figure 1 and 2) we expect that all organisms will be everywhere depending on their nutritional and light requirements.

Selected marine benthic (seafloor) invertebrates will be collected from shallow water (<30m) coastal regions of the Antarctic, via scuba diving, for use in experiments to determine the impacts of ocean acidification on their condition and function. We will target early life history stages (e.g., juveniles), but adults may also be collected. Should the adults spawn, due to the considerable differences in water temperatures between the Antarctic collection sites (-1.9°C) and coastal New Zealand (>10°C), we consider it highly unlikely that any offspring would survive, let alone create a self sustaining population.

The marine bacteria and archaeabacteria we plan to collect, culture and identify will inhabit every possible ecological niche in the environment. There are very few, if any, unifying characteristics for these organisms, except that they are isolated from the marine environment and will have a salt requirement as the oceans are 33 to 37 ppt (3.5-3.7%) salt. Marine bacteria are in relatively high concentrations in near shore, upwelling and estuarine waters but sparser in the open oceans (Atlas and Bartha, 1993; Rheinheimer, 1992). Heterotrophic marine bacteria in the open oceans are generally associated with algae or detrital particles as they offer a nutritional advantage and can be classed as both primary producers and heterotrophic consumers (Fuhrman et al., 1993). Marine bacteria generally grow slower than their terrestrial counterparts and show pleomorphism (the occurrence of two or more structural forms during a life cycle) in culture (Atlas and Bartha, 1993; Rheinheimer, 1992). They are well known for their ability to degrade natural organic compounds, such as cellulose, agar, alginate, chitin, hydrocarbons and phenols to name a few. Eighty percent of marine bacteria in seawater assemblages stain gram-negative and 95% stain gram-negative after isolation on agar although the proportion of gram positives bacteria is thought to be higher. A recent study has shown that up to 20 000 species of marine bacteria exits in one litre of seawater (Sogin et al 2006). Most marine bacteria are motile by means of flagella. Spore formers are not widely seen in seawater but can be found in marine sediments (Atlas and Bartha, 1993; Rheinheimer, 1992). For example heat resistant spore formers represented only a small fraction (0.0001%) of the total bacteria population isolated from the bay of Vigo, Spain (Zdanowski and Figueiras, 1997). Therefore we anticipate that we will isolate few spore formers from the samples

that we collect. Some coastal bacteria such as *Vibrio cholera, V. parahaemolyticus* are known to cause infection in humans, but few if any oceanic bacteria have caused infections in humans. Considering we are isolating bacteria from mainly oceanic and sediment samples we anticipate that we will isolate very few, if indeed any, human pathogens. Bacteria pathogenic to other aquatic organisms are common in the marine environment and these will be isolated during this work. We will not be assessing the organisms for their pathogenic potential but we assume that we will isolate *Vibrio* spp. *Cytophaga* spp., *Flexibacter* spp. *Shewanella* spp., *Alteromonas* spp. and a number of strains/species within in these genera are know to cause disease in aquatic organisms.

Very few archaeabacteria have been isolated into pure culture from the marine environment. The few that have been isolated have been isolated mainly from extreme environments such as hydrothermal vents or have fastidious growth requirements, ie methane, methanol, sulphur etc. Their presence in the marine environment has been proven by molecular methods. Due to the fastidious growth requirements of archaeabacteria we anticipate that we will rarely isolate these. No members of the archaeabacteria, at this time, are known to cause disease of humans, plants or animals.

Eukaryotic marine organism, including yeasts, fungi, protozoa, thraustochytrids and microalgae will be isolated from the marine environment. Yeast populations are known to be the dominant fungi in the open oceans (Nagahama, 2006). Yeast populations are sparser in marine water than in fresh water, and decrease with increasing depth and increasing distance from land (Hagler & Ahearn 1987). The yeasts found in the marine environment include members of the genera *Candida, Cryptococcus, Trichosporon,* and *Rhodotorula* to name a few (Nagahama, 2006). There are currently no known human pathogens among the marine yeasts however some can cause disease in other marine organisms.

Only about 500 fungi species, less than 1% of all known species, have been isolated from the marine environment. There are about 100 hundred species of marine zoo-spore forming fungi mostly *Pythiales* and Thraustochytrids; and additionally higher order fungi in the marine environment (Carlile, et al, 2001). The Thraustochytrids are described separately below. About 90% of the fungi require seawater for growth and sporulation (formation of fungal spores) and are therefore described as obligatory marine organisms (Carlile, et al, 2001). The distribution of fungi like other marine organism is determined by the availability of nutrients. The main food sources in the marine environment for fungi are aquatic pants such as seaweeds (or even wood in the deep sea), any other marine organism or sources of organic matter (Carlile, et al, 2001). Almost a third of all known marine fungi are associated with seaweed or plankton and they sometimes form symbiotic relationships with seaweed. There are currently no known obligatory marine fungi that cause disease in humans, however fungi do cause infections in marine fish e.g. fin rot caused by members of the oomycetes. We are currently not intending to isolate specifically for fungi other than the Thraustochytrids, however we may isolate fungi as contaminants on our bacterial agar plates.

Thraustochytrids are marine, biflagellate protists (Phylum Sagenista, kingdom Protista or Chromista). They typically produce a network of filaments or tubes, which serve as tracks for the cells to glide along and absorb nutrients for them. They are commonly found on alga and seagrass and as decomposers on dead plant material, and also include some parasites of marine

invertebrates. Two genera in particular will be targeted for isolation, *Thraustochytrium* and *Schizochytrium,* however other genera may also be isolated. Both these genera exhibit a similar life cycle whereby mature sporangium (approximately 50µm diameter) release biflagellate zoospores (approximately 3-6µm diameter) into the water, which mature over a few hours, and in turn develop into sporangium. They are not known to be pathogenic or allergenic to humans. In culture, they do not require special conditions for growth other than ambient temperature and basic macronutrients (carbohydrate and nitrate sources). No thraustochytrids to date are known to produce toxic blooms and the only reports of contamination have come from sponge cell isolation experiments (whereby the sponge has ingested thraustochytrids from the water column prior to collection). Most species of thraustochytrids, including those already identified in New Zealand, occur abundantly in seawater and sediments worldwide (Raghukumar, S 1988; Raghukumar, S 2002)

Microalgae are the ocean's primary producers. During the year, this group only occurs in low concentrations at non-bloom conditions in Antarctic waters, the Southern Ocean and Oceanic parts of the Exclusive Economic Zone (EEZ) around New Zealand. They can under the right conditions form algal blooms, i.e. spring condition when nutrients are higher and the conditions in general are more favourable. Even though the 'extreme' environmental conditions in the Antarctic are not favourable to many species, representatives of most algal classes may be found in these ocean assemblages. As a group they span a size ranging from <2 µm in most prokaryotes to several millimetres long in some eukaryotes such as diatoms. In this study attention will be paid to larger eukaryote species such as microflagellates (5 – 20 µm), dinoflagellates and diatoms (20 – 60 µm). In the 'warmer' waters, e.g., in tropical and temperate regions of the EEZ, there are a small number of marine microalgal species known to associate with harmful algal blooms (HAB) or toxic outbreaks. These include the fish-killing microflagellate species (e.g., *Chattonella* spp., *Prymnesium* spp. *Chrysochromulina* spp.), PSP- (e.g., *Alexandrium* spp., *Gymnodinium* sp.), DSP- (e.g., *Dinophysis* spp., *Prorocentrum* spp.) and NSP- producing (e.g., *Karenia* spp.) dinoflagellate and an ASP-producing *(Pseudo-nitzschia* spp.) diatom species. All of these species have already been isolated from coastal waters around New Zealand. Only a very few of such 'cold water' counterparts are known to exist in Antarctic waters. Moreover, only a small number of the Antarctic dinoflagellate species are known to produce cysts as part of their life histories. Thus we do not anticipate collecting and culturing many representatives of the group of harmful/toxic algal species for biodiversity studies from the Ross Sea, Southern Ocean or Oceanic EEZ. We anticipate culturing, particularly those relatively less well known/undescribed species for further taxonomic studies.

The marine invertebrates we wish to collect are restricted by size, they are all described as marine and will not survive in freshwater. A specific example of an organism we wish to collect is the Antarctic scallop *Adamussium colbecki*, these have planktotrophic larvae. Timing of their reproduction is strongly dependent on availability of food (Chiantore, et al. 2002); spawning varies in the Ross Sea dependent on location, and has been reported to occur in October (Berkman, et al. 2001) and February (Chiantore, et al. 2002). Larval life is thought to be around 100 days (Tyler et al. 2003). *Adamussium* are benthic (seafloor) dwelling organisms and, like scallops in temperate systems, they are capable of 'swimming'. They are a long lived species (i.e., to around 90 years old). Another example is *Laternula elliptica*, a soft shell Antarctic clam which has larvae that are released into the water column at an advanced juvenile stage at the beginning of winter (Berkman

et al. 1991). *Laternula* live buried in the sediment. Around the wider Antarctic, their larvae are released in the summer, autumn and winter months (refs in Powell, et al. 2001). *Laternula* live to in excess of 20 years. Neither of these species poses any risk to humans or other fauna. As Antarctic organisms they are adapted to living in very cold water (generally around -1.92°C in McMurdo Sound), and it is highly unlikely that these individuals would survive in more temperate waters. The organism we wish to collect from the Southern Ocean and within the Oceanic EEZ have similar life cycles and stages, although it is hard to generalise. Again we anticipate that either they will not survive as the environmental conditions (i.e. water temperature or nutrients) in coastal waters around New Zealand will not allow them to survive or reproduce, and if they could survive they would already be here due to current flows around New Zealand.

Section Four – The Proposed Containment System and its Effectiveness

4.1 Describe the proposed containment system (physical and operational) and the ability of the organism(s) to escape from this system.

The adequacy of the containment regime is a principal consideration so you need to provide comprehensive information on the containment system. Containment facilities must be registered by MAF, and you should provide documentary evidence of this. Refer to relevant containment manuals as appropriate. Please also ensure that ERMA New Zealand has an up-to-date copy of the containment manual relating to this facility. Identify possible pathways of escape of the organism(s) from containment, including through lapses of security or sabotage. Describe the biological features of the organism(s) that might affect its ability to escape from containment.

> All marine organism will be collected by one of two sampling methods, either shipboard on either the RV *Tangaroa* or NIWA's other research vessel or scuba diving in the Ross Sea, Antarctica. The shipboard methods will involve sampling the water column or marine sediments, by a variety of methods i.e. niskin bottles, multicore, beam trawl or grab. These samples will mainly be used for direct plating on agar or inoculating enrichment media with a small sub-sample for the isolation of organisms. This research will be conducted in laboratories on board the RV *Tangaroa* or other NIWA research vessels. The laboratories on board are to PC1 standard. All incubations on board will occur in airtight containers and will not be opened inside the 12 mile limit and will be transferred on land to a containment facility (Table 1). The small marine invertebrates (<30mm) that are collected on board will be kept in closed containers with regular water changes. This water will be disinfected on board by either chemical sterilisation or autoclaving before disposal. A small amount (<100g sediment or <250mL of oceanic water per sampling container) may be bought back to one of the containment facilities for the isolation of organisms on land. These samples will be collected on board and transferred to sterile sealable containers which will be transferred to one of the containment facilities for processing. The containment facilities will be approved under the MAF Biosecurity New Zealand and ERMA New Zealand Standard Facilities for Microorganisms and Cell Cultures: 2007 at minimum physical containment level 1 (PC1). Access to the containment facilities at all sites is restricted by security at the front desk; no visitor can gain entry to NIWA sites unless accompanied by a staff member. The laboratories are locked when not in use, so there is no uncontrolled access to any of the samples and/or organisms. These procedures will prevent the accidental escape of organisms. Spore forming organisms, if isolated, will be cultured in sealed container and will not be allow to go through to sporulation by carefully monitoring of the growth phase. If spores

are formed the organisms will only be worked on in a class II biological safety cabinet. Marine fungi, other than thraustochytrids, if isolated will either be destroyed or only be worked on in class II Biological Safety Cabinet. Thraustochytrids produce spores in liquid culture which does not pose a risk for spores to be released in the form of aerosols, therefore thraustochytrids will only be allowed to sporulate in liquid culture.

The second sampling method will involve scuba diving and the collection of larger (<120mm) marine invertebrates. The organism will be inspected for any associated encrusting organisms which will be removed in Antarctica. The marine invertebrates will be kept in sealable containers with a small amount of water so that they can be transported live back to New Zealand via air. Once in New Zealand they will be transferred to one of the containment facilities operating under the 154.02.06 Transitional Facilities for Ornamental fish and Marine Invertebrates and 154.02.08 Transitional and Containment Facilities for Invertebrates, for further experiments. Approved by MAF for operations at PC1. These experiments will be carried out in small closed system aquaria in a cold room in a containment facility. The waste water from these aquaria will be sterilised using either chemical sterilisation or autoclaving prior to disposal. The marine invertebrates we wish to collect are unable to fly and will be kept in aquaria. They cannot climb out of these aquaria and will only be able to escape due to a hole or breakage of the aquaria. Therefore escape of these organisms is highly unlikely. However, if they did manage to escape from the aquaria, they would end up in freshwater, i.e. drains, and would not be able survive in freshwater and the sewage treatment system.

Samples will be taken from the dead marine organism for biochemical and/or chemical analysis by non-containment laboratories. Most samples will be destroyed as part of the analysis, should any sample(s) remain this will be returned or disposed off via autoclaving by the analysing laboratory.

All materials entering the different containment/transitional facilities will be logged as outlined in each facility's manual and will be tracked in each of the individual facilities. All waste material will be sterilised either through chemical sterilisation or autoclaving prior to disposal as outlined in each facility's manual. Security at each NIWA site is through a controlled reception area with security card entrance for all other doors. All containment/transitional facilities comply with MAF security requirements and will be updated if required to meet the requirements of this approval.

Table 1: NIWA Transitional and Containment facilities to which standard they operate, the level of containment and the operator.

Facility Code	Facility Type	Standards	Location	Operator
563	Transitional Facility	Transitional for Biological products MAF Standard 154.02.17 (Currently upgrading to microbiology standard and PC2)	NIWA Wellington (Mahanga Bay)	S Allen
568	Transitional Facility and Containment Facility	Transitional for Biological products MAF Standard 154.02.17 and Transitional and Containment for Invertebrates MAF Standard 154.02.08 to PC1	NIWA Hamilton	J Hall
569	Transitional Facility and Containment Facility	Transitional facility for biological products, and Containment Facility PC2 MAF standard 154.03.02 for microorganisms and cell cultures.	NIWA Christchurch	D Sutherland
3117	Transitional Facility and Containment Facility	Operating to MAF standard 154.03.02 Containment for micro-organisms and ASNZS 2243.3:2002 PC2 laboratories.	NIWA Wellington (Greta Point)	V Webb

Section Five - Identification and Assessment of Risks, Costs, and Benefits

This section must include information on the beneficial and adverse effects referred to in the HSNO Act. It is easier to regard risks and costs as being adverse (or negative) and benefits as being positive. You should consider costs and benefits with respect to both non-monetary and monetary (dollar) terms and also consider the distribution of this incidence. Provide a brief description of where the information in the application has been sourced from, e.g. from in-house research, independent research, technical literature, community or other consultation.

5.1 Ability of organism(s) to establish a self-sustaining population.

Discuss the ability of the organism(s) to establish an undesirable self-sustaining population, should an escape from containment occur, and the ease with which such a population could be eradicated. You should consider the ability of the organism(s) to survive and reproduce if it did escape from containment.

> The micro-organisms isolated from the sediment and water samples are likely to be in New Zealand or are unlikely to establish in New Zealand due to water temperature and/or nutritional requirements. A study by Baldwin et al. 2005 analysed water samples across a transect from the Antarctic to the Arctic, across the Pacific. The results showed that some phylotypes, both prokaryotic and eukaryotic, were ubiquitously distributed and that some were restricted to certain broad zones, i.e. polar regions, but not to a specific area. They hypothesised that the environmental conditions i.e. water temperature and organic mater selected for certain phylotypes in specific regions, i.e. Temperate, Tropical or Polar Regions. Therefore, we hypothesise that due to the currents around New Zealand, if it could be here and survive it will be here and if it is not here it cannot survive as the conditions are unfavourable for it to establish and grow. Figure 1 shows the currents around Antarctica and in the Southern Ocean. Figure 2 shows the New Zealand's EEZ and the currents around New Zealand. The marine organisms we collect may be able to survive in lower salt concentrations, but it is unlikely that they will survive in freshwater. Some bacteria maybe able to survive in freshwater as some bacterial genera are ubiquitously distributed in both terrestrial and marine habitats such as *Pseudomonas* spp. and *Micrococcus* spp.

The phytoplankton samples we plan to bring back are probably already within the 12 mile zone due to the currents around New Zealand, except some specifically cold-water adapted phytoplankton species. These cold adapted species will not be able to survive in New Zealand's waters as the difference in water temperatures is too great (+10 - 20°C). Some of those cold-water adapted species will not, however, survive as the range of water temperatures experienced in New Zealand exceed the upper thermal tolerance (above 6 - 9°C) limit of these species (Fiala and Oriol, 1990).

The other marine eukaryotes, such as fungi, yeast, invertebrates and thraustochytrids will either not be able to survive around coastal New Zealand due to the differences in temperatures and/or nutrients, or if they could survive they would already be here due to the current flows from Antarctica to New Zealand and the currents around New Zealand.

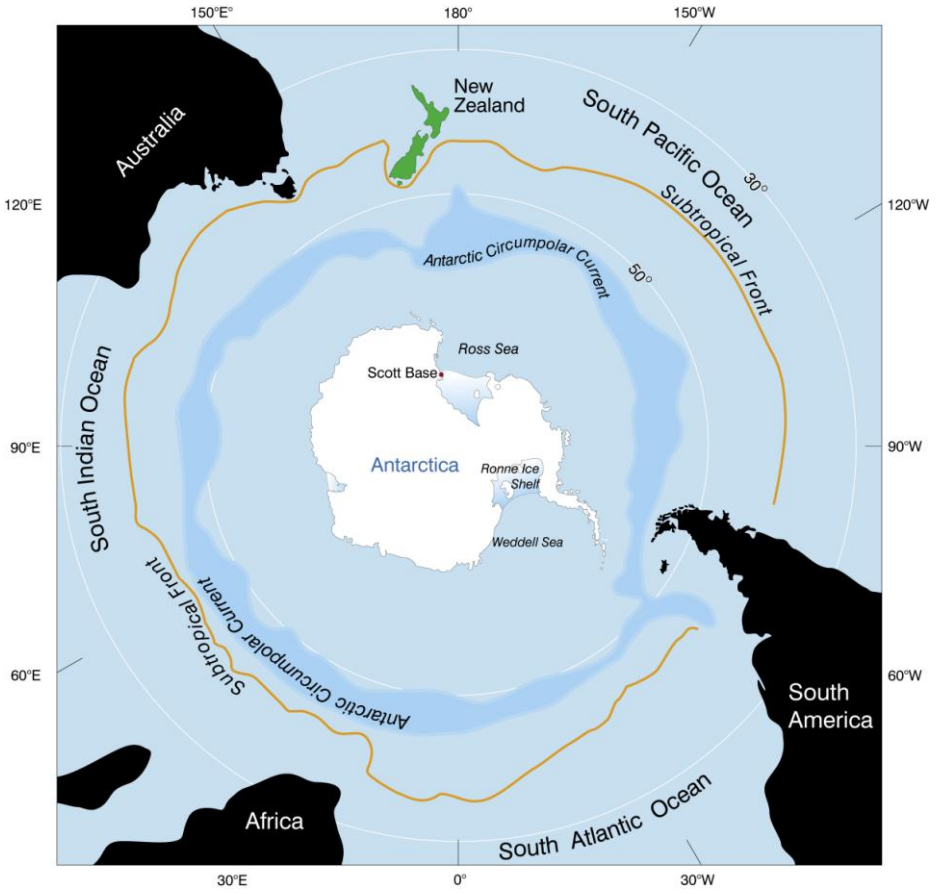

Figure 1: Currents around Antarctica showing the Antarctic Circumpolar Current and Subtropical front

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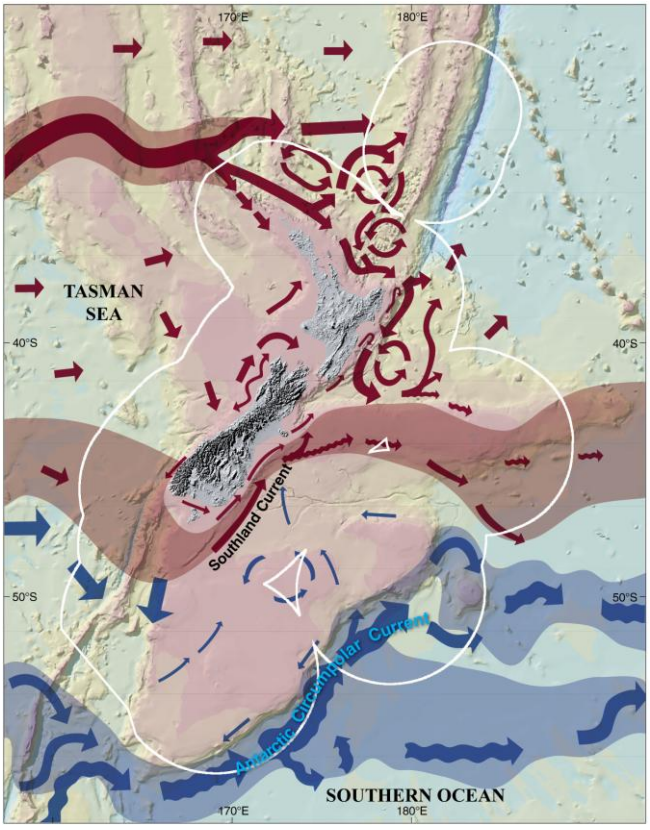

Figure 2: Current around New Zealand and extend of New Zealand's current Exclusive Economic Zone (EEZ) (white line)

5.2 Identify all potential adverse effects of the organism(s). Identify potential adverse effects associated with the organism(s) and with any inseparable organisms, both within containment, and outside of containment (should an escape occur). Consider effects on the environment, and human health and safety (e.g. of workers in the containment facility), and any ethical and cultural effects. It is important to think about the source of the risk, i.e. the way in which the risk is created (the exposure pathway), and then the consequences of exposure. Adverse effects should be identified for the following categories:

A. Potential adverse effects on the environment, in particular on ecosystems and their constituent parts (e.g. adverse effects on: life supporting capacity of air, water, soil and ecosystems; native and valued introduced flora and fauna; natural habitats and the intrinsic value of ecosystems; New Zealand's inherent genetic diversity; animal or plant health)

> Bacteria and Archaeabacetria: The risk of either the bacteria or archaebacteria on the environment are mainly competition for resources (nutrients) and displacement of other microbes from the ecosystem. The potential exist for the micro-organism to cause infection/disease in both terrestrial and marine; plants and animals.

Microscopic Eukaryotes (eg thraustochytrids and micro-algae): The micro-algae could form an algal bloom causing a change in the ecosystem. Due to the fast growing nature of a bloom event the environment could be depleted of nutrients and oxygen adversely effecting aquatic plants and animals. The potential exist for some of the microscopic eukaryotes, e.g. fungi, to cause disease in aquatic animals or plants. Some of these animals or plants could be native species.

Marine Invertebrates: The risk of the marine invertebrate on the environments is the competition for ecological niches in the marine environment. They could potentially displace the current marine invertebrates both native and introduced by competing for resources, nutrients and space.

B. Potential adverse effects on public health (including occupational exposure)

>Bacteria and Archaeabacetria: Very few, if any, marine micro-organisms are known to cause human diseases. Therefore the risk to public health is very small. The risk for opportunistic infections does exist but only to small sections of the community such immuno-compromised people (i.e. HIV positive or chemotherapy patients). The risk to laboratory works is also small as all organisms are risk group 1 and not known to cause human infections.

Microscopic Eukaryotes (eg thraustochytrids and micro-algae): There are some potential adverse effects from these organisms. Micro-algae are known to produce some potent toxins under bloom forming conditions. These toxins can adversely affect the public and laboratory workers. Terrestrial fungi, are known to produce human allergens, marine fungi to date have not been shown to produce these. However, the potential exist that we isolate a marine fungi that is able to produce an allergen.

Marine Invertebrates: NIL. Marine invertebrates are not known to adversely affect public health.

C. Potential adverse effects on the relationship of Māori and their culture and traditions with their ancestral lands, water, sites, waahi tapu, valued flora and fauna and other taonga (taking into account the principles of the Treaty of Waitangi). For example, you should consider whether the organism(s) would have an effect on specific native flora or fauna if they escaped from containment.

>Bacteria and Archaeabacetria: No different from the effects outlined in A and B, except that some of the aquatic plant or animals maybe Maori food sources.

Microscopic Eukaryotes (eg thraustochytrids and micro-algae): No different from the effects outlined in A and B, except that some of the aquatic plant or animals maybe Maori food sources.

Marine Invertebrates: No different from the effects outlined in A and B, except that some of the aquatic plant or animals maybe Maori food sources.

D. Other potential adverse effects (such as New Zealand's international obligations, social or economic adverse effects, ethical issues)

> Bacteria and Archaeabacetria: None

Microscopic Eukaryotes (eg thraustochytrids and micro-algae): None

Marine Invertebrates: None

5.3 Provide an assessment of the adverse effects identified in Sections 5.2.

The assessment should include the nature, likelihood or probability of occurrence, and magnitude of each adverse effect (i.e. **the risk**), and the value (in monetary or non-monetary terms) of a particular adverse effect (i.e. **the cost**). The uncertainty bounds of the information contained in the assessment should also be discussed.
Adverse effects should be assessed in relationship to:

A. Potential adverse effects on the environment, in particular on ecosystems and their constituent parts (e.g. adverse effects on: life supporting capacity of air, water, soil and ecosystems; native and valued introduced flora and fauna; natural habitats and the intrinsic value of ecosystems; New Zealand's inherent genetic diversity; animal or plant health)

> Bacteria and Archaeabacetria: The chances of escape are small for the marine micro-organisms as they are worked on only in containment facilities. In addition, we expect that the organisms that could survive around coastal New Zealand are already here and that any others would not survive.

Microscopic Eukaryotes (eg thraustochytrids and micro-algae): The chances of escape are small for the marine microscopic eukaryotes as they are worked on only in containment facilities. In addition, we expect that the organisms that could survive around coastal New Zealand are already here and that any others would not survive. Chances of marine micro-algae building up to affect native, 'warm-water' species are considered to be pretty slim.

Marine Invertebrates: The risk of the marine invertebrates escaping containment is very slim, given that they cannot fly and cannot climb out of their aquaria. In addition, we expect that the organisms that could survive around coastal New Zealand are already here and that any others would not survive. Therefore the chances of adverse effects are very slim.

B. Potential adverse effects on public health (including occupational exposure)

>As for A

C. Potential adverse effects on the relationship of Māori and their culture and traditions with their ancestral lands, water, sites, waahi tapu, valued flora and fauna and other taonga (taking into account the principles of the Treaty of Waitangi). For example, you should consider whether the organism(s) would have an effect on specific native flora or fauna if they escaped from containment. If consultation with Maori has been undertaken, provide details of the process used and the outcome.

> As for A

D. Other potential adverse effects (such as New Zealand's international obligations, social or economic adverse effects, ethical issues)

> As for A

5.4 Identification of beneficial effects (benefits)

Identify and describe monetary and non-monetary benefits associated with importing the organism(s) into containment. Outline and discuss the purpose(s) for the importation and the potential use of the organism(s). Focus on the immediate benefits, as well as longer-term benefits. For example, "increase in scientific knowledge", "increased production of agricultural produce". Substantiate claims by reference to sources of information. Specify whether the benefits identified are environmental, public health or economic benefits; and/or are specific benefits to Maori.

> The central theme of collecting and identifying the organisms in this application is to understand the current biodiversity in New Zealand, Southern Ocean and the Ross Sea and the physiology and biochemistry of these organism. Currently we know very little about the microscopic organisms in these water masses. If we are to be able to predict how large scale climatic changes will affect the ecosystem we first need to understand which organisms are there and their role(s) in the ecosystem. Water masses exhibit different biological signatures in productivity, seasonality and in their relative contribution to a wide range of biogeochemical processes. To advance NIWA biological and biogeochemical modelling of oceanic biophysical processes knowledge of phytoplankton biogeochemical signals are needed. Isolation of phytoplankton from waters close to New Zealand and their subsequent culture in the laboratory in specified media under controlled physical conditions allows experiments to be performed where any differences in phytoplankton characteristics caused by changing these conditions can be measured. This will increase our understanding of the factors that control temporal and spatial trends of the phytoplankton community and the community's resilience to change of pelagic ecosystems in offshore waters. In the Southern Ocean around Antarctica microalgae are a diverse group of marine organisms. As well as forming the base of the food chain, this group also plays a role in influencing climate. The Southern Ocean is a key area of exchange of $C0_2$ with the Atmosphere. Seasonal and spatial variability in the build-up of this group is a principal determinant of whether the ocean is drawing down atmospheric CO_2. Studies of Antarctic microalgae, including those that can be cultured, would help us to better understand: the diversity of this group in the Southern Ocean and also the role plays by a group of calcium carbonate ($CaCO_3$)-producing species such as coccolithophorids in the 'regulation' of polar atmosphere. Ocean acidification associated with increased atmospheric CO_2 levels is a real and current threat to coastal and oceanic ecosystems worldwide. Absorption of this excess atmospheric CO_2 by the oceans has already decreased, and will continue to decrease, ocean pH. This 'acidification' of the oceans has important implications for organisms that depend on calcium carbonate for shell generation due to its affects on shell formation and dissolution, and on physiological processes such as growth and reproduction. Cold water regions such as the Ross Sea are particularly vulnerable due to increased solubility of $CaCO_3$. Information on likely effects of ocean acidification is scarce, and nothing is known of its effects on Antarctic seafloor organisms and communities. It is essential that we improve this knowledge if we are to predict how (and whether) these valuable ecosystems might respond and adapt to this climate-change associated impact. Ocean acidification on other organism is even less studied and part of the research to be conducted with the organisms isolated will address this knowledge gap. The benefits of this application are wide; they will provide fundamental biodiversity information of organism that we

know very little about (ie bacteria, archaeabacteria, marine microscopic eukaryotes and micro-algae) and especially the oceanic species of these organisms and provide insights into their biochemical and physiological activities by studying them in pure culture in the laboratory. Once we have some of this fundamental information we can study how the effect of climate change through increasing ocean temperature and acidification will affect the organisms and the cycling of major elements (carbon, nitrogen, phosphorus, iron and sulphur) in the oceans. In addition, Under the Convention on Biological Diversity, New Zealand has a particular responsibility for conserving our ecosystems, including the Ross Sea. The New Zealand Biodiversity Strategy requires that all ecosystems in our nation (and this includes the Ross Dependency) be maintained. We cannot full fill this obligation unless we understand what biodiversity is currently there. The organisms that we wish to collect and study as part of this application will be a first step in filling knowledge gaps in the diversity of in particular microscopic organisms.

5.5 Provide an assessment of the benefits identified in Section 5.4.

Estimate the likelihood that the benefits will be realised, the magnitude of benefits associated with importing the organism(s) into containment, and any uncertainties associated with this assessment. You should also indicate who would receive the benefits and the expected time-course of delivery of the benefits.

>The benefit through the scientific knowledge gained by studying the organisms collected as part of this application will be wide reaching. It will provide new scientific knowledge about how the oceans and the organism in them might change due to climate change. NIWA has been conducting marine research for many decades and has contributed to many international initiatives, for example IPCC report on climate change, Global Ocean Observing System (GOOS), and the information gained will be disseminated through the various FRST funded programme, eg 'Ice CUBE' 'Biogeochemical & Carbon cycles', 'Biophysical Time-series' projects in the 'Consequences of Earth-Ocean Change' and 'Coasts and Oceans' 'Biodiversity & Biosecurity' OBI programmes. In addition, the knowledge gained will contribute to the Census of Antarctic Marine Life voyage to the Ross Sea as part of International Polar Year (IPY) funded through the Ministry of Fisheries and Land Information New Zealand.

5.6 Overall evaluation of risks, costs, and benefits

This overall evaluation is the main task of the Authority. The Authority has to decide whether the beneficial effects of having the organism in containment outweigh the adverse effects of the organism and any associated inseparable organisms. The Authority must also be satisfied that the organism can be safely contained. You may wish to express a view on the relative importance of the different risks, costs and benefits and how they should be brought together in making a decision.

> The benefits of the application outweigh the potential risks.

Section Six – Additional Information

6.1 Do any of the organism(s) need approvals under any other New Zealand legislation or are affected by international obligations?

For example, indicate whether the organism is subject to other New Zealand legislation, e.g. the Biosecurity Act 1993, or Animal Welfare Act 1999; or if the organism(s) are listed in CITES, then approval is required from both the importing and exporting countries.

> NIWA already holds Permits for this work, however some specific permits need to be renewed for each specific research voyage or collection trip.

Current:

High Seas Fishing Permit. Pursuant to Section 113H of the Fisheries Act 1996. Allows NIWA to take and transport fish, aquatic life and seaweed on the high seas.

Ministry of Fisheries Special Permit. Section 97(I) (i) and (ii) of the Fisheries Act, 1996. Allows NIWA to take fish aquatic life and seaweed irrespective of either size, state, site, method or time of fishing, for the purpose of education and investigative research. Restricted to the EEZ.

MAF Import permits. NIWA holds a number of MAF import permits which will have to be updated to include the marine organism included in this approval.

In progress: (needed for each individual voyage or collection trip)

IEE Initial Environmental Evaluation in accordance with the Antarctica (Environmental Protection) Act 1994. This permit will allow NIWA to operate its research vessel RV *Tangaroa* in the Ross Sea and lists the conditions under which we must operate.

Ministry of Fisheries Collection permit for the Ross Sea, Antarctica. Collection permit under the Antarctic Marine Living Resources Act 1981. This permit will allow NIWA to collect aquatic life in the Ross Sea, Antarctica

6.2 Have any of the new organism(s) in this application previously been considered in New Zealand or elsewhere?

For example, has the organism(s) been previously considered for import (e.g. under the Plants Act)?

> Some of the organisms that are covered by this application are approved for import into containment through application NOC01005 (water and marine samples from Antarctica) and application NOC04016 (unidentified microorganisms from Antarctic water, soil, sediment and rocks). Both required containment at PC1.

6.3 Is there any additional information that you consider relevant to this application that has not already been included?

>

6.4 Provide a glossary of scientific and technical terms used in the application.

>

6.5 List of appendices.

List any appendices included with this application. Any information that is commercially sensitive, or additional material included with the application (such as details of consultations, referenced articles) should be contained in appendices. The main application should refer to the relevant appendices but be able to be read as a stand-alone document.

> List of possible organisms that maybe collected.
Scientific references

Section Seven – Application Summary

Summarise the application in clear, simple language that can be understood by the general public. Include a description of the organism(s) to be imported into containment, and any risks and benefits associated with their importation. This summary will be used to provide information for those people and agencies who will be notified of the application (e.g Ministry of Agriculture and Forestry, Department of Conservation, Crown Research Institutes) and for members of the public who request information. Do not include any commercially sensitive information in this summary.

> This application seeks approval to import into containment marine organisms, and sediment/water samples for the isolation of marine organisms, from the oceans around New Zealand, the Southern Ocean and Antarctic waters for scientific research.

One major group of organisms we wish to import are microorganisms (<30mm) which include bacteria, fungi, yeasts and algae. These organisms form the base of the food web and are involved in the cycling of nutrients in the oceans. There is currently limited knowledge about the numbers, identity and activities of these organisms. To try and understand this we wish to bring these organisms back to the laboratory so that we can identify and study them. These microorganisms will all be marine organisms, and will be studied in containment, at a minimum of physical containment level 1 (PC1). There is very little risk to humans, plants or land based animals from these organisms, and it is anticipated that many of the organisms we will isolate will also be present in the coastal environment around New Zealand.

We also wish to import marine invertebrates (<120mm) to study their physiology and biochemistry. In particular, we wish to study animals that may be affected by climate change and ocean acidification. Again these organisms will be studied in a containment facility (PC1). These organisms are not known to cause any adverse effects to human health, animals or plants.

The benefits of this research are a better understanding of the biodiversity of the macro- and micro- organisms in the oceans around New Zealand, Southern Ocean and the Ross Sea. Secondly we will study the effect of climate change on these organisms and how they might adapt to increases in ocean temperature and acidification. This will help us predict the possible consequences of climate change on oceans around New Zealand, Southern ocean and Antarctic waters.

Application for approval to import into containment any new organism that is not genetically modified, under Section 40 of the Hazardous Substances and New Organisms Act 1996

Checklist

Please check and complete the following before submitting your application:

All sections completed	Yes
Appendices enclosed	Yes/ NA*
Confidential information identified and enclosed separately	Yes/NA
Copies of additional references attached	Yes/NA
Cheque for initial fee (incl. GST) enclosed	Yes/No
If "yes", state amount:	$…….
Direct credit made to ERMA bank account:	Yes/No
If "yes" give date of direct credit …/…/… and amount deposited:	$…….
Application signed and dated	Yes
Electronic copy of application e-mailed to ERMA New Zealand	Yes

*NA – not applicable

Signed: **Date:**

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ENVIRONMENTAL RISK MANAGEMENT AUTHORITY
NGĀ KAIWHAKATŪPATO WHAKARARU TAIAO

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Appendix 1

Examples of organisms we may find, this list is not exhaustive and only gives an indication of the diversity we may encounter. The list is complied using the 6 Kingdoms as proposed by Woese et al 1977.

Kingdom Bacteria (including cyanobacteria) (Domain Bacteria)
Examples of Genera**:** *Acidobacter* spp., *Acinetobacter* spp., *Actinobacteria* spp., *Aneurinibacillus* spp., *Arthrobacter* spp., *Bacillus* spp., *Brachybacterium* spp., *Cellulophaga* spp., *Coliwellia* spp., *Curtobacterium* spp.,. *Cytophaga* spp., *Exiguobacterium* spp., *Flavobacterium* spp., *Flexibacter* spp., *Gemmimonas* spp., *Glaciecola* spp., *Halomonas* spp., *Isoptericola* spp., *Kitasato*spo*ra* spp., *Leeuwenhoekiella* spp., *Listonella* spp., *Marinobacter* spp., *Marinomonas* spp., *Methylophaga* spp., *Microbacterium* spp., *Microbulbifer* spp., *Micrococcus* spp., *Nesterenkonia* spp., *Oceano*spp*irillum* spp., *Octadecabacter* spp., *Paenibacillus* spp., *Pelobacter* spp., *Planctomycetes* spp., *Planococcus* spp., *Polaribacter* spp., *Pseudoalteromonas* spp., *Pseudomonas* spp., *Psychrobacter* spp., *Psychrobacter* spp., *Psychroflexus* spp., *Psychromonas* spp., *Psychroserpens* spp., *Rhodococcus* spp.,. *Roseobacter* spp., *Salegentibacter* spp., *Sanguibacter* spp., *Shewanella* spp., *Sphingomonas* spp., *Stappia* spp., *Stenotrophomonas* spp., *Streptomyces* spp., *Sulfitobacter* spp., *Terredinibacter* spp., *Verrucomicrobia* spp., *Vibrio* spp.

Kingdom Archaebacteria (Domain Archaea)
Marine archaebacteria, currently there are very few cultured representatives but they fall into three main groups: Methanogens, *Crenarchaeota* and planktonic *Euryarchaeota*

Kingdom Protista (Including protozoa, fungi and yeasts) (Domain Eukarya)
Examples of Genera: *Schizochytrium* spp., *Thraustochytrium spp*., *Japonochytrium spp*. *Labyrinthula spp*., *Diplophrys* spp.

Kingdom Animalia (Domain Eukarya)
Invertebrates:
Molluscs:
Class Bivalvia, members of the following orders Pholadomyoida and Pteroida e.g., *Adamussium colbecki, Laternula elliptica*

Echinoderms:
Members of the following classes Echinoidea and Stelleroidea e.g., *Sterechinus neumayeri*, *Odontaster validus*.

Arthropoda, Crustacea, eg the following orders Amphipoda, Isopoda, Tanaidacea, Cumacea
Arthropoda, Crustacea, Rhizocephalidea
Arthropoda; Pycnogonida (Sea spiders)

Kingdom Plantae (Domain Eukarya)
Eukaryotic Phytoplankton
Diatoms: in Class Bacillariophyceae

Examples of Genera: *Thalasiosira* spp., *Rhizosolenia* spp., *Thalassionema* spp., *Pleurosigma* spp., *Cylindrotheca* spp., *Fragilariopsis* spp., *Pseudonitzschia* spp., *Nitzschia* spp.

Dinoflagellates: Class Dinophyceae
Examples of Genera: *Prorocentrum* spp., *Dinophysis* spp., *Gymnodinium* spp., *Gyrodinium* spp., *Polykrikos* spp., *Ceratium* spp., *Alexandrium* spp., *Gonyaulax* spp.
Protoperidinium spp.

Microflagellates: 3 classes
Class Prymnesiophyceae
For example: *Phaeocystis* spp. and Coccolithophores

Class Prasinophyceae,
For example: *Pyramimonas* spp.

Class Cryptophyceae
For example: Cryptomonads