Native oyster (*Ostrea edulis*) reintroduction trials

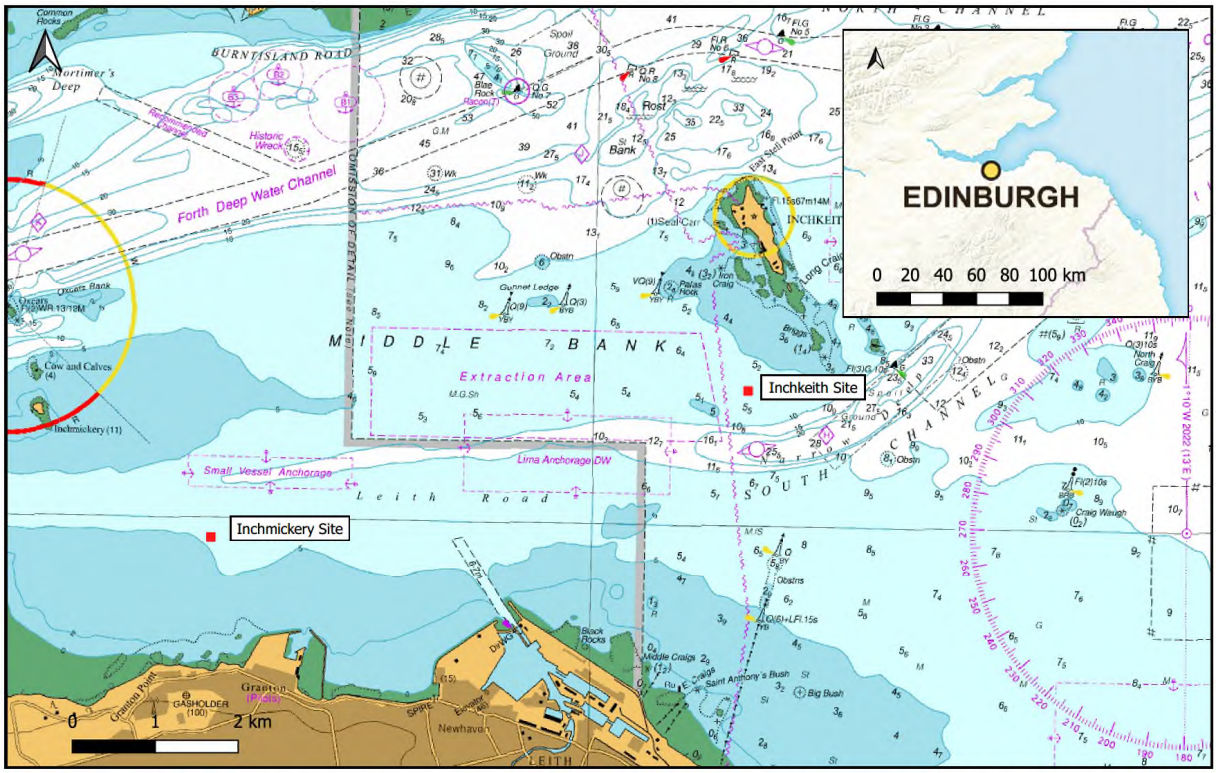
Firth of Forth

Biosecurity Plan - Reviewed December 2023

Background

Restoration Forth is a collaborative project which includes World Wildlife Fund, Marine Conservation Society, and Heriot-Watt University and others. Overall, the project aims to enhance populations of the seagrasses *Zostera marina* and *Z. noltii* and to reintroduce native oysters (*Ostrea edulis*) at selected sites within the Firth of Forth.

This Biosecurity Plan relates to the reintroduction of native oysters to two 100m x 100m plots situated north of Leith in the Firth of Forth and close to Inchkeith and Inchmickery.



Heriot-Watt University - Oyster Growth Trials - Firth of Forth
Figure 1:
Location Plan Showing Translocation Plots

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Following the deployment of oysters to the plots they will be visited annually by scientific dive teams for *in-situ* monitoring and small numbers of oysters will be removed for further laboratory analysis.

This biosecurity plan is based on existing documents, which have previously been provided to, and agreed by, regulatory bodies (including Marine Directorate Science and NatureScot) in relation to other projects and relate to the source and movements of oysters, staff, and their Personal Protective Equipment. These documents are presented as follows:

Annex 1 – Biosecurity Measures at the Heriot-Watt University Aquarium. Previous versions of Annex 1 have been considered by Marine Directorate Science, as a Standard Operating Procedures document relating to holding and screening of oyster sourced for restoration. Operating in accordance with this SOP document is a requirement of Marine Directorate Science, Fish Health Inspectorate in its role in registering the laboratory as a Shellfish Holding Facility.

Annex 2 – Deals with cleaning and biosecurity measures for Dive Equipment and PPE. Previous versions of this annex have been included within biosecurity statements prepared in compliance with planning permissions for trial sites in the Dornoch Firth and were approved by NatureScot and Marine Scotland to accompany the Translocation Licence for this project in the Firth of Forth. Operating in accordance with this document is aimed at avoiding the transfer of disease, pathogens and invasive non-native species (INNS) via contaminated equipment.

Annex 3 – Summarised Biosecurity Measures for Project Volunteers. This document establishes the biosecurity protocols to be followed by volunteers external to the project and those working with them.

Annex 4 – This Annex reviews marine and brackish Invasive Non-Native Species sourced from the published list prepared by UK TAG to inform River Basin Management Planning under the Water Framework Directive. This Annex summarises the UK distribution of the listed species, identifies whether a species is known to be present at or close to either donor or recipient site and if present at a donor site, whether it would prevent the relocation of oysters despite the presence of cleaning and inspection protocols. It identifies three species, *Didenum vexillum*, *Crepidula fornicata* and *Urosalpinx cinaria* which, if present on the donor site, would prevent its use in the project.

Biosecurity Measures Statement

Over the course of the conservation translocation ten to fifteen thousand native oysters (*Ostrea edulis*) will be deployed to each of the two sites in the Firth of Forth. The experimental population will incorporate both part grown and adult oysters. Stock will be sourced from existing oyster farms holding native oysters on site at Little Loch Broom (Charron Ltd (trading as Maorach Beag) SS0877) from Sea Wilding project at Loch Craignish (SS0935), and Islay Oysters, Loch Gruinart, Islay (SS0452). In addition oysters will be sourced from wild fishery at Loch Ryan

The Code of Practice for Non-Native Species for Scotland has been carefully considered.

1. All source populations will be sampled and screened, prior to transfer, for notifiable pathogens, *Bonamia ostreae*, *Marteilia refringens* and Oyster Herpes Virus (OsHV1), by quantitative real-time PCR (qPCR) and the methods recommended by the Diagnostic Group at Marine Directorate Science in Aberdeen. The number of oysters representing 1% of the total consignment will be tested for each pathogen respectively.

2. Each donor site will be inspected by Prof. Bill Sanderson and his team for visible signs of invasive non-native species (INNS) on the screening list set out in Appendix 4. Any biota on site suspected of being INNS but not identified to species *in situ* will be sampled and referred to a recognised expert in the field if necessary. To prevent the spread of *Didemnum vexillum*, or other high impact INNS, Dr Dan Harries will be consulted on suspicious specimens and a sample will be sent to Marine Directorate Science for species identification by DNA sequencing. No stock will be moved from any site where *D. vexillum, C. fornicata* or *U. cineria* are found based on the assessment of species on the UK TAG list and other, emerging INNS lists (Annex 4).

3. An audit report will be produced for each supply site (1&2 above). This Audit trail will be maintained for batches of oysters as they pass through the subsequent stages of cleaning, inspection, holding and deployment.

4. Native oysters destined for transfer/relocation will be inspected at the donor site by Prof. Bill Sanderson and team before being transferred in sealed, watertight containers to goods-in yard at John Muir Building, Heriot Watt University. On receipt in the yard oysters will be cleaned of external epibiota. Experience has shown that the predominant epibiota fouling on donor oysters from any site are barnacles. These will be scraped from each donor shell with a blunt edged knife, prior to scrubbing with a small stiff bristled brush and any material removed from the shells will be retained, double bagged and consigned for disposal within the biological waste stream at Heriot Watt University. If time constraints prevent a whole consignment of oysters being cleaned on the day of arrival, they will be held temporarily in an aquarium facility dedicated for this purpose and separated from quarantine facilities described below.

   Following cleaning, the donor oysters will be further inspected to identify the presence of any

residual epibiota any shells with remaining visible external biota will be fed back for further cleaning and inspection.

5. During this inspection particular attention will be given to screening for the unlikely presence of Pacific oysters (*Magallana gigas*) from any consignment of native oysters. Oysters <10g will not be accepted for translocation due to the difficulty of differentiating between *Ostrea edulis* and *Magallana gigas* at small sizes. Any pacific oysters identified will be removed and destroyed.

6. The cleaned and inspected consignment will be transferred into Heriot-Watt University's Home Office licensed, bio-secure containment aquarium facilities at HWU Edinburgh. The oysters will be further scrubbed if required, and the shell surfaces sterilised in a formalin solution to remove any residual risk of the transfer of INNS or hitchhiker species (Annex 1 for further details).
7. Consignments will be held and depurated for at least 5 days in U/V sterilised artificial sea water then surfaces inspected and sterilised again if there is any doubt there are organisms remaining on the shell surfaces.

8. The purpose of the oyster biosecurity 'fire-wall' in HWU is to assure that hitchhiker species including Invasive Non-Native Species are <u>not</u> transferred from the donor site to the recipient site and to pre-screen and avoid transfer of oyster diseases. Although the biosecurity measures are specifically intended to prevent the transfer of High Impact Marine INNS as included in the UK TAG list (UKTAG classification of alien species working paper v8.pdf wfduk.org; reproduced in Annex 4) it is impractical to inspect each of 10-15,000 oysters of between 10mm and 70mm shell length to identify epibiota to species level and to then determine whether or not it is a species on the INNS list. The emphasis is on cleaning and subsequent inspection to remove ALL external biota at three control points: 1) On arrival at HWU, 2) whilst within biosecure holding aquarium and, 3) prior to transfer to the recipient site.

9. Native oysters will then be transferred from the HWU aquarium to chartered workboat for transfer to the experimental sites in the Firth of Forth. All transfers will take place in sealed watertight containers.

10. Deposit methodology will be to release oysters from the support vessel at the sea surface to the seabed c 5m below. Subsequent monitoring involves the use of divers and associated equipment. Biosecurity measures related to movement of equipment into and out of the Firth of Forth are referred to in Annex 2.

Post Deployment Monitoring

1. During site visits to service the seabed plots, annual inspections will be made for the presence of INNS as part of routine biodiversity monitoring. Records will be logged by a trained surveyor, and the presence of any INNS observed or suspected will be sampled and reported to NatureScot and Marine Directorate and advice will be sought to discuss next steps. Confirmed records will be entered to NBN Atlas via iRecord.
2. In the event that INNS are discovered within the experimental plots, efforts will be taken to remove INNS from the area and further investigation will be undertaken to ascertain the extent of INNS population and whether the project or another source has been the vector for the INNS. These efforts will be made in consultation with relevant government agencies and follow appropriate guidance.

Staff Training

1. All project staff (including volunteer contributors) will be made aware of these biosecurity measures as part of induction to the project. They will also be familiarised with material from the Non-Native Species Secretariat and the Clyde Marine Plan booklet with flip guide DangleBookLowRes30-7-12.pdf (clydemarineplan.scot).
2. From time to time, it may be necessary to engage external vessels and contractors to assist in aspects of the experimental deployment, recovery, and monitoring. In such situations the biosecurity measures required for operation on the site will be communicated to the contractor as part of the procurement process and contractors will be required to demonstrate compliance with biosecurity measures. Compliance will be demonstrated by completion of a checklist detailing areas previously worked, information provided to the contractor, and cleaning operations undertaken prior to the deployment to the project site.

ANNEX 1

Biosecurity Measures at the Heriot Watt University aquarium

Activities within this Annex are only to be undertaken by trained and authorised Staff.

Set-up of depuration tanks in secure controlled temperature room (CT) Room G36, John Muir Building, Heriot-Watt University, Edinburgh, (SS0917):

If the tanks are dirty and were unused for a long time start at point 1. Otherwise, start in point 4 after rinsing the tank with tap water.

1. Run through with tap water to start with; then
2. Clean/brush tank surfaces with a solution of soapy water or Sodium Hypochlorite solution made up to 0.1% (1000ppm) of Sodium Hypochlorite with a minimum contact time of 10min (e.g. 10ml of 14-15% w/v Sodium Hypochlorite solution made up to 1.5L with tap water or 30ml of c.5% w/v Sodium Hypochlorite made up to 1.5l with tap water). Manually (by gravity), pour the same solution into the pipework and pumps.
3. Rinse the tanks with tap water. Fill the tanks with tap water leave for ~1hr then empty, repeat if required.
4. Using a spray bottle, disinfect tank surfaces with a Virkon™ Solution, (1% w/v), using a minimum contact time of 10 min. Use the same treatment for the pipework and pump.
5. Open the available valves to remove the Virkon solution from the pipework and pumps. Fill and run through with fresh tap water (as many times as required, until there is no foam present. Usually 2 times).
6. Clean and disinfect any *ancillary equipment* within the CT room in depuration tanks (1-5 above) or other suitable container, following the same procedures (above).
7. Make-up the depuration unit with a 15kg bag of Peacock Seamix Artificial Seawater
8. Check and adjust salinity to 30-36 ppt before introduction of oysters (may require another 10% salt).
9. Wash floors and walls with any suitable cleaning product or Sodium hypochlorite, made-up to 0.05% w/v Sodium Hydroxide, with a minimum contact time of 15-20min (e.g. 5ml of 14-15% w/v Sodium Hypochlorite solution made up to 1.5L with tap water or 15ml of c.5% w/v Sodium Hypochlorite made up to 1.5l with tap water). After cleaning the surfaces, they must be disinfected, for example using a spray bottle with Virkon solution 1%, and then rinsed with tap water and wiped with a wet cloth after that.
10. Set-up CT room tread-pad with 10L of 1% Virkon™ S solution (disinfectant dilution rate is 1:100, i.e. 10 grams of Virkon™ S to every 1 litre of water) per foot-bath.
11. Refresh CT room footbath each five days (Virkon directions for use state that product is stable for 5 days once prepared).
12. Keep CT room locked during depuration with single key access and restrict entry to only trained and authorised personnel.
13. Maintain an auditable record of staff entry and exit from CT room.
14. Use CT room specific PPE (wellies / lab coat / nitrile gloves).

15. Disinfect reception area corridors from CT room to JM Yard with bleach (Sodium hypochlorite), made-up to 0.05%w/v for surface cleaning as described above.
16. Adjust temperature of the room.

Biosecurity:

1. Before the oyster consignment is transferred to Heriot Watt University, visit supplier and:
   a. Search site for invasive / non-native species (INNS) and any signs of ill-health (e.g., >20% shells permanently gaping following handling, strong "off" smell, apparent low weight obvious higher than expected mortalities).
   b. Send samples of suspicious potential INNS to Marine Scotland Science for species identification, with special emphasis to *Didemnum vexillum*.
   c. Reject supplier if specified high impact INNS are found on site (see above).
   d. Randomly select a number of oysters, representing 1% of the total consignment and send for independent qPCR screening for *Bonamia*, *Martellia* and Oyster Herpes virus to Marine Scotland Science.
2. Native oysters selected for transfer off site will be transported in sealed, leakproof containers.
3. Oysters will arrive at a designated oyster reception area in JM yard and will be subject to cleaning before transfer into the CT room. Cleaning will involve removal of fouling epibiota (with particular attention to INNS on the screening list) from shells of oysters using a blunt, hard edge. Material removed is to be double bagged and consigned for disposal as biological waste.
4. Scrub shell surface with clean seawater and a stiff fine bristled brush.
5. Inspect cleaned oysters for remaining fouling organisms and reject or re-clean and reinspect before acceptance.
6. When consignment of oysters arrives at Heriot-Watt University, place access notices to restrict corridor traffic between CT Room and oyster reception area in JM yard. Restricted access request to be lifted after cleaning and disinfection of the area is complete (below).
7. Inspect and remove remaining epifauna from shells of oysters using a blunt, hard edge in the JM yard. Use 50L water-tight plastic trays that have been sterilised /disinfected using the procedure specified under *ancillary equipment* (6 above). Wear eye protection, lab coat and disposable nitrile gloves specific to this task.
8. Count all oysters entering the process.
9. Waste from epifauna will be scraped into 50L water-tight plastic trays and dealt with as biological waste (below).
10. Any oysters with excessive shell damage or deformity such that they cannot be reliably surface sterilised to be removed from consignment, double-bagged and frozen in the exterior freezer in JM Yard next to back door (before transfer to biological waste (below). All rejected oysters to be counted and records kept.
11. Individual oyster will be inspected to identify potential accidental transfer of Pacific oyster (*Magallana gigas*). If any Pacific oysters found, they should be rejected at the point of identification. Pacific oysters removed from consignment in JM yard, are to be double-bagged and frozen in the exterior freezer in JM Yard next to back door (before transfer to

biological waste (below). All rejected oysters to be counted and records kept. If identification to species is not possible, or if there is any doubt over identification, shells in question should also be rejected.

12. Make-up 4% Formalin solution in seawater in a suitable 40-50L container in fume-hood in JMG 49
13. Make-up adjacent similar container of tap water in fume hood as rinse-bath.
14. Place oysters in mesh container inside leak-proof box and transfer to fume-hood in JMG49.
15. Using task-specific lab coat, rubber gauntlets, eye protection and with fume-hood extractor 'on', submerge mesh container of oysters in the 4% formalin for 2 minutes.
16. Remove oysters from formalin and submerge in tap water to rinse for 2 minutes
17. Rinse container and oysters in running tap water for 2 minutes
18. Repeat from stage '9' for subsequent batches
19. At end, empty 4% Formalin in fume hood to 25L disinfected containers (see *ancillary equipment* above) and disinfect external surfaces with 4% Formalin wipe in fume hood, followed by rinse of container exterior, marked-up with contents and store in Formalin store in JM yard before transfer to chemical waste disposal contractor (below).
20. Remove oysters from fume hood and transfer to racks in the depuration units in CT Room JM G36 using leak-proof containers. Enumerate oysters using a regular grid in the racks.
21. Clean and disinfect reception area from CT room to JM Yard using decontamination process (below). Disinfect ancillary equipment used for animal transfer/handling using procedure in Set-up (point 6). Disposable gloves to clinical waste (Decontamination point 6). Lab coats to cleaning contractor (clinical) using JM Stores.

Maintaining oysters in biosecure facilities:

1. Maintain Virkon tread-pad in entrance to CT room with 10L of 1% Virkon™ S solution (disinfectant dilution rate is 1:100, ie 10 grams of Virkon™ S to every 1 litre of water) per foot-bath.
2. Refresh CT room footbath each five days.
3. Keep CT room locked during depuration with single key access and minimise entry.
4. Maintain an auditable record of staff entry/exit.
5. Use CT room specific PPE (wellies / lab coat / nitrile gloves).
6. Fill depuration unit with dechlorinated tap water and approx. 15kg of artificial seawater salt and turn pumps and UV 'on'.
7. TMC depuration units utilise a twin 25W splash-proof UV steriliser that allows a double-pass to guarantee an effective dose-rate. Use lamp endcaps to inspect daily and ensure they are both still operating. Replace bulb if necessary.
8. Allow depuration units to stabilise for at least 24hours, checking salinity and adjusting salt / freshwater content to ensure close to full salinity (30-36psu).
9. Introduce racks with oysters stacked umbo facing downwards so they open upwards.
10. Turn-off depuration unit and make-up 1L *Nannochloropsis* algal mix from 10ml concentrate (ZMSystems) and pour over oyster racks each day. Leave for 1 hour before turning the depuration unit 'on' again.

11. Each day, remove all racks from depuration tanks and inspect for moribund or dead oysters (gaping and un-responsive). Remove dead animals from tank and double-bag and place in freezer in JM Yard after wipe-down external surfaces with 1% Virkon™ S. Mark for later disposal (below).
12. Each day, check salinity and temperature and log in the record sheet. Note number of mortalities for each depuration unit and log number of people accessing the room (to be minimised).
13. Keep CT room otherwise locked.
14. Every second day, or if excessive protein froth has developed, (or if any other water parameter like O2, Ammonia, NO_2, NO_3, Ph, suspended solids are not acceptable) move oysters to a tank with freshly-made-up artificial seawater (above).
15. When tanks are drained, rinse the tank with tap water. Clean/brush the tank with a solution of water + soap + bleach. Rinse the tank. Disinfect tank surfaces with a Vircon solution (see details above). Manually (by gravity), fill the pumps and pipework with the same solution (contact time >10 min). Drain the pumps and pipework using the valves. Rinse the tank with tap water. Refill tank with tap water and turn on the pumps to rinse all the system. Drain and repeat this step until there is no foam present (usually 2 times). Fill the tank with dechlorinated tap water and make-up with artificial seawater as above.
16. Clean and disinfect ancillary equipment using procedure in Set-up (point 6). Disposable gloves to clinical waste (Decontamination point 6). Lab coats to cleaning contractor (clinical) using JM Stores at end.

Aquarium Decontamination / Clean-up:

1. At end of the biosecurity procedure (at least 5-7 days) and when oysters are no longer in the CT room or if consignment rejected due to disease status, ensure all tanks are drained-down according to the following procedure:
    - Add Sodium hypochlorite, made-up to 0.1% w/v contact time of 15-20 minutes.
    - Drain to main sewer.
    - Rinse the tank with tap water. Clean/brush the surfaces with a solution of water + soap + bleach. Disinfect the pipework and pumps with the same solution or a solution of bleach as previously described (not always required). Rinse the tanks. Drain the pipework and pumps. Rinse pipework and pumps thoroughly if you filled them with any kind of solution.
    - Using a spray bottle, disinfect tank surfaces with a Virkon solution (1% w/v). Fill the pipework and pumps as previously described. Contact time >10min.
    - Rinse the surfaces of the tank with tap water.
    - Drain the pipework and pumps using the valves available. Refill the tank with tap water and turn on the pumps (leave the pumps running for some time). Drain the tank and repeat the operation (filling the tank, running the pumps and draining the water) until there is no foam present (usually 2 times in total).

2. All surfaces and floors in the CT room to be washed-down with bleach (Sodium hypochlorite), made-up to 0.1175 g/L), contact time of 15-20 minutes following producer-guideline for general disinfection.
3. All surfaced to be rinsed-off with tap water.
4. All surfaces to be rinsed with Virkon™ S, 400mg/40L of water (1% w/v). Contact time approx. 40 min, minimum contact time 10 min. 1 % solution is stable for 5 days.
5. All surfaces to be rinsed-off with tap water.
6. All nitrile gloves and other process waste in CT room bin to be bagged and disposed of to clinical waste contractor (below) after wipe-down of external bag surfaces with 1% Virkon™ S.
7. Wash-down floors from CT room to JM yard (tread space) using same process above.

Biological waste disposal:

Waste oysters (mortalities, rejects or diseased) and epifauna to be transferred from JM yard freezer facility to the Astell Autoclave in J.M./F43. Staff using the autoclave must be trained in its use:

1. Waste oysters (mortalities, rejects or diseased) and epifauna to be transferred to sealed autoclave bio-hazard bags that will not melt during autoclaving. Sleeved within 10L autoclave buckets where appropriate. Wipe-down external surfaces with 1% Virkon™ S (see above). Direct transfer to autoclave or storage in CT room at point of origin before direct transfer. PPE: lab coat and disposable nitrile gloves.
2. Autoclave at 121 degrees Celsius for 15 minutes (whole cycle takes approximately 2 hours. Temp of 121°C is only maintained for stated time of 15min)
3. Transfer autoclaved waste to clinical waste disposal bags.
4. Autoclaving shellfish waste it is then bagged and boxed in snap-lock boxes, stored in CT room, then collected for incineration by the authorised clinical waste removal firm.
5. Obtain waste transfer note from the licenced waste carrier and retain records for 2 years.

Chemical waste disposal:

1. Formalin waste from surface sterilisation procedure (above) to be kept in Formalin store (JM Yard) prior to removal by licenced chemical waste disposal contractor such as TradeBe / Avanti (current).
2. Arrange transfer to chemical waste disposal contractor. Record destination and disposal method.
3. Obtain waste transfer note from the licenced waste carrier and retain records for 2 years.

ANNEX 2 –Biosecurity Measures at the restoration site

This document covers processes related to the deposit and recovery of oysters from the restoration site and they are outlined as:

1. Depositing of *O. edulis* of size classes from 10mm – 75mm (10-89g) for growth and survival trials from a boat.
2. Recovery of ancillary equipment such as cable ties and bags.
3. Removal of samples of oysters (100 animals) to measure growth from previous deployments and ecotoxicology screening. Oysters will be rendered biologically inert by freezing on site prior to transport to laboratory for oven dry and dry weight analysis.
4. Removal of 4L of seawater and dispatch to Marine Scotland Laboratories in Aberdeen for molecular INNS and disease screening.

The majority of oysters will be distributed to the seabed by deposit from the surface with four sub-samples of 100 oysters glued to biodegradable line 15m in length and deployed to the seabed by scientific diving.

Biosecurity at access/launch point prior to travel to site

1. Vehicle wheels and tyres and external surfaces of RIB (if used) and all PPE will be sprayed with Virkon™ S, 400mg/40L of water (1% w/v). 1 % solution (stable for 5 days). Care should be taken to avoid spraying Virkon on exposed brake surfaces and callipers on road vehicles.
2. Submerge ancillary equipment (including boat kit, snips, zip ties, blue recovery bags) in 5L 'dunk-tank' with Virkon™ S, 400mg/40L of water (1% w/v). 1 % solution is stable for 5 days with contact time of approx. 40 min but minimum of 10 min.
3. Spray-down boat, deck vehicle wheels and undercarriage with Virkon™ S, 400mg/40L of water (1% w/v) on departure from access point.
4. For 1-3, PPE: lab coat and disposable nitrile gloves (disposal route detailed below). Dust mask and eye protection when operating sprayer (4).

Biosecurity on deployment/recovery vessel:

1. Designated team members to use: lab coat, nitrile gloves and disinfectable wellies that remain in the designated deck area.
2. Fill 75L 'dunk-tank' with Virkon™ S, 400mg/40L of water (1% w/v). 1 % solution is stable for 5 days.
3. Submerge equipment into Virkon™ 'dunk-tank' for 10 minutes.

4. Transfer associated waste such as cable ties, paper towels and all used disposable PPE to biological waste bag. Clearly label bag. Waste disposal as detailed below.
5. Transfer samples of oysters to a sample bag with nitrile gloves. Clearly label bag.
6. Transfer 8 x 500ml bottles of samples of seawater water to sample bag with nitrile gloves. Clearly label bag.
7. Wipe bottles with 1% Virkon, dry with paper towel and double-bag.
8. After landing, double bag lab coats and exterior surfaces wiped with 1% Virkon. Transferred to designated clinical laundry routes at EGIS Stores in John Muir Building, Heriot-Watt. Place all used gloves and paper towels into biohazard bag.
9. At access / landing point, use tap water in dunk-tank to rinse everything before departure from site.
10. Freeze oyster samples using portable freezer unit at access / landing point. Return to Heriot-Watt University and directly transfer oysters from freezer to 100 degree oven for dry weight analysis and also to -80 degrees storage freezer pending ecotoxicology screening. Use nitrile gloves and lab coat as above. Waste to disposal as detailed below.
11. Refrigerate water samples on site. Exterior surfaces wiped bags with 1% Virkon and post direct to Marine Scotland laboratories in Aberdeen for molecular analysis of INNS and disease agents (Dr Iveta Matejusova).

Biological waste disposal:

1. All bagged and surface sterilised biological waste material will be transported to HW facility in snap-lock boxes and then collected for Incineration by the PHS Group.
2. Obtain waste transfer note from the carrier and retain records for 2 years.

ANNEX 3

Summarised Biosecurity Measures For Project Volunteers.

Restoration Forth: Biosecurity Protocol for Movement of People, Equipment and Vehicles

Why: Prevention of the transfer of disease, pathogens and invasive non-native species (INNS) between water bodies which could have an overall detrimental effect on restoration efforts of both native oysters (*Ostrea edulis*) and seagrass (*Zostera marina* and *Zostera noltii*) in the Firth of Forth.

Who: All members of staff, volunteers, and members of public involved in any Restoration Forth event which takes place on/in/or near seawater (e.g. on the beach). This could be engagement events (protocol for members of public personal equipment provided in section 2.5). Site visits, beach cleans, fieldwork or any other direct interaction with the Firth of Forth estuary.

One staff member (or volunteer if no staff present) should be appointed the *'biosecurity hero'* for the event, this should be decided in advance of event and '*biosecurity hero's* name should be listed in the risk assessment. This person is responsible for implementing the biosecurity protocol below in preparation, during and after the event and is responsible for ensuring all items needed to fulfil protocol are available. This person is also responsible for ensuring that biosecurity is included as part of the risk assessment.

What: Any item which has had contact with seawater or materials from oyster and seagrass habitats (including items which have had possible but not confirmed contact with water). A non-exhaustive list of items is provided in Table 1 below. This provides an example of some of the more common items which may require decontamination but may not include all items. *Biosecurity hero* should use good sense when deciding which items need to be decontaminated.

How: By implementing the below protocols when visiting any site (not just the Firth of Forth) as part of the Restoration Forth project. Protocol should be implemented before arrival and departure of any site visited.

'If in doubt, decontaminate it out!'

1. Preparation of Virkon™ S Solution and PPE Use

Nitrile gloves/rubber gloves, protective glasses, and masks to be worn in preparation and use of Virkon™ S* and all other chemicals mentioned in the below protocol.
Care taken when using this chemical specially in the powder format in high wind conditions.
A 1% solution of Virkon™ S should be used for general disinfection

1. Using measuring cup provided measure a quarter cup of Virkon™ S powder for every litre of tepid tap water used (~8.5g in 1 litre of tap water).
2. Mix with a long mixing device (a long thin item should suffice) to ensure all powder is dissolved. Mix gently to prevent formation of bubbles.
3. Leave solution in open container for a few minutes to allow all powder to activate.

Solution is stable for 5 days so prepare solution as close to intended decontamination event as possible.
IMPORTANT: Do not mix Virkon™ S with any other chemical, including, bleach.

Adaptations to protocol have been provided in section 4 if Virkon™ S is not available

2. Decontamination

Remove any visible species, substrate and collected water prior to chemical decontamination. This material should be retained, double bagged and transferred into the biological waste stream. This includes the removal of macroalgae species, sand and mud and any seawater which might have pooled in any equipment (unless vital to data collection).
Where seawater must be transferred between geographical regions (i.e. for data collection or organism translocation) it should not be allowed to enter a different water body and should be disposed of via mains sewerage system.

2.1 Large, Non-Sensitive Equipment inc. Vehicles

1. Fill pressurised spray bottles with Virkon™ S solution to the ratio provided in section 1 (i.e. if 2L pressurised garden sprayer use half cup provided).
2. Spray items with Virkon™ S solution from pressurised sprayer *NOTE: Spray solution downwind of other people and oneself*.
3. Cover as much ground / water contacting surface area as possible (eg tyres)
4. Leave Virkon™ S on item for minimum of ten minutes (do not leave metal objects in solution for more than ten minutes avoid contact with brake surfaces and calipers).
5. Rinse items thoroughly with tap water.
6. Allow items to dry fully before use on another site.

2.2 Small (< 50cmx50cm), Non-Sensitive Equipment

If dunk tank is available follow below protocol, if unavailable follow protocol in section 2.1.

1. Prepare Virkon™ S solution following section 1 in 75L (or similar) dunk tank.
2. Submerge equipment in Virkon™ S solution for a minimum of ten minutes (do not leave metal objects in solution for more than ten minutes).
3. Remove equipment from solution and rinse thoroughly with tap water,
4. Allow items to dry fully before use on another site,

2.3 Sensitive Equipment

Milton™ sterilising solution for sensitive items, these include items which are in direct contact with face, mouth and hands (see Table 1 below).

1. Prepare solution with 30ml Milton™ (one cap full) to 5L of tap water (0.6% v/v).
2. Place items in Milton™ solution until completely submerged.
3. Leave for minimum of 15 minutes.
4. After 15 minutes remove from solution and leave to dry.

2.4 Fabric Clothing

All clothing which has gotten wet with saltwater should be washed on a high heat with detergent and left to dry fully before being worn to visit another site.

2.5 Personal Equipment for Public Engagement Events

The following refers to members of the public attending engagement events in and around the Firth of Forth where it cannot be established if equipment/clothing has been properly decontaminated before attending.

In Advance of event:

- Encourage attendees to wear waterproof footwear preferably waders or wellington boots.
- Preparation of Virkon™ S footbath solution using protocol in section 1 in a ~ 50L flat plastic storage container with a lockable lid.
- Fill footbath to be at least ankle depth in lockable flat plastic storage container.
- Storage container to be sealed in black plastic refuse bags for transportation to site.
- If event requires use of attendee's personal equipment (i.e. wetsuits, masks, snorkels etc.) please ask attendees to follow the biosecurity infographic (provided shortly) before arriving onsite.
- All staff and volunteers are to make sure their clothing is clean and has been previously decontaminated following protocol in section 2.4 if required.

During Event:

- Ask attendees to step both feet into the footbath before stepping onsite.
- Where non-waterproof footwear is worn ask attendees to place the soles of their shoes (one at a time) into the solution, assisting with their balance where required.
- Any equipment which has not been decontaminated previously should be placed in footbath as well before arrival and departure from site.

After Event:

- Once all attendees have used footbath after site visit lock footbath up with lid and place back in refuse bags for transportation away from site.

- **IMPORTANT**: Do not empty footbath solution on site unless able to empty into a general use drain (i.e. sink, toilet etc).
- Transport to disposal site and follow chemical disposal protocol in section 3.1.
- Where attendees' personal equipment has been used (i.e. wetsuits, boots etc.) please remind them to follow the biosecurity protocol in the infographic (provided shortly) once at home.

2.6 Water Sensitive Items

1. Prepare Virkon™ S solution according to section 1 (dilute as necessary for more sensitive items such as electronics at user's discretion).
2. Using appropriate PPE soak a cloth in solution and wring cloth well to rid of excess solution.
3. Wipe over item gently with cloth wearing protective gloves.
4. Leave to dry.

2.7 Items with High Possibility of Disease/INNS Transfer

This refers to items which used to host/attach to living organisms such as shells and rocks. If removing them from the site in which they were found please first check the item does not still host a living organisms (unless needed for data collection).

1. First scrape off any attached organisms such as sponges, algae, hydroids, etc. anything which is attached which is not part of the original structure. This can be done using a household butter knife, spoon, or oyster shucking knife (CARE MUST BE TAKEN HERE TO AVOID INJURY).
2. Submerge the item in a bleach solution above 500ppm (section 4) for 24 hours in a sealed container.
3. Rinse items in tap water and leave to dry for 48 hours before taking to another site.
4. Capture the scraped biological material into biohazard bag placed in the leak-proof container and dispose appropriately or leave at the site of collection.

3. Disposal

3.1 Disposal of Chemicals

All containers of chemicals should be discarded of in a drainage area that connects to a water treatment plant, e.g. down a toilet or bathroom sink.

If chemical solution has been sprayed onto items on the ground, ensure the area has been rinsed with tap water before departing site.

Where possible decontaminate items in an area were run off will flow into a drain and not into a water course.

3.2 Disposal of Biological Material

All PPE and any waste from decontamination activities must be binned or cleaned before leaving site.

Any disposable PPE which has been used during decontamination must be binned in a local public bin at site location before departure. Ensure that waste is disposed of in a manner that prevents it finding its way back to the marine environment.
it is preferable practice where possible to bag all PPE used in this process and incinerate however this is not deemed appropriate for this protocol due to inaccessibility of appropriate incineration facility

4. Adaptations to Protocol

Where Virkon™ S is not available a solution made from household bleach would suffice. Mix bleach with tap water to ensure 1% sodium hypochlorite. All other aspects of protocol remain the same including use of PPE and amount of time before rinsing.

IMPORTANT: Do not mix bleach and Virkon™ S.

THE BAREST MINIMUM: If for some reason Virkon™ S and bleach are not available the barest minimum cleaning is a thorough rinse in tap water and complete drying of item before it is in contact with water from another site. This is a least preferable option.

Table 1: Non-exhaustive list of equipment which might require decontamination after a site visit/event and prior to visiting another site with reference to relevant decontamination protocol (these provide examples and do not list every item which may need to be decontaminated).

Category	Further Details	Protocol
Diving /Snorkel Equipment – Large	Dry suit, wet suit, BCDs, cylinders,	2.1
Clothing – Non-Waterproof	T-shirts, trousers, jumpers, trainers (non-waterproof), thermals, undersuits, gloves, hats, scarfs, socks, underwear,	2.4
Diving/Snorkel Equipment – Small	Weight belts, SMB/DSMB, wetsuit boots, fins, shot line, net bag, slates and pencils, knife/cutting tools, torch, weights, swim tow/float,	2.2
Diving/Snorkel Equipment – Sensitive	Mask, snorkel, regulators, hood, gloves, go pro housing, bathyscope, dive computer,	2.3
Clothing – Waterproof	Waders, wellington boots, waterproof trousers, waterproof jackets, salopettes, life jackets (not automatically activated)	2.1
Scientific Equipment	Reels, tapes, quadrats, buckets, corers, box corers, seagrass planting tools,	2.2
Vehicles	Boat, boat, trailer, wheels, wheel arches, tow bar, lower bumper, parking blocks,	2.1
Water Sensitive Items	Automatically activated life jackets, electronic items, personal bags, wallets/purses etc, laminated paper items,	2.6
Heavily Contaminated Items	Shells from site, rocks, calcified algae (e.g. maerl), crustacean shells (e.g. crabs),	2.7
Public Member's Personal Equipment	Wellington boots, waders, buckets, hiking boots, trainers, spades,	2.5

Annex 4

Invasive Non-Native Species, distribution and action if present at Donor site

This Annex reviews marine and brackish Invasive Non-Native Species sourced from the published list prepared by UK TAG to inform River Basin Management Planning under the Water Framework Directive. This Annex summarises the UK distribution of the listed species and identifies, based on records accessed via the National Biodiversity Network Atlas, whether a species is known to be present at, or close to either donor or recipient site. It identifies three species, *Didenum vexillum*, *Crepidula fornicata* and *Urosalpinx cinaria* which, if present on the donor site, would prevent its use in the project.

Species and (Impact)	Comment	Reject donor site if present
Slipper limpet, *Crepidula fornicata* (High)	Not know to be present on donor sites. Record from outer Clyde Estuary Commercially damaging to oyster farmers but unlikely to be transferred into donor site from commercial hatchery where it is not present.	Yes
Carpet sea squirt, *Didemnum vexillum* (High)	Listed as colonial sea squirt, Didemnum spp (non-native) in UK-TAG list which we take as meaning all non-native Didemnids. Known to be present in marinas in Clyde Estuary and at sites within Argyll and Bute but not known to be present at donor sites.	Yes
Asian shore crab, *Hemigrapsus sanguineus* (High)	Recorded presence on the UK south and southeast coasts and in south Wales, also coasts of Sweden and Denmark. No known presence at donor sites or risk of project activities creating connectivity between existing locations and donor sites is low.	No, larvae present no risk, post larvae would be readily removed by cleaning and inspection.
Asian shore crab, *Hemigrapsus takanoi* (High)	Recorded presence in the UK in south east around Suffolk, Kent and Essex, also coast of Denmark, No known presence at either donor	No, larvae present no risk, post larvae would be readily removed by cleaning and inspection.

	site or risk of project activities creating connectivity between existing locations and donor sites is low.	
American lobster, *Homarus americanus* (High)	UK records from south and southeast coast and single record from Banff, Moray Firth. No known presence at either donor site, or risk of project activities creating connectivity between existing locations and donor sites is low.	No, larvae present no risk, post larvae would be readily removed by cleaning and inspection.
Leathery sea squirt, *Styela clava* (High)	Records from several sites on west coast of Scotland. Recorded in Craignish and Loch Ryan, within the water bodies containing donor sites.	No - large specimens would be readily visible and removable, small specimens would be removed through cleaning and inspection.
American oyster drill, *Urosalpinx cinerea* (High)	All UK records from south and southeast coasts of England. Commercially damaging to oyster farmers but unlikely to be transferred into donor site from commercial hatchery where it is not present. If present on donor site would be likely to cause significant mortalities of the stock held on site reducing the number of shells available for transfer. No known presence at either donor site, risk of project activities creating connectivity between existing locations and donor sites is low.	Yes
Common cord-grass / Townsend's grass / rice grass, *Spartina anglica* (High)	Present at sites throughout UK including areas of the Firth of Forth (recipient site). Not known to be present at donor sites.	No – plant inhabits the upper foreshore; no risk of vegetation or seed being transferred on donor stock. Risk of seeds or vegetation being transferred on equipment will be eliminated through equipment cleaning protocol.

Japanese kelp, *Undaria pinnatifida* (High)	No records from donor sites, record from 2016 from Port Edgar Marina, within the Firth of Forth within 30 km of receiving site.	No, adults and sporophytes would be removed during cleaning and inspection.
Marine tubeworm, *Ficopomatus enigmaticus* (High)	Listed as a brackish water on UKTAG list but noted as marine by NBN atlas, no records in Scotland, but record from Cumbria (1959).	No, larvae unlikely to present risk, adult individuals construct fragile tubes and would be removed during cleaning and inspection.
Chinese mitten crab, *Eriocheir sinensis* (High)	Listed as a brackish/freshwater species on UKTAG list. No records from donor sites. Record from outer Clyde Estuary.	No, larvae present in brackish water, unlikely to present a risk to full marine donor and recipient sites. Any post larvae or adults would be readily removed through cleaning and inspection.
Gulf wedge clam, *Rangia cuneata* (High)	Only UK records are from Lincolnshire in a slightly brackish canalised river. Established in Netherlands. No records at donor sites, donor and recipient sites full marine.	No, if present would be burrowed within sediment. Sediment not intentionally removed during translocation process. Cleaning of equipment would prevent transfer of sediment on PPE.
Japanese skeleton shrimp, *Caprella mutica* * (Moderate)	Record of presence close to both Donor Sites, no records in the vicinity of the recipient site.	No, would be removed by cleaning and inspection.
Pacific oyster, *Magallana gigas* (Moderate)	Present in containment on Little Loch Broom and Islay donor sites. Not present in Loch Craignish. Recognised risk of existing farmed populations becoming feral and self-sustaining through global climate change. LLB donor site subject to planning condition requiring environmental monitoring to minimise the risk of feral populations developing. Several records of live *M. gigas* at sites throughout Scotland, most of which are likely to be escaped	No, Adoption of policy which prevents the movement of *O. edulis* seed from sites containing *M. gigas* would remove most nursery sites and seed suppliers from rewilding projects. Not practicable. Risk of transfer of *M. gigas* with *O. edulis* is low and further reduced by inspection of oyster seed prior to transfer from donor sites and rejection of all shells which are or are suspected to be *M. gigas*. Minimum size for transplanted

	stock from oyster farming operations. Record from Firth of Forth. Extensive feral populations on European coasts of the North Sea. Although *O. edulis* and *M. gigas* both present on the Little Loch Broom and Islay donor sites they are held in separate containers and stocks are not mixed. Small risk native *O. edulis* seed being contaminated with *M. gigas* seed at hatchery prior to delivery.	oysters is 10mm to ensure that they are of a size that species can be distinguished. Further inspection and screening in holding tanks during shell cleaning.
Red seaweeds, *Bonnemaisonia hamifera* *(Moderate)	This species has records showing presence close to both Craignish and Little Loch Broom donor sites and in the outer Firth of Forth	No – If present on seed oysters will be removed by cleaning and inspection so not transferred. Any intertidal fragments that may contaminate PPE removed by equipment cleaning protocols.
Marine alga*, Gracilaria vermiculophylla* (Moderate)	UK records from south Wales and northern Irish Sea.	No – If present on seed oysters will be removed by cleaning and inspection so not transferred. Any intertidal fragments that may contaminate PPE removed by equipment cleaning protocols.
Marine copepod, *Acartia tonsa* (Low)	No known records at either donor site, historical record from upper Forth estuary	No – If present will be removed by cleaning and inspection
Magellan mussel, *Aulacomya ater* (Low)	No records of this species on NBN Atlas, known report of presence on oil and gas related equipment moored in Cromarty Firth in 1990's	No – If present will be removed by cleaning and inspection
Bamboo worm, *Clymenella torquate* (Low)	Scottish records from upper Loch Linnhe / Loch Eil distant from donor sites and outer Firth of Forth	No – If present on seed oysters will be removed by cleaning and inspection so not transferred.

	within the same water body as the recipient site.	Any polychaete on intertidal weed fragments that may contaminate PPE removed by equipment cleaning protocols.
Marine amphipod, *Corophium sextonae* (Low)	Recorded in Loch Gairloch (1990) and Loch Scotnish (2013) both within 50km of donor sites At Little Loch Broom and Craignish, not recorded at Islay.	No – If present on seed oysters will be removed by cleaning and inspection so not transferred. Any amphipods on intertidal weed fragments that may contaminate PPE removed by equipment cleaning protocols.
Barnacle species, *Austrominius modestus* (Low)	Numerous isolated records from Scottish west coast and widely distributed on the east coast including Firth of Forth	No – Established presence within the same water body as the recipient site. will be removed by cleaning and inspection.
Marine polychaete, *Goniadella gracilis* (Low)	Record in approaches to Loch Broom (2010) c15km from LLB donor site	No – If present on seed oysters will be removed by cleaning and inspection so not transferred. Any polychaete on intertidal weed fragments that may contaminate PPE removed by equipment cleaning protocols.
Marine hydrozoan, *Gonionemus vertens* (Low)	No records of live specimens on NBN Atlas	No – If present on seed oysters will be removed by cleaning and inspection so not transferred. Any hydrozoan on intertidal weed fragments that may contaminate PPE removed by equipment cleaning protocols.
Marine polychaete, *Marenzelleria viridis* (Low)	Recorded in Beauly Firth and Firth of Tay on the East Coasts. Adults and juveniles live as infauna in soft mud.	No, if present would be burrowed within sediment. Sediment not intentionally removed during translocation process. Cleaning of equipment would prevent transfer of sediment on PPE.

American hard-shell clam, *Mercenaria mercenaria* (Low)	Single record from Scottish west coast at Kentra Bay, Ardnamurchan, distant from donor sites	No, if present would be burrowed within sediment. Sediment not intentionally removed during translocation process. Cleaning of equipment would prevent transfer of sediment on PPE.
American piddock *Petricola pholadiformis* (Low)	NBN Atlas shows no records in Scotland, present in England and Wales. Bores in hard clay, limestone and solid mud as well as in pieces of peat and wood.	No – If present on seed oysters will be removed by cleaning and inspection so not transferred.
Zuiderzee or dwarf crab *Rhithropanopeus harrisii* (Low)	No records of live specimens on NBN Atlas. Museum records from SE England (1890)	No
Manilla Clam *Ruditapes philippinarum* (Low)	Single record in Scotland at Carbost, Loch Harport, Isle of Skye (2014)	No - if present would be burrowed within sediment. Sediment not intentionally removed during translocation process. Cleaning of equipment would prevent transfer of sediment on PPE.
New Zealand flat oyster *Tiostrea lutaria (Ostrea chiliensis)* (Low)	UK records confined to Menai Strait, North Wales. Small self-sustaining population resulting from deliberate introduction.	No – very low risk of being present on site due to lack of vectors.
Red seaweeds *Agardhiella subulate* (Low)	Single NBN Atlas record from Swanage, Dorset (2021)	No – If present on seed oysters will be removed by cleaning and inspection so not transferred. Any intertidal fragments that may contaminate PPE removed by equipment cleaning protocols.
Captain pike's weed *Pikea californica* (Low)	UK records confined to SW England and Scilly Isles	No – If present on seed oysters will be removed by cleaning and inspection so not transferred. Any intertidal fragments that may contaminate PPE removed by equipment cleaning protocols.

Japanese weed *Sargassum muticum* (Low)	Numerous records around UK coastline but not within donor sites.	No – If present on seed oysters will be removed by cleaning and inspection so not transferred. Any intertidal fragments that may contaminate PPE removed by equipment cleaning protocols.
Tapegrass *Vallisneria spiralis* (Low)	Shows as a Marine Species on UKTAG list but listed as FW species present at inland waterways on NBN Atlas, confined to central England	No – If present on seed oysters will be removed by cleaning and inspection so not transferred. Any intertidal fragments that may contaminate PPE removed by equipment cleaning protocols.
Orange Striped Sea Anemone, *Diadumene lineata*	Recorded from 1980s MNCR review from several sites in Loch Sween approx. 15km south of the Cragnish donor site.	No – If present on seed oysters will be removed by cleaning and inspection so not transferred.
Marine hydrazoan *Tricellaria inopinata*	Record from Loch Finnart, Firth of Clyde, 2017	No – If present on seed oysters will be removed by cleaning and inspection so not transferred. Any hydrozoan on intertidal weed fragments that may contaminate PPE removed by equipment cleaning protocols.
Red Algae, *Dasysiphonia japonica*	Record of presence in Little Loch Broom. Isolated records on east coast, eg Cromarty Harbour but distant from recipient sites.	No – If present on seed oysters will be removed by cleaning and inspection so not transferred. Any intertidal fragments that may contaminate PPE removed by equipment cleaning protocols.

DOCUMENT CHANGE LOG

Version	Amendment	Author(s)	Date
Draft	Document created by adapting document approved for DEEP project Dornoch Firth. References to locations within Dornoch Firth and DEEP project removed. Annex relating to source and biosecurity of shell cultch removed (not required in the current project)	Jim Bromham (JB) and Bill Sanderson (WGS)	15/03/2023
Draft	Document reviewed and minor amendments. Distributed as draft with suggested refinements to Cass Bromley CB (NatureScot) and Naomi Arnold (NA) (WWF)	WGS	20/03/2023
V1	Site map added, Annex 3 added incorporating biosecurity summary for project volunteers authored by Emmy Cooper-Young (ECY), page footer amended, change log added. Document distributed to NA, CB, Licensing@nature.scot, and Bernadette Moloughney (Marine Scotland)	JB and ECY	23/03/2023
V1.1	Collated Comments from Scottish Ministers and NatureScot	Scottish Ministers and NatureScot	03/04/2023
V1.2	Document amended to respond to comments from Scottish Ministers and NatureScot	JB and WGS	24/04/2023
V1.3	Document with further comments from Scottish Ministers and NatureScot	Scottish Ministers and NatureScot	10/05/2023
V1.4	Document amended to respond to further comments from Scottish Ministers and NatureScot. Revised List of INNS species with supporting comments removed from body of document and added as Annex 4. Document approved by NS and MD in support of application for translocation licence for movement of native oysters into the Firth of Forth.	JB and WGS	29/05/2023
V2.0	This version. Full review and amendment prior to submission of the document in support of Marine Licence Applications. Addition of new donor sites at Islay and wild fishery at Loch Ryan. Review of Annex 4 to assess presence of INNS at all donor sites. Rewrite of cleaning protocols to account for cleaning on arrival at HWU.	JB and WGS	18/12/2023 Finalised 23/01/24