Native oyster (*Ostrea edulis*) Large Scale Habitat Restoration

Dornoch Firth

Biosecurity Plan - Reviewed August 2024

Background

The Dornoch Environmental Enhancement Project (DEEP), a collaboration between Glenmorangie Distillery, Heriot-Watt University and The Marine Conservation Society has been undertaking growth and survivability trial deployments of native oysters (*Ostrea edulis*) on to seabed plots in the Dornoch Firth since late 2016. The plots incorporate various experimental treatment including the introduction of imported shell cultch, and native oysters some of which are deployed to the cultch whilst others are enclosed within net bags or basket enclosures.

Two initial plots granted a full suite of permissions in 2016 were relocated onto East and West plots with new permissions during winter 2018. Whilst the intention was that the experiments and licences/permissions ran until September 2020 the onset of national movement and work restrictions due to the COVID-19 pandemic led to the project seeking an extension to the timescale of these licences to September 2022.

During Autumn 2022 the project sought further extension in the cessation date of the permissions and the addition of a third site closer to Glenmorangie Distillery. As of August 2023 Planning Permissions, Marine Licences and Crown Estate Leases have been granted for all three plots.

The DEEP project partnership now proposes the implementation of a large-scale habitat enhancement project which seeks to restore and stock c40Ha of Native oyster habitat on defined plots within the Dornoch Firth.

Biosecurity Plans have previously been agreed with regulatory bodies for the two initial experimental plots. These documents were reviewed and an enhanced biosecurity plan was approved for the third experimental plot in January 2023. On the advice of NatureScot and Marine Scotland sections of the document were rewritten during preparation of a biosecurity plan to support applications for conservation translocation licences on a related projects in the Firth of Forth. (June 2023, reviewed April 2024). The present document is a further evolution of the Dornoch Firth Biosecurity plan incorporating feedback from the Forth project.

The Biosecurity Plan references additional documents.

Annex 1 – Biosecurity Measures at the Heriot-Watt University Aquarium. Previous versions of Annex 1 have been considered by Marine Directorate of Scottish Government's Fish Health Inspectorate (FHI), 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 FHI in its role in registering the laboratory as a Shellfish Holding Facility. This section of the document was previously approved by Marine Scotland Science / Fish Health Inspectorate in 2018 but was subject to substantive amendments on advice of NatureScot and Marine Scotland in June 2023, and minor amendments following official inspection of holding facility in February 2024.

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 (now Marine Directorate) to accompany the Translocation Licence for Oyster deployment in the Firth of Forth as part of Restoration 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 – DEEP project Shell Cultch Biosecurity Measures. Operating in accordance with this document provides for the initial inspection of transport weathering and handling of shell cultch and the process in which it is transferred from being a Category 3 Animal By-Product to an inert product that can be deposited on the seabed. The document was required by Marine Scotland prior to its approval of an application to deposit shell cultch into the Loch Ryan Oyster Fishery in Dumfries and Galloway. This deposit was also part of the DEEP project and the same shell weathering transport and audit process is utilised throughout the project.

Annex 5 – 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, it identifies possible vectors or means of translocation and 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 records show a historical presence on a donor site, would prevent its use in the project unless further investigation confirms it is not present at the time of the translocation.

Biosecurity Measures Statement

Over the course of the large scale enhancement project four Million native oysters (*Ostrea edulis*) will be deployed across multiple receiving plots in the Dornoch Firth. The experimental population will incorporate both part grown and adult oysters distributed to the seabed as individuals, or as the project matures may incorporate the introduction of pre-seeded cultch material direct from commercial hatchery. Stock will be sourced from existing oyster farms holding native oysters on site at:

Little Loch Broom (Charron Ltd (trading as Maorach Beag) SS0877)
Sea Wilding project at Loch Craignish (SS0935),
Islay Oysters, Loch Gruinart, Islay (SS0452).

In addition, wild native oysters will be sourced from the managed wild fishery in Loch Ryan.

Oysters will be randomly deposited on to enhanced sea bed habitat consisting of weathered and partly crushed scallop shell and crushed rock sourced from local quarries. The scallop shell will be subject to biosecurity measures as set out in Annex 4 below, the crushed rock is biologically inert and is not subject to any additional biosecurity measures.

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 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. When samples cannot be identified in field they will be sent to Dr Dan Harries for identification and also sent to Marine Directorate 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 with screening comments. 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 workboat for transfer to the translocation plots in the Dornoch Firth. 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 5-10 m below. Subsequent monitoring involves the use of divers and associated equipment. Biosecurity measures related to movement of equipment into and out of the Dornoch Firth are referred to in Annex 2.

Post Deployment Monitoring

1. During site visits to service the recipient plots, annual inspections, during summer months 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 any unidentified potential INNS to Marine Directorate for identification by DNA sequencing.
   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 Directorate of the Scottish Government.
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. (Fresh solution to be made up prior to use)
    - 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°C 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.

In the event of increased and unexplained mortality:

1. All onward movement of oysters will be halted

2. A sample of oysters will be sent to the diagnostics laboratory (presently Scottish Government Diagnostics Laboratory in Aberdeen) for disease screening.

3. The Government Diagnostics Laboratory has a statutory duty to subsequently notify Scottish ministers in the event of a notifiable disease being identified.

4. In the event of a catastrophic mortality event (50% +), the whole consignment in the aquarium will also be destroyed following the clinical waste disposal method identified in the biosecurity protocols.

5. Onward movement from the APB site will only resume if increased unexplained mortality stops and if diagnostics laboratory samples are negative for notifiable diseases.

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 (subtidal site) or by hand (intertidal site)
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.

On subtidal sites 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 a leak proof container with nitrile gloves. Clearly label container.
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 Directorate 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.

DEEP: 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 native oysters (*Ostrea edulis*) in the Dornoch Firth.

Who: All members of staff, volunteers, and members of public involved in any DEEP event which takes place on/in/or near seawater (e.g. on the beach). These 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 Dornoch Firth 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 Dornoch Firth) as part of the DEEP 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 Dornoch Firth 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.

ANNEX 3 - Shell Cultch Biosecurity Measures

Procedure for shell re-use in DEEP
Oysters need shell to settle and grow on. Shells from shellfish processors are classified under Animal By-Product (ABP) Regulations as Category 3. Traces of residual shellfish biology are removed through outside 'weathering' (e.g. Billion Oyster Project), which is enhanced when shells are 'turned-over' during this time. Twelve months of weathering is typically used within the shellfish industry as a suitable procedure and has been adopted by DEEP to 'do no harm' during restoration work.

The following Steps are used to validate supply:

Step 1: Shellfish producer to ensure that shell waste as clean as practicable prior to transport from processing plant to weathering site.

Step 2: Transport from processing plant to weathering site is to be undertaken by licenced waste carrier with movement documented by waste transfer note. At this stage the shells are still considered as Category 3 Animal By-Product and should be recorded on the transfer note with the appropriate European Waste Code as set out in SEPA document WM3. It is suggested that the most appropriate code would be 02 02 99 for material coming directly from shellfish processing facility.

https://www.sepa.org.uk/media/162771/waste-classification-technical-guidance-wm3.pdf

Step 3: Ensure shell waste is weathered for twelve months minimum: *Promote protocol of weathering site standards (Appendix 1) and consult 'stock weathering record' (Appendix 2). Ensure use of 'shell weathering sites' working to agreed standards and ensure weathering sites have mechanisms in place to ensure that shell loads are deposited into the correct weathering pile.*

Step 4: PROCEDURE FOR SHELL RE-USE AND CHAIN OF CUSTODY *(see Appendix 3*)

a) **Shell supply inspected/ sampled prior to consignment movement off-site:** *Take representative samples prior to movement from the weathering site, to validate that there is no residual biology and no live external epibiota. Record representative documentary evidence. NOTE: Movement from the weathering site to high water mark prior to loading to seagoing vessel is also required to be carried*

out by licenced waste carrier with movement documented by waste transfer note. At this time it is suggested that a satisfactory waste code would be 19 02 99

b) Shell supply inspected/ sampled post movement off-site but prior to vessel loading:*prior to deployment / loading onto vessel, take representative samples of the consignment, and scrutinise to establish no residual biology or epifauna. Ensure that the consignment has not become contaminated during off-site transportation, with other sources[1].*

NOTE: If certified as having no residual biology or epifauna the shells are no longer considered to be waste at this point. Any shell found to be contaminated as described above should be remain as waste and returned to the weathering site, any shell material considered as end of waste but not subsequently deposited should be reconsigned as waste and retained at storage area at high water mark or returned to weathering area as appropriate.

c) Vigilance during *deployment* *During deployment to the deposition site, remain vigilant that the consignment has not become contaminated[1] and periodically inspect.*

This procedure will periodically be reviewed to ensure best practice.

[1] *It has been HWU experience that off-site transport of clean shell from the processor can sometime become contaminated with more problematic material buried in the transport sacks.*

APPENDIX 1: Protocol/ Agreed Standards for Shell Weathering Sites
Guidelines for the separation of shells within the 'weathering site'.

- A 'shell weathering site' is used for the storage and weathering of different bivalve shells discarded from shellfish processing industries.
- Figure 1 represents a simple six plot area (Plots A-F). However, material deposited into each plot should be bivalve group specific. Therefore, more plots will be needed if a site is processing multiple types of bivalve shells (e.g. mussel, oyster, clam, scallop).
- Plots should be spatially and physically separated to avoid cross contamination.
- Plot A will be filled with shells of a specific type (e.g. scallops) for three months; at which point, the plot is closed for further use and weathering commences. Plot B is then used for the following three months. This pattern continues until plots A-F are filled.
- At the fifteen-month stage, Plot A will be ready for consignment, subject to appropriate sampling and testing.
- Each plot should be allowed to rest as fallow for three months before further use.
- At a given 'weathering site' the number of plots may be reduced, but this will increase the storage time (e.g. deposition into Plot A for six months). Alternatively, the number of plots could be increased, and this would decrease the storage time (e.g. deposition into Plot A for one month only)

Figure 1: Plan of a 'shell weathering area': One shell type (e.g. Scallops)

Plot A Deposit Scallop shells in this plot during (Year 1) Months 1-3 *After fifteen months – consign to use and clean/leave fallow for three months* *Re-use Plot A (Year 2) Months 19-21*	**Plot B** Deposit Scallop shells in this plot during (Year 1) Months 4-6 *After fifteen months – consign to use and clean/leave fallow for three months* *Re-use Plot B (Year 2) Months 22-24*
Plot C Deposit Scallop shells in this plot during (Year 1) Months 7-9 *After fifteen months – consign to use and clean/leave fallow for three months* *Re-use Plot C (Year 3) Months 1-3*	**Plot D** Deposit Scallop shells in this plot during (Year 1) Months 10-12 *After fifteen months – consign to use and clean/leave fallow for three months* *Re-use Plot D (Year 3) Months 4-6*
Plot E Deposit Scallop shells in this plot during (Year 2) Months 13-15 *After fifteen months – consign to use and clean/leave fallow for three months* *Re-use Plot E (Year 3) Months 7-9*	**Plot F** Deposit Scallop shells in this plot during (Year 2) Months 16-18 *After fifteen months – consign to use and clean/leave fallow for three months* *Re-use Plot F (Year 3) Months 10-12*

APPENDIX 2: Shell Supplier: Stock Weathering Record

Name Business (*holding weathering shell stock***)……**

Site Name (*place where weathering stock located***)...……..**

Site ID/ Licence Number……..

1. Plot Record

Plot A: (Description: Shell type)
……
Date Plot Opened*: e.g. Year 1: Months 1-3*

Date Delivery	Volume shell deposited	Origin of shell material	Authorised Signature

2. Plot A Maintenance Record

Date Turned	Observations/ Comments	Authorised Signature

APPENDIX 3: DOCUMENTATION OF PROCEDURE FOR SHELL RE-USE AND CHAIN OF CUSTODY

When completed, form to be emailed to the Duty Inspector at MS.fishhealth@gov.scot at least two weeks prior to the proposed final movement.
When complete, copy form to Duty Inspector at MS.fishhealth@gov.scot for records

1. DETAILS OF MOVEMENT TO HIGH WATER MARK

Start date:		Completion date:	
Material being moved:		Details of movement to High Water:	
Reason for movement:			
Consignment location and custodian:			
Consignment transfer to 'at sea'[1] custodian:			
Origin of shell material:		Delivery date:	

2. DESTINATION OF SHELL MATERIAL

Site name:		Site / license ID number:	
Business name:			

3. INSPECTION PROCEDURE DETAILS AT SUPPLY POINT

(Please use additional page if necessary)

3.1. Documenting evidence of weathering-period Provide evidence that shell material has been weathered for at least 1 year. E.g. *Plot records as highlighted in Appendix 2*
3.2. Inspection of weathered material prior to movement to high water mark Inspect consignment and take representative samples through-out to validate that there is: • No residual biology, • No closed-shell material with unknown biotic content and • No live external epibiota. Secure representative documentary evidence. If contamination or biological residue is identified – Complete Part 4.

[1] The high-water mark

i. CONFIRMATION OF INSPECTION: NAME: ORGANISATION: DATE:

4. Details of contamination or identification of biological residue:
Details of ‘action taken’ based on Part 4 (above).

APPENDIX 4: DOCUMENTATION OF PROCEDURE FOR SHELL RE-USE AND CHAIN OF CUSTODY - MOVEMENT FROM HIGH WATER MARK TO DEPOSIT

1. Prior to deployment / loading to vessel: Inspect consignment and take representative samples through-out to validate that there is: • No residual biology, • No closed-shell material with unknown biotic content and • No live external epibiota. Secure representative documentary evidence. If contamination or biological residue is identified – Complete Part 3 CONFIRMATION OF INSPECTION: NAME: ORGANISATION: DATE:
2. During deployment: remain vigilant that the consignment is not becoming contaminatedPeriodic inspection to validate that there is: • No residual biology, • No closed-shell material with unknown biotic content and • No live external epibiota. Secure representative documentary evidence. If contamination or biological residue is identified – Complete Part 3 CONFIRMATION OF INSPECTION: NAME: ORGANISATION: DATE:
3. Details of contamination or identification of biological residue:
Details of 'action taken' based on Part 3 (above).

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 (Working Paper V8.0 29/01/2021) 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, *Didemnum vexillum*, *Crepidula fornicata* and *Urosalpinx cinaria* which, if present on the donor site, would prevent its use in the project unless further investigations confirm that the donor stock is free of these species. Where a species has been renamed since the publication of the TAG document the revised name is used in the table.

Where the presence of an INNS is identified in a donor water body the table cell is coloured RED to indicate that increased vigilance for this species is required during the various stages of the cleaning and inspection process.

Cells are coloured ORANGE where an INNS is present in a nearby water body where there is an identified vector. For example Loch Ryan may be susceptible to transfer of INNS via shipping for those species present in Northern Ireland.

Species and (Impact)	Comment	Reject donor site if present	Loch Ryan	Islay	LL Broom	Craignish
Slipper limpet, *Crepidula fornicata* (High)	Commercially damaging to oyster farmers but unlikely to be transferred into donor site from commercial hatchery where it is not present.	Yes – if further investigations confirm recent presence	NBN Atlas records 2013 presence at west of Loch Ryan. Record investigated and confirmed to be a data error. Location extensively surveyed with no further identification. Not considered to be present at donor site.	No known presence at donor site		

Species and (Impact)	Comment	Reject donor site if present	Loch Ryan	Islay	LL Broom	Craignish
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 – if further investigation confirm recent presence	Suspicious potential Didemnid investigated by eDNA screening March 2024 D. vexillum confirmed not present in the sample analysed.	No known presence at donor site		
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	No known presence at donor site Larvae present no risk, Post larvae would be readily removed by cleaning and inspection			

Species and (Impact)	Comment	Reject donor site if present	Loch Ryan	Islay	LL Broom	Craignish
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 site or risk of project activities creating connectivity between existing locations and donor sites is low.	No	No known presence at donor site Larvae present no risk, Post larvae would be readily removed by cleaning and inspection			
American lobster, *Homarus americanus* (High)	UK records from south and southeast coast and single record from Banff, Moray Firth. or	No	No known presence at donor site Larvae present no risk, Post larvae would be readily removed by cleaning and inspection			

Species and (Impact)	Comment	Reject donor site if present	Loch Ryan	Islay	LL Broom	Craignish
Leathery sea squirt, *Styela clava* (High)	Records from several sites on west coast of Scotland. Recorded in Loch Ryan, within the water bodies containing donor sites.	No	Known presence in the same water body as donor site. Increased vigilance during cleaning to ensure rejection of any shells carrying S. clava	No known presence at donor site		
			Larvae present no risk, Post larvae (small specimens) would be readily removed by cleaning and inspection, large specimens would be readily visible and result in rejection on if individual donor animals.			
American oyster drill, *Urosalpinx cinerea* (High)	All UK records from south and southeast coasts of England.	Yes – if further investigation confirm recent presence	No known presence at donor site 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 animals available for transfer.			

Species and (Impact)	Comment	Reject donor site if present	Loch Ryan	Islay	LL Broom	Craignish
Common cord-grass / Townsend's grass / rice grass, *Spartina anglica* (High)	Present at sites throughout UK including areas of the Cromarty Firth approximately 20km sw of the recipient site. Not known to be present at donor sites.	No	No known presence at donor site 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 on NBN Atlas.	No	No presence recorded in NBN Atlas but present in Northern Ireland with marine shipping traffic a potential vector for introduction. NatureScot report presence at Stranraer Marina in the same water body as the donor site.			

			Adults and sporophytes would be removed during cleaning and inspection any microscopic gametophytes would be removed by formalin treatments.

Species and (Impact)	Comment	Reject donor site if present	Loch Ryan	Islay	LL Broom	Craignish
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	No known presence at donor site 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	No known presence at donor site. 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	No known presence at donor site. 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, Recorded presence in the Cromarty Firth approximately 20km sw of the recipient site.	No	No known presence at Donor Site.		Record within 5km of donor site.	
			If present on donor stock would be readily removed by cleaning and inspection.			

<table>
<tr><th>Species and (Impact)</th><th>Comment</th><th>Reject donor site if present</th><th>Loch Ryan</th><th>Islay</th><th>LL Broom</th><th>Craignish</th></tr>
<tr><td rowspan="3">Pacific oyster, Magallana gigas (Moderate)</td><td rowspan="3">Recognised risk of existing farmed populations becoming feral and self-sustaining through global climate change.

Several records of live M. gigas at sites throughout Scotland, most of which are likely to be escaped stock from oyster farming operations. Extensive feral populations on European coasts of the North Sea.

Small risk native O. edulis seed being contaminated with M. gigas seed at hatchery prior to delivery.</td><td rowspan="3"></td><td rowspan="2">One live wild specimen located by HWU survey but not at donor site.</td><td colspan="3">Present on site in containment (commercial oyster farm) but in separate containers and not mixed with O. edulis

Low risk of O. edulis seed being contaminated with M. gigas seed at hatchery prior to delivery to donor site.</td></tr>
<tr><td></td><td>Site subject to planning condition requiring monitoring to minimise the risk of feral populations developing</td><td></td></tr>
<tr><td colspan="4">Low risk of transfer with O. edulis further reduced by inspection prior to transfer from donor site and rejection of all suspected to be M. gigas. Min. size for transplanted oysters 10mm to ensure that species can be distinguished. Further inspection and screening in holding tanks during cleaning.</td></tr>
</table>

Species and (Impact)	Comment	Reject donor site if present	Loch Ryan	Islay	LL Broom	Craignish
Red seaweeds, *Bonnemaisonia hamifera* *(Moderate)	Present at Donar Sites and records from harbours within Moray Firth	No	Present within the same water body as the donor site	No known presence on site.	Present within the same water body as the donor site	
			If present on donor stock 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	No Known Presence on site but present in Northern Ireland with marine shipping traffic a potential vector for introduction.	No Known Presence on site If present on donor stock will be removed by cleaning and inspection		
			If present on donor stock will be removed by cleaning and inspection so not transferred. Any intertidal fragments that may contaminate PPE removed by equipment cleaning protocols.			

Species and (Impact)	Comment	Reject donor site if present	Loch Ryan	Islay	LL Broom	Craignish
Marine copepod, *Acartia tonsa* (Low)	No known records at either donor site.	No	Historical (1955) record from northern Solway Firth	No Known Presence on site		
			If present on donor stock 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	No Known Presence on site If present on donor stock will be removed by cleaning and inspection			
Bamboo worm, *Clymenella torquate* (Low)	Scottish records from upper Loch Linnhe / Loch Eil distant from donor sites.	No	No Known Presence on site 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 amphipod, *Corophium sextonae* (Low)	Recorded in Loch Gairloch (1990) and Loch Scotnish (2013) and Northern Ireland within 50km of donor sites	No	Known presence within 50km of donor site	No Known Presence on Site	Known presence within 50km of donor site	
			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.			

Species and (Impact)	Comment	Reject donor site if present	Loch Ryan	Islay	LL Broom	Craignish
Barnacle species, *Austrominius modestus* (Low)	Numerous isolated records from Scottish west coast and widely distributed on the east coast including Cromarty Firth approximately 20km sw of the recipient site.	No Established presence within the same water body as the recipient site.	Known record from the same waterbody as the donor site	No known presence at the donor site		
			If present on seed oysters will be removed by cleaning and inspection so not transferred.			
Marine polychaete, *Goniadella gracilis* (Low)	Record in approaches to Loch Ewe c15km from LLB donor site	No	No known presence at the donor site 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	No known presence at the donor site 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.			

Species and (Impact)	Comment	Reject donor site if present	Loch Ryan	Islay	LL Broom	Craignish
Marine polychaete, *Marenzelleria viridis* (Low)	Recorded in Beauly Firth (c.35km from recipient site) and Firth of Tay on the East Coasts. Adults and juveniles live as infauna in soft mud.	No	No known presence on donor site but present in Northern Ireland with marine shipping traffic a potential vector for introduction.	No known presence at the donor site		
			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	No known presence at donor site. 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	No known presence at donor site. If present would be burrowed within hard clay sediment, limestone and solid mud. Sediment not intentionally removed during translocation process. Cleaning of equipment would prevent transfer of sediment on PPE.			
Zuiderzee or dwarf crab *Rhithropanopeus harrisii* (Low)	No records of live specimens on NBN Atlas. Museum records from SE England (1890)	No	No known live specimens in UK			

Species and (Impact)	Comment	Reject donor site if present	Loch Ryan	Islay	LL Broom	Craignish
Manilla Clam *Ruditapes philippinarum* (Low)	Single record in Scotland at Carbost, Loch Harport, Isle of Skye (2014)	No	No known presence at the donor site 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	No known presence on donor site. 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	No known presence on donor site. 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	No known presence on donor site. 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			

Species and (Impact)	Comment	Reject donor site if present	Loch Ryan	Islay	LL Broom	Craignish
Japanese weed *Sargassum muticum* (Low)	Numerous records around UK coastline particularly the west coast, no known presence within Dornoch Firth	No	Known presence in Loch Ryan	No known presence at donor site.		
			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	No known presence at donor site. 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	No known presence at donor site. 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.			

Species and (Impact)	Comment	Reject donor site if present	Loch Ryan	Islay	LL Broom	Craignish
Marine hydrazoan *Tricellaria inopinata*	Record from Loch Finnart, Firth of Clyde, 2017	No	No known presence on site but present in Northern Ireland with marine shipping traffic a potential vector for introduction.	No known presence on donor site.		
			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.			

Species and (Impact)	Comment	Reject donor site if present	Loch Ryan	Islay	LL Broom	Craignish
Red Algae, *Dasysiphonia japonica*	Record of presence in Little Loch Broom. Isolated records on east coast, eg Cromarty Harbour c 20km from recipient site.	No	No known Presence on site but present in Northern Ireland with marine shipping traffic a potential vector for introduction.	No known presence on donor site.	Known record from this donor site	No known presence on donor site.
			If present on donor oysters will be removed by cleaning and inspection so not transferred. Any intertidal fragments that may contaminate PPE removed by equipment cleaning protocols.			

Species and (Impact)	Comment	Reject donor site if present	Loch Ryan	Islay	LL Broom	Craignish
Corella Eumoyota	This species is not present on UK-TAG list. It is not reported as present at donor sites in the NBN atlas	No	Presence in Loch Ryan reported by NatureScot, but not shown in NBN Atlas. Present in Northern Ireland with marine shipping traffic a potential vector for introduction.	No known presence on donor site.		

DOCUMENT CHANGE LOG

Version	Amendment	Author(s)	Date
Original	Document Approved by Highland Council with input from SNH during November 2018. Prepared in response to planning condition for original small scale trial sites and incorporating cultch biosecurity measures from related project in Loch Ryan.	Jim Bromham (JB) and Bill Sanderson (WGS)	05/10/20218
Revision1	Extensive revision to include analysis of INNS present. Agreed by Highland Council and NatureScot. Revisions required to comply with Planning Condition for new experimental plot in the Dornoch Firth.	Jim Bromham (JB) and Bill Sanderson (WGS)	November 2022
Revision2	Document created by adapting document approved for related restoration translocation projects in the Firth of Forth. (Restoration Forth)	Jim Bromham (JB) and Bill Sanderson (WGS)	Aug 2023
Revision3	This Version Document Reviewed and updated to include changes to Annexes within Restoration Forth Documents.	JB WGS	16/08/2024 09/09/2024