2.3 Strategies to reduce norovirus (NoV) contamination from bivalve molluscs

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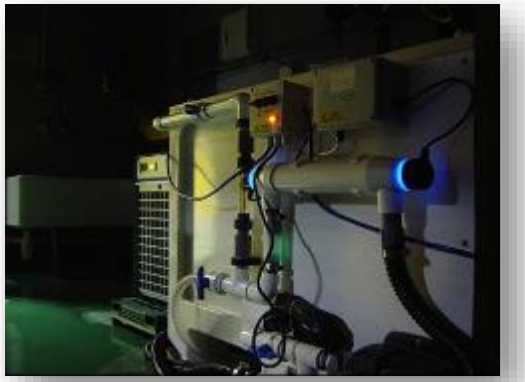

Stakeholder Event 15.04.2021

Nutritious, safe, and sustainable seafood for consumers

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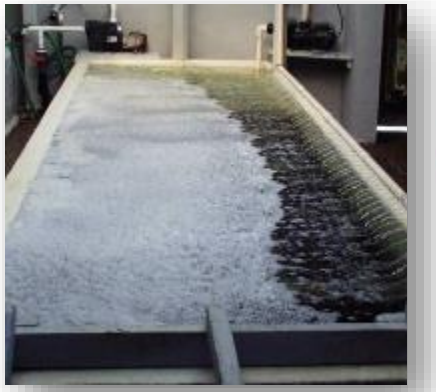
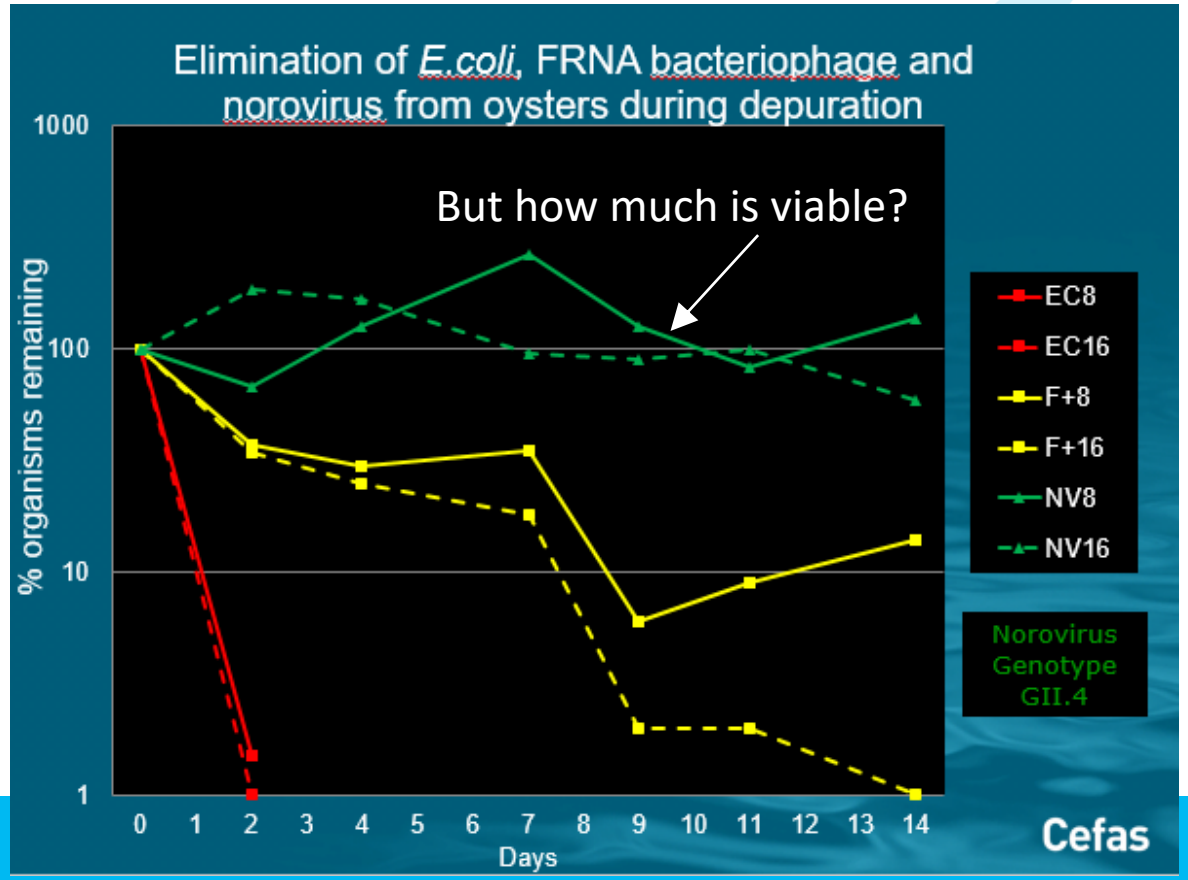
Introduction



- Depuration (purification)
  - Treatment for class B shellfish under EU legislation
  - Purified in clean seawater tanks
  - For microbiological contaminants

  - Generally effective for bacteria
  - **Does not work so well for viruses e.g. norovirus (NoV) - (EFSA, 2012)**
  - No NoV standard in EU legislation (yet)
  - Depurated shellfish free from *E. coli* can still cause NoV illness outbreaks
    - Diarrhoea and vomiting
    - Lost sales

Findings from previous studies:



Aim of study

- Develop enhanced depuration based on NoV **removal** and **infectivity**
  - Needed by the shellfish industry, particularly in winter (peak NoV season)

Challenges

- Current routine NoV test methods (i.e. PCR) cannot indicate 'live' or infectious virus (risk to consumer)
- Surrogate virus F-specific RNA bacteriophage (F-RNA phage) can be used to indicate infective NoV risk(?)
- Advantage of using F-RNA phage is that it's easy to 'grow' and quantify

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Methods

- Pacific oysters (*Crassostrea gigas*)
- Environmentally contaminated
- Factors tested during depuration:
  - Increased water temperature 18°C vs 8°C
  - Feeding - microalgae and shellfish diet
  - Light vs dark regimes
  - Disturbance vs non-disturbance
  - Continuous flow-through vs recirculated unfiltered seawater

Oysters tested for:

- NoV (PCR) – checking for genogroups I and II (most associated with human illness)
- F-RNA phage (culture), F-RNA phage (PCR)
- *E. coli* (statutory indicator)

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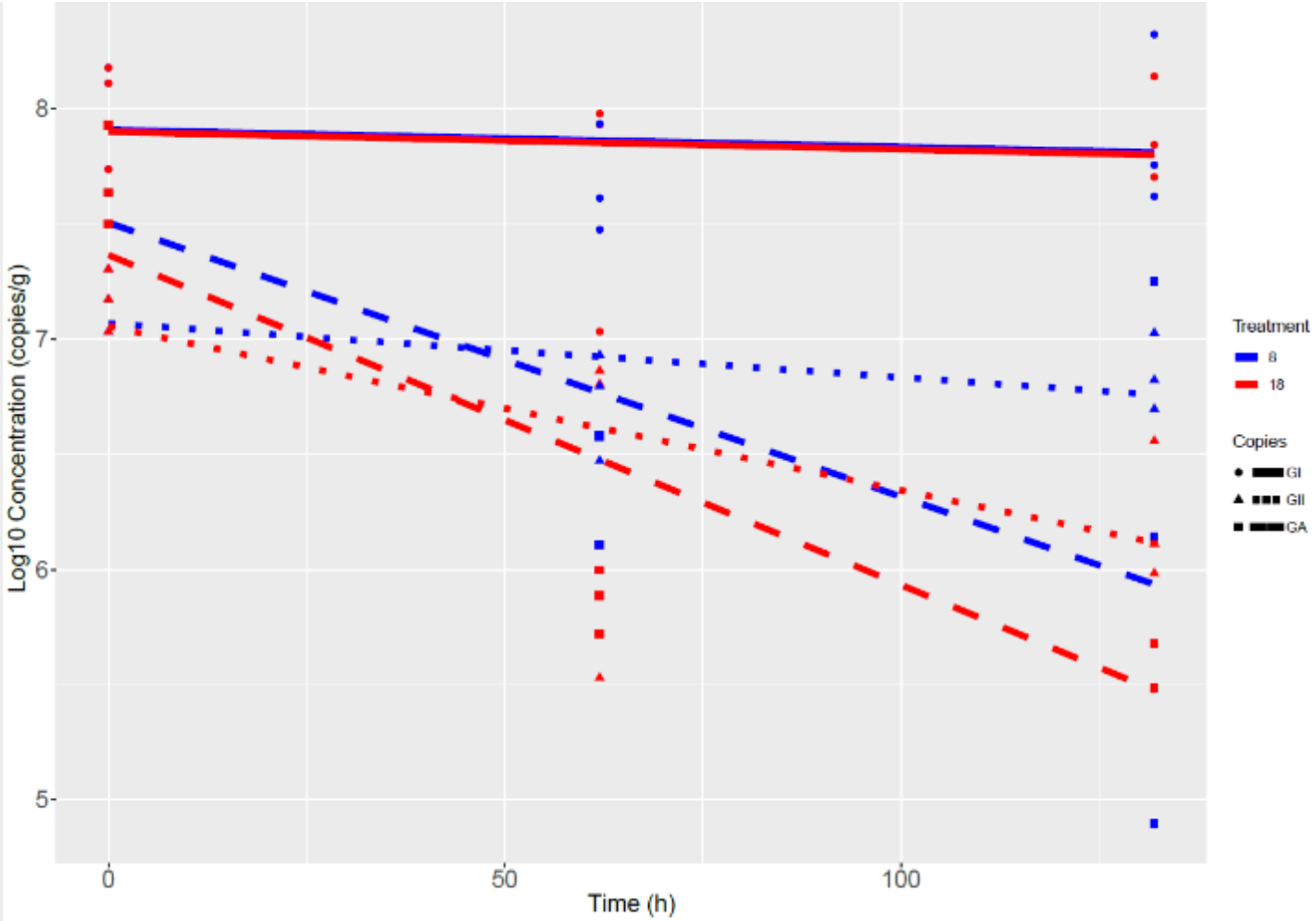
Results

NoV removal from oysters over 6 days : 8 vs 18°C



- NoV GII (dotted lines) better removed than NoV GI (continuous lines)
- Best results at 18°C
- F- RNA phage (dashed lines) better removed than all – suggests not a good indicator for NoV depuration?
- To be confirmed by further work

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Conclusions from study

- Temperature: depuration **2x better at 18°C** than at 8°C after 5 days
- NoV genogroup II better removed than GI
  - NoV GI removal levels were negligible (binding of NoV GI to oyster gut?)
  - NoV GII levels were reduced up to 60%
- Suggests depuration useful for some strains of NoV
- Salinity needs to be matched to natural environment to which shellfish are acclimatised (+/- 20%)
- Other factors trialled, including feeding, had no significant improved effect on NoV removal
- F-RNA phage better removed in our study than both NoV GI and GII – further investigation planned
- *E. coli* consistently removed in all trials (as expected)

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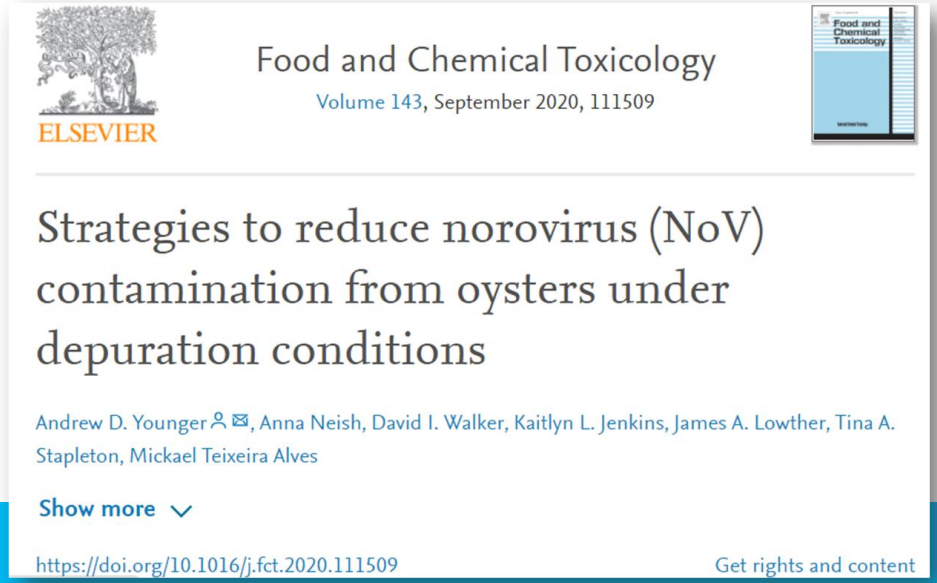
Summary outputs



- Elevated depuration temperatures can lower NoV levels from contaminated class B oysters (assuming temperate climate similar to the UK)
  - Relatively cheap to add heating to systems (e.g. 35 to 450 Euros for 90kg to 750kg systems)
- Our method may reduce health risks by improving seafood safety
- Seafood producers and processors can use the method with benefits for consumers worldwide

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Deliverable – Protocol for Industry

Enhanced depuration of Norovirus (NoV) from Pacific oysters (Crassostrea gigas) – recommendations for the shellfish industry

Trials conducted from January to March 2019 by the Centre for Environment, Fisheries and Aquaculture Science, Weymouth Laboratory under the EU funded 'Seafood Tomorrow' project.

Norovirus and depuration

Noroviruses can genetically be classified into at least seven different genogroups, each of which can be further divided into different genotypes. Genogroups I, II and IV are those associated with human illness (Ramirez et al 2008). Most noroviruses that infect humans belong to genogroups GI and GII (Vinjé et al 2000). Noroviruses from Genogroup II, genotype 4 (abbreviated as GII.4) account for the majority of adult outbreaks of gastroenteritis.

Across most of Europe, Norovirus (NoV) illness associated with the consumption of oysters eaten raw or lightly cooked is generally a winter problem (mainly November to March). Experience in the UK has shown that there is a close association between low seasonal environmental temperatures and NoV presence in oysters i.e. colder winters tend to be associated with a greater prevalence of NoV in the community and in bivalve shellfish. Conversely, NoV levels generally decline markedly in the summer months. An enhanced depuration procedure would therefore be most beneficial in the winter months.

Depurating Pacific oysters

Commercial scale systems at Cefas

Summary findings and recommendations from these trials

The trials carried out in this study were run at Cefas Weymouth in 600 Litre commercial scale depuration tanks. The findings from these trials were as follows:

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Most beneficial

- *Elevated temperature:* It is known that purification is effective for removal of bacteria but known to be less effective for viruses. These trials provide evidence that elevated temperature during depuration can achieve significant removal of NoV*, however, the extent of removal appears to depend on the strains of NoV present. Our trials showed consistently better removal of NoV Genogroup II (GII) compared with Genogroup I (GI). We found approximately 46% removal of NoV GII at 18°C after 2 days and 80% after 5 days compared with a maximum of 16% NoV GI removal. We found no difference in shelf-life between oysters from trials held at 8 or 18°C for up to 12 days post depuration.
- Twice the rate of NoV GII removal was achieved at 18°C compared with 8°C after 6 days.
- *Trials show that salinity levels can make a difference* and this is likely to relate to the range of salinities that the oysters have experienced in the production area from which they have been harvested. We would therefore suggest that the Seafish** recommendation to depurate in seawater with salinity that is within 20% of that in the shellfish harvesting area should be followed.

No obvious effect

- *No effect of feeding:* in fact our trials suggest that NoV removal may be better with no feeding at all.
- *No obvious difference between depurating shellfish in a light or dark environment*
- *No benefit of filtered vs unfiltered water* i.e. No difference between natural seawater flowing through the system on a constant renewal basis and recirculated seawater in a closed system.
- *No benefit of disturbance vs no disturbance* i.e. vibration from the pump attached to the tank did not appear to make any difference to NoV removal.

**Some strains appear more difficult to remove than others and this may, at least in part, be attributable to NoV binding to the gut of the oysters. This effect has been observed by researchers at IFREMER, France (Maalouf et al, 2011).*

***Seafish is a Non-Departmental Public Body set up to support the UK seafood industry. Cefas has historically worked closely with Seafish to develop depuration guidelines for the industry.*

Other recommended conditions based on this work and previous recommendations by Cefas/Seafish

- Avoid temperature shock as this may stress shellfish and/or cause spawning, particularly if they are in a seasonally conditioned state. Introduce shellfish to water of a similar temperature to that in the harvesting area from which they have come and then gradually change temperature of water to 18°C to allow the shellfish to acclimatise.
- Pacific oysters should be held in trays in no more than a double layer of shellfish.

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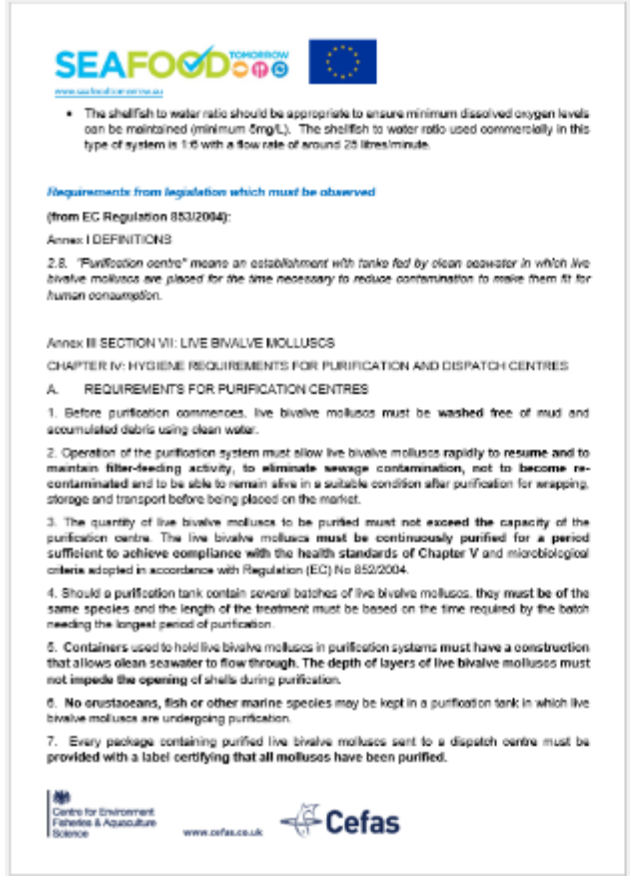

- The shellfish to water ratio should be appropriate to ensure minimum dissolved oxygen levels can be maintained (minimum 5mg/L). The shellfish to water ratio used commercially in this type of system is 1:6 with a flow rate of around 25 litres/minute.

Requirements from legislation which must be observed

(from EC Regulation 853/2004):

Annex I DEFINITIONS

2.8. *"Purification centre" means an establishment with tanks fed by clean seawater in which live bivalve molluscs are placed for the time necessary to reduce contamination to make them fit for human consumption.*

Annex III SECTION VII: LIVE BIVALVE MOLLUSCS

CHAPTER IV: HYGIENE REQUIREMENTS FOR PURIFICATION AND DISPATCH CENTRES

A. REQUIREMENTS FOR PURIFICATION CENTRES

1. Before purification commences, live bivalve molluscs must be **washed** free of mud and accumulated debris using clean water.

2. Operation of the purification system must allow live bivalve molluscs **rapidly to resume and to maintain filter-feeding activity, to eliminate sewage contamination, not to become re-contaminated** and to be able to remain alive in a suitable condition after purification for wrapping, storage and transport before being placed on the market.

3. The quantity of live bivalve molluscs to be purified must not **exceed the capacity** of the purification centre. The live bivalve molluscs **must be continuously purified for a period sufficient to achieve compliance with the health standards of Chapter V** and microbiological criteria adopted in accordance with Regulation (EC) No 852/2004.

4. Should a purification tank contain several batches of live bivalve molluscs, they must be of the **same species** and the length of the treatment must be based on the time required by the batch needing the **longest** period of purification.

5. **Containers** used to hold live bivalve molluscs in purification systems must have a construction that allows **clean** seawater to flow through. **The depth of layers of live bivalve molluscs must not impede the opening of shells** during purification.

6. **No crustaceans, fish or other marine species** may be kept in a purification tank in which live bivalve molluscs are undergoing purification.

7. Every package containing purified live bivalve molluscs sent to a dispatch centre must be **provided with a label certifying that all molluscs have been purified.**

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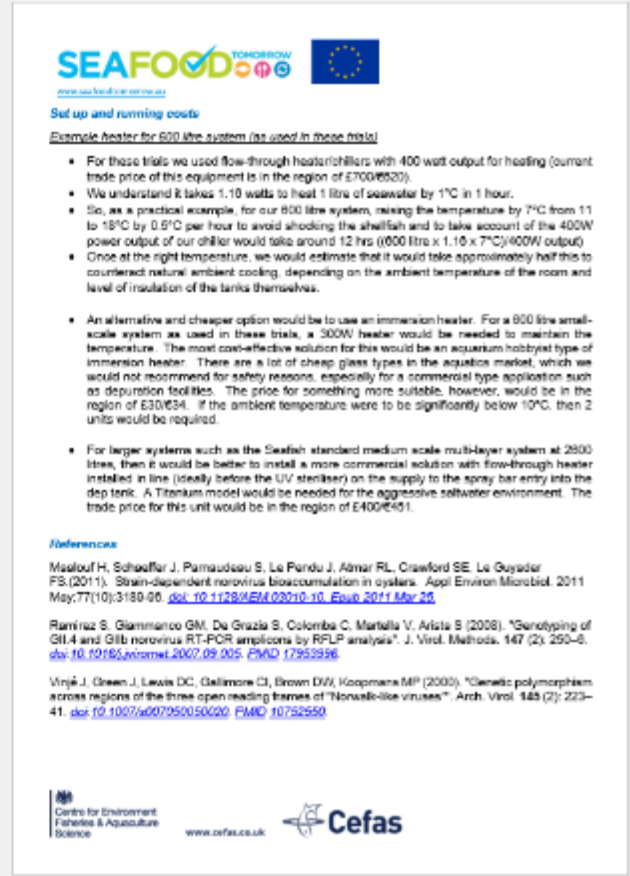

Set up and running costs

Example heater for 600 litre system (as used in these trials)

- For these trials we used flow-through heater/chillers with 400 watt output for heating (current trade price of this equipment is in the region of £700/€820).
- We understand it takes 1.16 watts to heat 1 litre of seawater by 1°C in 1 hour.
- So, as a practical example, for our 600 litre system, raising the temperature by 7°C from 11 to 18°C by 0.5°C per hour to avoid shocking the shellfish and to take account of the 400W power output of our chiller would take around 12 hrs ((600 litre x 1.16 x 7°C)/400W output)
- Once at the right temperature, we would estimate that it would take approximately half this to counteract natural ambient cooling, depending on the ambient temperature of the room and level of insulation of the tanks themselves.
- An alternative and cheaper option would be to use an immersion heater. For a 600 litre small-scale system as used in these trials, a 300W heater would be needed to maintain the temperature. The most cost-effective solution for this would be an aquarium hobbyist type of immersion heater. There are a lot of cheap glass types in the aquatics market, which we would not recommend for safety reasons, especially for a commercial type application such as depuration facilities. The price for something more suitable, however, would be in the region of £30/€34. If the ambient temperature were to be significantly below 10°C, then 2 units would be required.
- For larger systems such as the Seafish standard medium scale multi-layer system at 2800 litres, then it would be better to install a more commercial solution with flow-through heater installed in line (ideally before the UV steriliser) on the supply to the spray bar entry into the dep tank. A Titanium model would be needed for the aggressive saltwater environment. The trade price for this unit would be in the region of £400/€451.

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Thank You

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