Contract Reference C2472

LOADING CAPACITIES AND CRITERIA FOR THE SUCCESSFUL DEPURATION OF COCKLES, *CARDIUM EDULE* IN SMALL SCALE STANDARD DESIGN DEPURATION SYSTEMS

Programme of Cockle Purification Trials Jointly Funded by Cefas and Seafish

S Bark, A Younger, R Lee. Weymouth Laboratory Report 2005

The Centre for Environment, Fisheries & Aquaculture Science
Weymouth Laboratory
Barrack Road
The Nothe
Weymouth

Tel +44 (0) 1305 206600
Fax +44 (0) 1305 206601
www.cefas.co.uk

Cefas is an executive agency of Defra

1. SUMMARY

These trials set out to investigate whether increased loadings of cockles could be successfully depurated using the current standard of 30kg for comparison. Initial trials also involved a comparison between hand-raked cockles and cockles harvested by a towed-cage fluidized-bed dredge and concluded that this form of mechanised harvesting yielded cockles that were suitable for depuration. Results indicated that successful depuration could indeed be achieved with 90kg loading at the current minimum depuration temperature of 7°C. The results at 16°C for both 30kg and 90kg were inconclusive but suggest that depuration of cockles in general at this temperature warrants further study.

2. INTRODUCTION

To minimise the health risk associated with the consumption of raw or lightly cooked shellfish, European Community Legislation places controls on the harvesting and placing on the market of live bivalve shellfish. Under the current European Shellfish Hygiene Directive (91/492/EEC) (Anon 1991) and derived national legislation (Food Safety (Fishery Products and Live Shellfish) (Hygiene) Regulations 1998 (HMSO 1998)), the microbiological requirements for bivalve molluscs being placed on the market are based on testing for bacterial faecal indicators, namely *E. coli*. The classification category determines the level of treatment (if any) that bivalve shellfish must be subjected to prior to sale for human consumption. The four classification categories are given in table 1.

Classification category	*E. coli* / 100g flesh	Comment
A	Less than 230	Suitable for consumption without processing.
B	90% of samples less than 4,600	Depuration or relaying in a category A area or cooking by an approved method.
C	Less than 46,000	Relaying (minimum of 2 months) in category A or B area needed or cooking by an approved method.
Prohibited	Above 46,000	Cannot be placed on the market.

Table 1: Criteria for the classification of bivalve molluscan shellfish harvesting areas (Food Safety (Fishery Products and Live Shellfish) (Hygiene) Regulations 1998 (HMSO 1998)).

Depuration is one of the major treatment processes for controlling the public health risks associated with sewage-contaminated shellfish. Whilst current depuration procedures are unable to guarantee the production of shellfish free

from all microbiological contaminants, when performed correctly it nevertheless plays a significant role in eliminating bacteria and reducing the viral content of shellfish. This has undoubted public health benefits. Given the relative inefficiency of viral reduction during depuration and the significance of this control step, it is critical that system design and operation is optimised to gain the maximum benefit from the process.

Under the Food Safety (Fishery Products and Live Shellfish) (Hygiene) Regulations 1998, responsibility for the approval and ongoing control of depuration centres lies with the Local Food Authority. During the approval process a technical inspection is also made by CEFAS on behalf of the central competent authority (Food Standards Agency) to ensure that systems are capable of meeting the requirements for successful purification. A Conditions of Approval document is issued by CEFAS which sets out the operating parameters for each system. This document then forms part of the overall approval granted by the Local Food Authority. The Conditions of Approval document stipulates conditions which are not explicit in the legislation but which CEFAS consider must be met to ensure successful depuration. Some of these conditions are generic and apply to all systems whilst some are specific for the system in question and the species being depurated.

The Conditions of Approval are based on scientific work that has been undertaken historically by the Directorate of Fisheries Research, MAFF (Ministry of Agriculture, Fisheries and Food) and the Sea Fish Industry Authority (SFIA or 'Seafish'). Various studies were undertaken to establish the physiological requirements of individual species undergoing depuration and also to identify the concomitant physical constraints of the operating system that could negatively affect the efficacy of the depuration process. It is only with such details and experimental evidence on minimum temperature, salinity, flow rate, DO requirements and loading densities/arrangements that approval conditions can be set and subsequent approval granted.

The criteria for the depuration of cockles were established following trials conducted by SFIA between 1990 and 1992. These trials were conducted in Allibert type 21250 containers with a capacity of 250 litres, approximately half the size of the small-scale shallow tank standard design system commonly approved for use in England and Wales today. Trial systems held a single Allibert 41042 tray. Trials with reference to stocking density focused specifically on loading of cockles within trays rather than any stacking of trays within the system. As a consequence, for small-scale standard design systems, operators are currently approved to depurate a maximum of 30kg of cockles which equates to 15kg of cockles per tray with trays arranged in a single layer. The reason for the 30kg maximum stocking density is essentially due to a lack of experimental evidence in support of the depuration of cockles at higher stocking densities. Studies on increased loading levels have, however, been conducted with mussels and these have successfully demonstrated the depuration of mussels loaded in multiple layers.

Numerous operators, approved for the depuration of cockles, have commented on the evident disparity between the permitted stocking densities for mussels and cockles. It is for this reason that the current study was undertaken.

1.2 *Current approval conditions for cockles (Cardium edule)*

The conditions that are currently applied to the depuration of cockles are based largely on work carried out by Seafish (Boulter 1994). These include the stipulation that depuration should only be applied to hand-gathered cockles. It was concluded in the Seafish work that mechanically harvested cockles should not be submitted for purification until work has shown that the particular method of harvesting does not have a detrimental effect on the depuration process.

Additional requirements are as follows:

- Post-harvesting handling: Cockles intended for depuration must not show signs of damage to the shell. The maximum period between harvesting and purification must be 6 hours.

- Loading: Cockles may be loaded to a maximum depth of 80mm in trays. Trays should only be loaded one layer high unless proven otherwise for a specific system by microbiological testing.

- Minimum temperature: 7°C

- Minimum salinity: Seawater salinity must not be less than 20‰.

- Maximum shellfish to water ratio: 1 to 18

- Seawater re-use: Seawater should be replaced after each cycle until further study has shown that re-use would not adversely affect depuration efficiency.

1.3 *Objectives and Rationale*

The aim of this study was to investigate and assess the viability of purifying cockles *Cardium edule*, at loading densities over and above the levels which are currently approved. Investigations focused on the depuration of cockles in small–scale standard design systems primarily at 7°C and 16°C (the temperature range criteria recommended by SFIA - Seafish Report N:443, 1992) with some initial trials being carried out at 17°C, employing densities of 30, 60 and 90kg for comparative analysis. In addition to this, a new type of mechanical dredge was assessed to facilitate the acquisition of the large numbers of cockles required for this study.

3. METHOD

Cockles were sourced from Poole harbour and were harvested by two methods, hand raking and dredging.

The dredging was carried out using a purpose-made fluidised bed dredge towed from a winching rig assembly located to one side of the boat. Ski-like runners on either side of the dredge enable it to be towed across the surface of the substrate with relative ease (figure 1a). High pressure jets of water are directed at the sand/silt via a spray bar located above and immediately in front of the 40° angled metal scoop that projects approximately three inches below the runners. The water jets essentially fluidise the sand/silt in front of the scoop. All shellfish are then directed by the scoop blade into the collection basket. Undersized animals and suspended sediment pass through the grid-like basket, which retains only the larger mature cockles. Once full, the dredge is winched out of the water (figure 1b), and its contents emptied out on to a riddle for further grading and sorting.

3.1 Facilities

All trials were conducted at Cefas Weymouth laboratory in the experimental facility, using two identical small-scale standard design depuration systems (Figure 2).

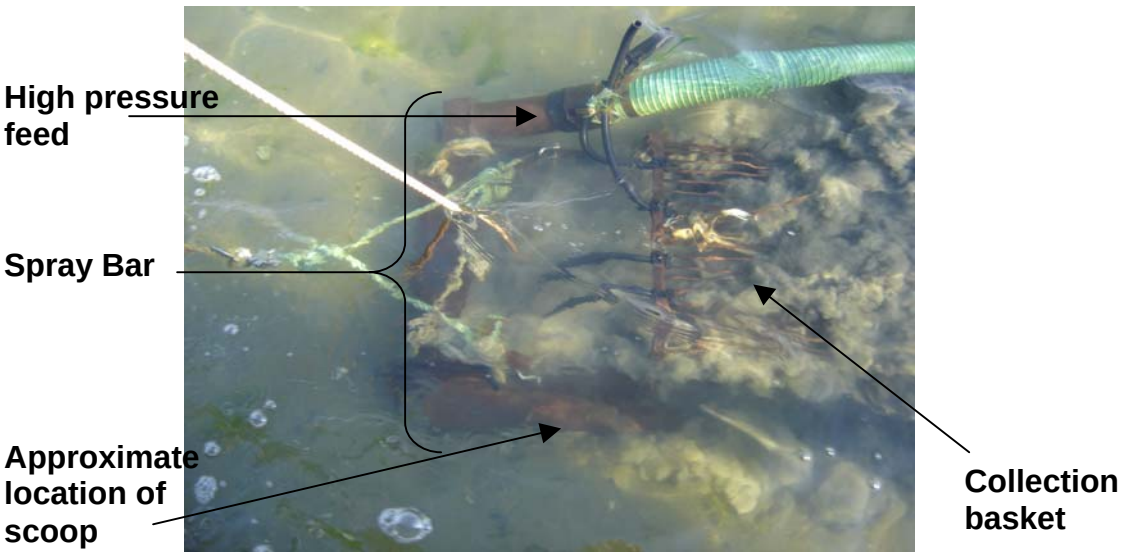


Figure 1a: Fluidising bed dredge in operation, being towed from right to left of the picture.

Figure 1b: Full dredge being winched back on-board

Experimental depuration systems used

Each experimental depuration unit consisted of a commercially available tank measuring 1375 x 1120 x 725mm containing a minimum water volume of 550 litres. Each system was fitted with a 25 watt UV steriliser through which all re-circulating water passed before re-entering the tank at a rate of 20L/min. Each system was fitted with a temperature probe calibrated against a UKAS accredited thermometer, connected to a 'Honeywell BMS' system, which recorded the temperature of the operating system continually. Temperature maintenance of re-circulating water was achieved by the use of a 'TECO' custom-built heater/chiller unit. In-line temperature regulation maintained tank water temperature to within ±0.4°C of the desired set point. All trials utilised natural seawater, salinity 33-35ppt, with a turbidity of <10NTU. Cockles were subject to 8.25h of daylight per 24h period at an intensity of ≈ 420 Lux.

Figure 2: Experimental Depuration unit in operation, 90 kg of cockles at 16^{o}C at 41h into cycle.

3.2 Bioaccumulation.

Unless otherwise stated the contamination procedure was as follows:

Screened effluent was collected from Wessex Water's Dorchester treatment works between 8 and 8:30 am on the morning of each trial. The *E. coli* concentration was found to vary between 1.6 x 10^5 – 6.8 x 10^6/100ml over the 8 trials conducted. In the first trial, 210ml of effluent was added to 550L of re-circulating seawater within the experimental depuration unit (with the UV unit switched off) and allowed to mix for a period of 15 minutes prior to the addition of shellfish.

All cockles sourced were re-immersed within six hours of harvesting. 60kg (plus extras to allow for sampling) of cockles (four Allibert 41042 trays each containing 15kg of cockles) was then added to the tank. Shellfish were immersed in the contaminated seawater for a period of 4h at 17°C ± 0.4°C. The system was then drained down.

Once drainage was complete, shellfish were left until 'spouting' had ceased and were then rinsed with fresh water. For each batch of shellfish artificially contaminated, three representative samples were taken. One sample from the shellfish nearest the spray bar (Top), one from shellfish occupying the middle (Middle) of the unit and third sample from the shellfish closest to the suction bar (Bottom) – see Figure 3. Each sample consisted of 30 cockles. The enumeration of *E. coli* was performed in accordance with ISO16649-3 and was undertaken by the on-site UKAS accredited laboratory.

Water / sewage samples were examined for *E. coli* according to the standard potable water method as detailed in the Report on Public Health and Medical Subjects No 71.

For trials requiring >60kg of shellfish, cockles were bio-accumulated in two parallel batches of 60kg for practical purposes. Cockles were then sourced from these two tanks in equal proportion to make up the loads required for any given trial described.

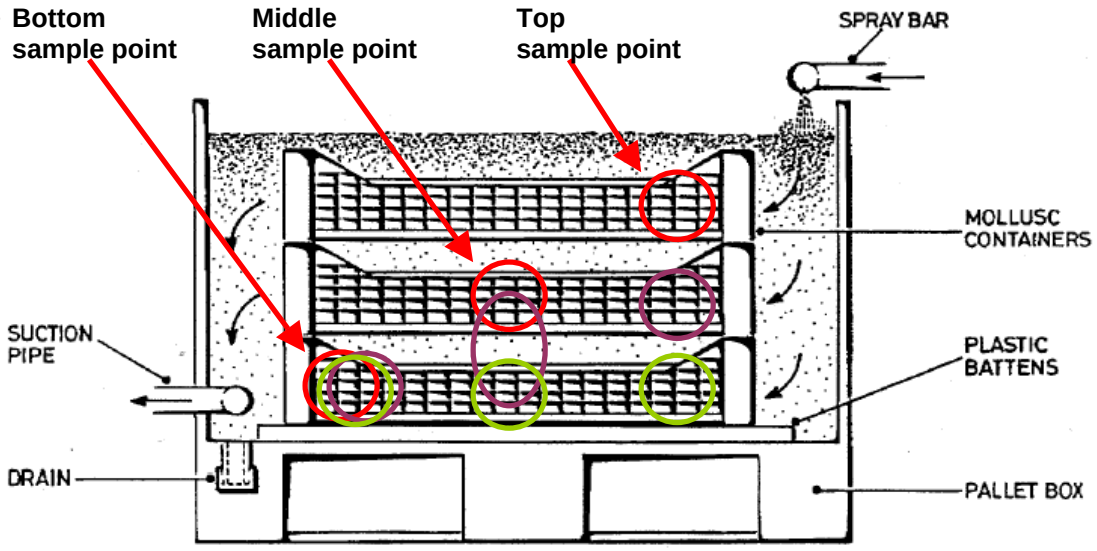


Figure 3: Experimental Depuration unit schematic in cross section identifying sample zones and orientation of trays.

Red zones denote sample regions for units loaded with 6 trays (90kg), purple zones for units loaded with 4 trays (60kg) and green for units loaded in a single layer, two trays (30kg). (Original diagram taken from SFIA operating manual for the Small Scale Shallow Tank Purification System).

3.3 Depuration challenge assessment

Firstly, the experimental depuration units were loaded with the desired number of trays (2 (30kg), 4 (60kg) or 6 (90kg)). All trays located on the bottom of the system were raised off the floor of the tank by ≥ 25mm. Purification units were then filled with seawater and the pump, chiller and UV system for each system switched on. The loaded experimental unit was then left undisturbed (i.e. molluscs not added or removed) during the 42 hour trial depuration period.

After 42 hours, drain-down and subsequent sampling was carried out as described above in 3.2.

3.4 Assessment Criteria.

Three main criteria were used for the assessment of depuration efficacy during the trials. These were:

Dissolved Oxygen (DO). Dissolved oxygen (mg/L) was assessed during all trials mid way through the cycle and at the end of the 42h depuration period prior to drain down.

Visual Observation. Visual observations were made throughout each trial to ensure that systems were operating as desired. At the mid and end point of the trial period an assessment of cockle activity, water clarity foaming and any other relevant features was made .

E.coli clearance. Pre and post depuration results (*E.coli*/100g) were assessed and percentage reductions calculated for each trial scenario.

A single assessment of shelf-life was also undertaken for dredged and hand-gathered cockles post depuration at 4°C.

3.5 Experimental trials

The investigation was divided into two phases as detailed below.

Phase 1 - Comparison of hand-gathered against dredged cockles: The first trials conducted were designed to assess any relative difference in depuration efficacy of dredged and hand gathered cockles. For the purposes of this trial a single Allibert 41042 tray containing 15kg of dredged cockles and single Allibert 41042 tray containing 15kg of hand gathered cockles was placed in the experimental depuration unit arranged in a single layer. Bioaccumulation utilised 330ml of screened effluent to ensure a reasonable initial microbial loading. The depuration challenge assessment was conducted at ambient temperature (approx.14°C).

Shelf life: 100 randomly selected cockles were selected following the depuration of 90kg of cockles at 16°C and 7°C. Cockles were placed in a plastic open box and stored at 4°C. Mortality assessments were conducted every 24h until 50% mortality was observed.

Phase 2 - Loading density assessment: All trials in phase 2 were carried out using dredged cockles. Various attempts were made at achieving a consistent level of contamination in the pre-depuration shellfish using primary effluent. However, despite these attempts there was a large amount of variability. The reasons for this are unclear, however, effluent *E. coli* variability was one influencing factor. A further explanation for this observation may be the inherent natural variation associated with cockles,

perhaps further exacerbated by changes in environmental conditions over the protracted period of the trials (June to September).

Investigations focussed in the main on the depuration of cockles at 7°C and 16°C. Early trials were carried out at 17°C where also the contamination protocol was being developed. Each trial consisted of a control experimental depuration unit employing the standard approved loading of 30kg of cockles, run in parallel with a second identical experimental depuration unit containing the elevated stocking density i.e. 60 or 90kg.

4. RESULTS

4.1 Phase 1 – Comparison of hand-gathered against dredged cockles

The first two trials conducted as detailed in table 2, were undertaken to assess the relative difference in terms of depuration efficacy between hand gathered cockles and cockles harvested using the fluidised bed dredge described in section 3.

***E.Coli* / 100g**	**Hand Gathered Cockles**			**Dredged Cockles**		
	Pre Depuration	Post Depuration	% Reduction	Pre Depuration	Post Depuration	% Reduction
Trial 1						
Tsp	91000	250	***98***	35000	90	***98***
M	22000	700		5400	750	
Bsp	35000	1300		17000	250	
Trial 2						
Tsp	70000	1300	***98***	17000	220	***99***
M	22000	500		31000	430	
Bsp	54000	1100		35000	500	

Table 2: Comparative depuration efficacy of hand gathered and dredged cockles

Pre depuration levels of *E. coli* following bio-accumulation appeared to be slightly higher in hand-gathered cockles. This may indicate some degree of shocking of the shellfish that were mechanically harvested compared with those that were hand-gathered or may be purely due to natural variability. The highest recorded contamination following bio-accumulation was 91000 *E. coli*/100g. All pre trial cockle analysis identified contamination in excess of 17000 *E. coli* /100g with the exception of one sample (dredged cockles from trial 1) which returned a value of 5400 *E. coli* /100g. Due to the high starting values, total clearance of *E. coli* was not achieved and post depuration sample results ranged from 90 – 1300 *E. coli* /100g. Percentage reduction values calculated for each method of harvesting show little comparative difference in either trial and were all >98%. However, it should be noted that percentage clearance values need to be viewed with caution as clearance is

non-linear once lower *E. coli* values are reached i.e. it is harder to achieve higher percentage reduction figures with lower starting *E. coli* values.

Trial No.	Depuration Temperature °C	Cumulative % Mortality			
		168h	***192h***	***216h***	***240h***
1	7	4	8	23	52
2	16	1	10	17	50

Table 3: Post depuration mortalities of dredged cockles stored at 4°C.

50 % mortalities of dredged cockles stored at 4°C were recorded at 10 days post-depuration (table 3).

No apparent differences in mortality rate were observed in any of the trial replicates regardless of the harvesting method employed.

4.2 Phase 2 - Loading density assessment

In total, eight trials were used for the assessment of depuration efficacy. Trials were run with duplicate tanks to compare the standard 30kg stocking density with the elevated stocking density of 90kg at 7°C and 16°C (Table 5). The concentration of effluent utilised for bioaccumulation ranged from 1.8 x 10^5 – 6.8 x 10^6 E. coli/100ml.

Pre-depuration (i.e. post bioaccumulation) *E. coli* content ranged from 5400 - >180,000 *E.coli*/100g. The variations in pre-depuration levels do not follow the fluctuations in effluent concentration used for bioaccumulation suggesting that there was perhaps some temporally related physiological variation within the shellfish themselves occurring.

Percentage reduction rates in excess of 98.65% were achieved in all trials. All trials carried out at 7°C met the end-product standard of 230 *E. coli*/100g post-depuration. However, failure to achieve this standard was noted in trials conducted at 16°C at both 30 and 90kg loads from the middle tray of the experimental units. One failure occurred in unit 2 (30kg) from trial 4 and the other in unit 1 (90kg) from trial 6.

Trial no.	Unit No.	Loading (kg)	Temp (± 0.4 °C)	Pre-depuration			Post- depuration		
				Top	Middle	Bottom	Top	Middle	Bottom
1	1	60	17	1300	1300	1300	<20	70	<20
	2	60	17	500	1300	1300	70	70	40
2	1	60	17	9100	5400	16000	40	70	20
	2	60	17	5400	5400	5400	40	50	20
3	1	90	17	500	430	430	<20	20	40
	2	90	17	220	430	500	20	<20	40
4	1	90	16	9100	16000	9100	20	160	20
	2	30	16	9100	16000	16000	70	500	70
5	1	90	16	>180000	>180000	>180000	500	500	200
	2	30	16	>180000	>180000	>180000	1100	750	160
6	1	90	16	16000	>18000	16000	90	500	110
	2	30	16	5400	>18000	9100	140	130	110
7	1	90	7	24000	24000	17000	70	90	40
	2	30	7	7000	24000	35000	20	20	20
8	1	90	7	24000	24000	17000	90	20	20
	2	30	7	7000	35000	24000	70	40	20

Table 5. Summary results from phase 2

The shellfish in trial 5 at 16°C were obviously very heavily contaminated and so it is perhaps not surprising that end product standard levels were not met with this particular trial. As a result, this trial was repeated. Again, high input levels were evident (although they were significantly lower than in the previous trial) and one of the results for the 90kg test load failed to meet the end product standard (a result of 500 in the middle tray)

Tray position or sample location was not found to have had a significant influence on depuration efficacy (see graphs at Appendix 1).

4.3.1 Visual observation

Dissolved oxygen measurements at the three sampling points during the experimental depuration period varied. The highest DO levels recorded were for trials run at 7°C, with saturation values up to 9.1mg/L. DO levels in all systems employing stocking densities of 30kg were consistently higher than those containing 90kg of cockles regardless of temperature. The lowest recorded DO levels were observed in systems operated at 16°C containing stocking densities of 90kg. The lowest DO level recorded was 4mg/L.

Figure 4: Dredged Cockles actively filter feeding within an operational Experimental Unit.

During the trials it was noted that cockles showed evidence of activity within about five minutes of immersion. Normal activity was generally observed (figure 4) within twenty minutes - valves were open with siphons clearly visible. At no time during any of the trials conducted were cockles found to be inactive. Activity levels did not appear to be significantly different at the lower DO levels experienced.

The largest build-up of proteinacious scum was observed in experimental units depurating 90kg of cockles at 16°C (figure 5b). The amount of scum decreased with temperature. Experimental units operated at 7°C stocked with 30kg of cockles generated the least scum (figure 5a).

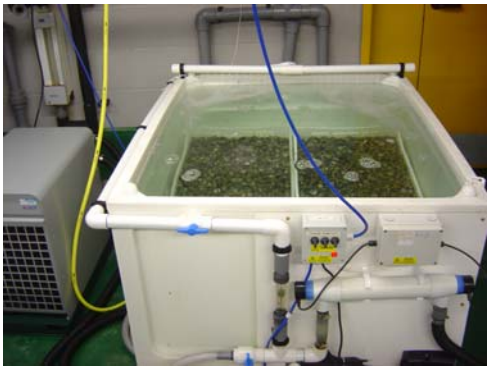


Figure 5a: 30kg of cockles mid cycle at 16°C, at 20h.

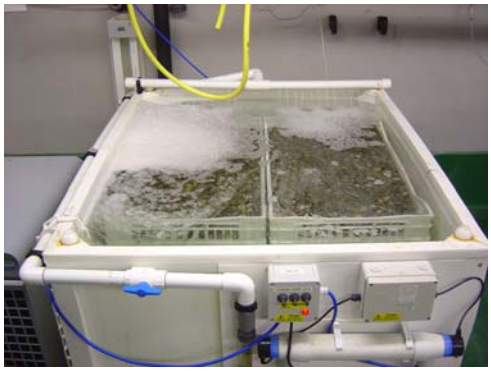


Figure 5b: 90kg of cockles mid cycle at 16°C, at 20h.

5. DISCUSSION

The acquisition of hand-gathered cockles in sufficient numbers given the time constraints placed on re-immersion of cockles for depuration, prompted preliminary investigations into the use of a new type of fluidised bed dredge (section 3) for this purpose. Boulter (1994) reported the occurrence of smashed and shocked cockles following traditional mechanical harvesting (suction dredging) techniques. It is as a result of the findings of these trials that only hand gathered cockles are currently permitted to be depurated. The current study found that harvesting using the fluidised bed method of dredging described above does not yield the high numbers of damaged cockles as previously reported for suction dredging.

Various attempts were made at achieving a consistent and realistic level of contamination in the pre-depuration shellfish using sewage effluent. However, despite these attempts there was still a large amount of variability. The reasons for this are unclear, however, effluent *E. coli* variability was one known influencing factor. A further factor may be some inherent natural variation associated with cockles, perhaps exacerbated by changes in environmental conditions and/or seasonal state over the protracted period of the trials (June to September).

Despite hand-gathered cockles apparently accumulating slightly higher levels of *E. coli* than dredged cockles (table 2), which may denote a possible shock response in the form of diminished activity, depuration rates as measured by percentage reduction and in achieving end product standard would appear to be acceptable at 7°C. There appears to be no appreciable difference between 30 and 90kg loading configurations at this temperature in terms of depuration performance.

As expected, both temperature and stocking density had a profound effect on the DO saturation within the operational system. Systems operated at the lowest densities (30kg) at 7°C did not fall below 8.6mg/L (approx. 80% saturation). The lowest recorded DO was 3.9mg/L in trial 5 suggesting perhaps an enhanced level of activity of cockles during this trial. Trial 6 conducted at the same temperature and employing the same loading of

cockles gave consistently high DO readings. From the data reported there is no evidence to suggest that DO levels of ≥ 3.9mg/L (approx. 50% at 16°C) impact negatively on the depuration activity of cockles, which concurs with the visual observations made by Boulter (1994).

Stocking densities of up to 90kg at 7°C have been shown to facilitate the reduction of *E.coli*/100g to well below end-product standards. However at 16°C, the occurrence of end-product failures has been noted for both the currently approved 30kg stocking density (trial 4) and the elevated stocking density of 90kg (trial 6) with single end product failures from each trial. Interestingly, if we look at trials 1 and 2 where duplicate batches of 60kg were depurated at 17°C pre-depuration, levels ranging from 5400 to 16000 *E.coli*/100g were all cleared to below the end-product standard after depuration. It could be argued that pre-depuration levels in many of the trials were unusually high and that given a more typical starting point end product standards would have been met. This may be true, however, occasional high results in B class shellfish can occur in the region of 4,600 to 18,000 and theoretically up to 46,000. As a consequence, systems should be able to demonstrate their ability to deal with occasional high levels of *E. coli*. Further work will be necessary to clarify the situation with regard to depurating cockles at higher temperatures and there may be a need to stipulate a maximum on the Conditions of Approval. This is something that has, so far, not been done for other species.

5.1 Conclusion and recommendations

From the results of this study, the depuration efficacy of hand-gathered and dredged cockles harvested by the towed-cage, fluidised-bed system as detailed would appear to be comparable. As a consequence it is recommended that purification should be approved for cockles harvested by this particular method.

This series of trials has demonstrated that stocking densities of cockles of up to 90kg will clear significant levels of *E. coli* at 7°C in small-scale standard design systems, employing minimum flow rates of 20L/min, with a DO of no less than 3.9mg/L. However the scientific evidence presented in this report in support of the depuration of cockles at 16°C is inconclusive. Whilst some work has been undertaken (Boulter, 1994) on the temperature criteria for the depuration of cockles, the current study has demonstrated the need for a further assessment into the effect of higher temperatures on their depuration efficacy.

5.1.1 *Summary of recommendations*

Based on previous Seafish work (indicated by an asterisk*) and the additional findings of this trial the following recommendations are made:

- Post-harvesting handling: Cockles harvested by certain forms of towed-cage fluidized-bed dredging may be suitable for depuration (the exact specification and description of the type of dredge deemed acceptable to be formally agreed after discussion with Seafish).

- Cockles intended for depuration must not show signs of damage to the shell*.

- The maximum period between harvesting and purification must be 6 hours*.

- Cockles may be loaded to a maximum depth of 80mm in trays*.

- The approval for the stacking of trays to 3 layers in the small-scale shallow tank system may be granted, providing the temperature of the operating system can be maintained at or above a minimum temperature of 7°C.

- Minimum temperature: 7°C*

- Maximum temperature: No maximum temperature can be stipulated at this stage but on the basis of this study may be less than 16°C (further study is needed to clarify this point).

- Minimum salinity: Seawater salinity must not be less than 20‰*.

- Maximum shellfish to water ratio: 1 to 6

- Seawater re-use: Seawater should be replaced after each cycle unless further study shows that re-use would not adversely affect depuration efficiency*.

6. REFERENCES

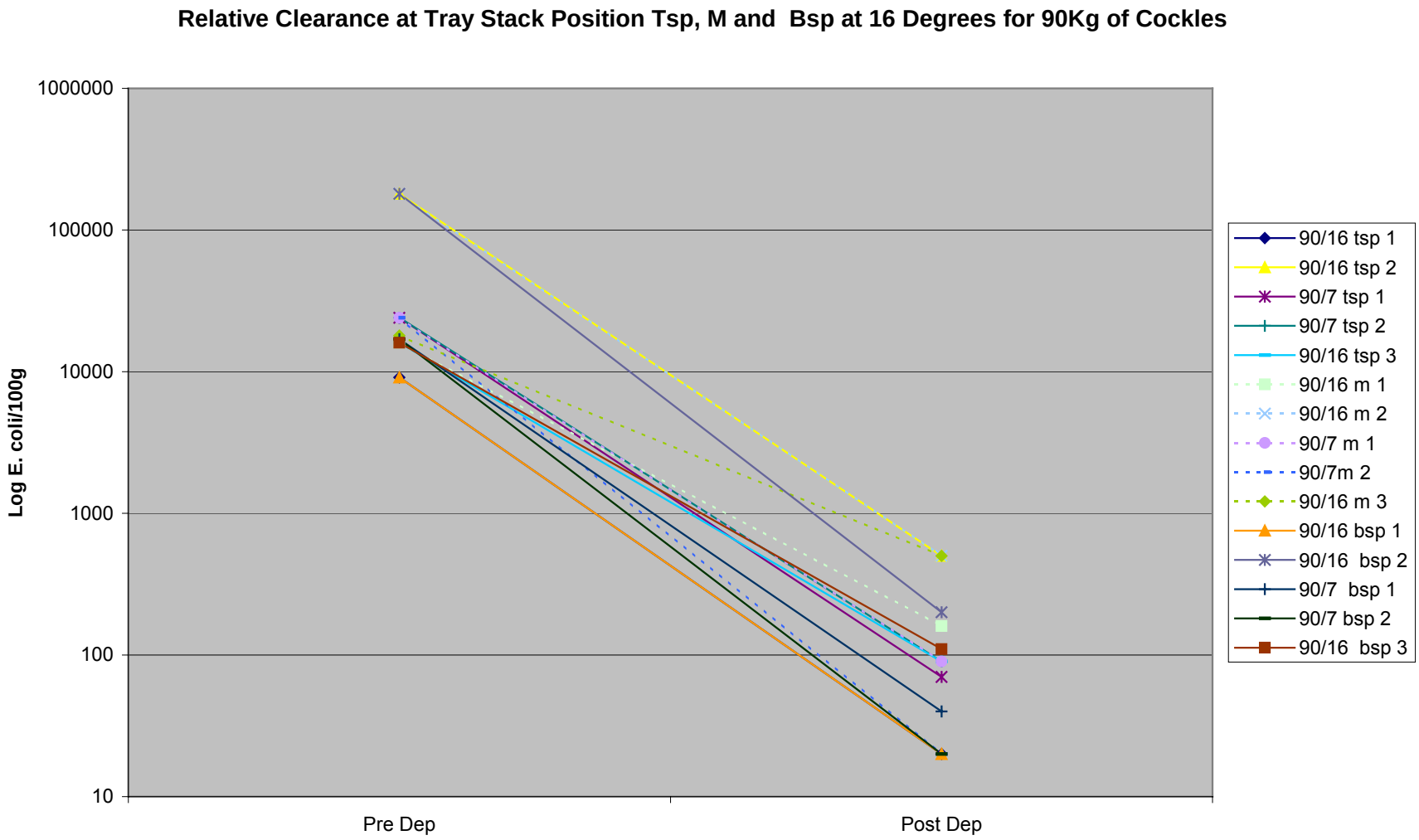
Anon., (1991). Council Directive of 15 July1991 laying down the health conditions for the production and placing on the market of live bivalve molluscs (91/492/EEC). **Offic. J. Eur. Comm**: 268, 1-14.

Boulter M., Wilson P., Denton W J. (1994).Trials to asses the viability of purifying cockles. **Seafish report** No.443. Hull, UK, Sea Fish Industry Authority.

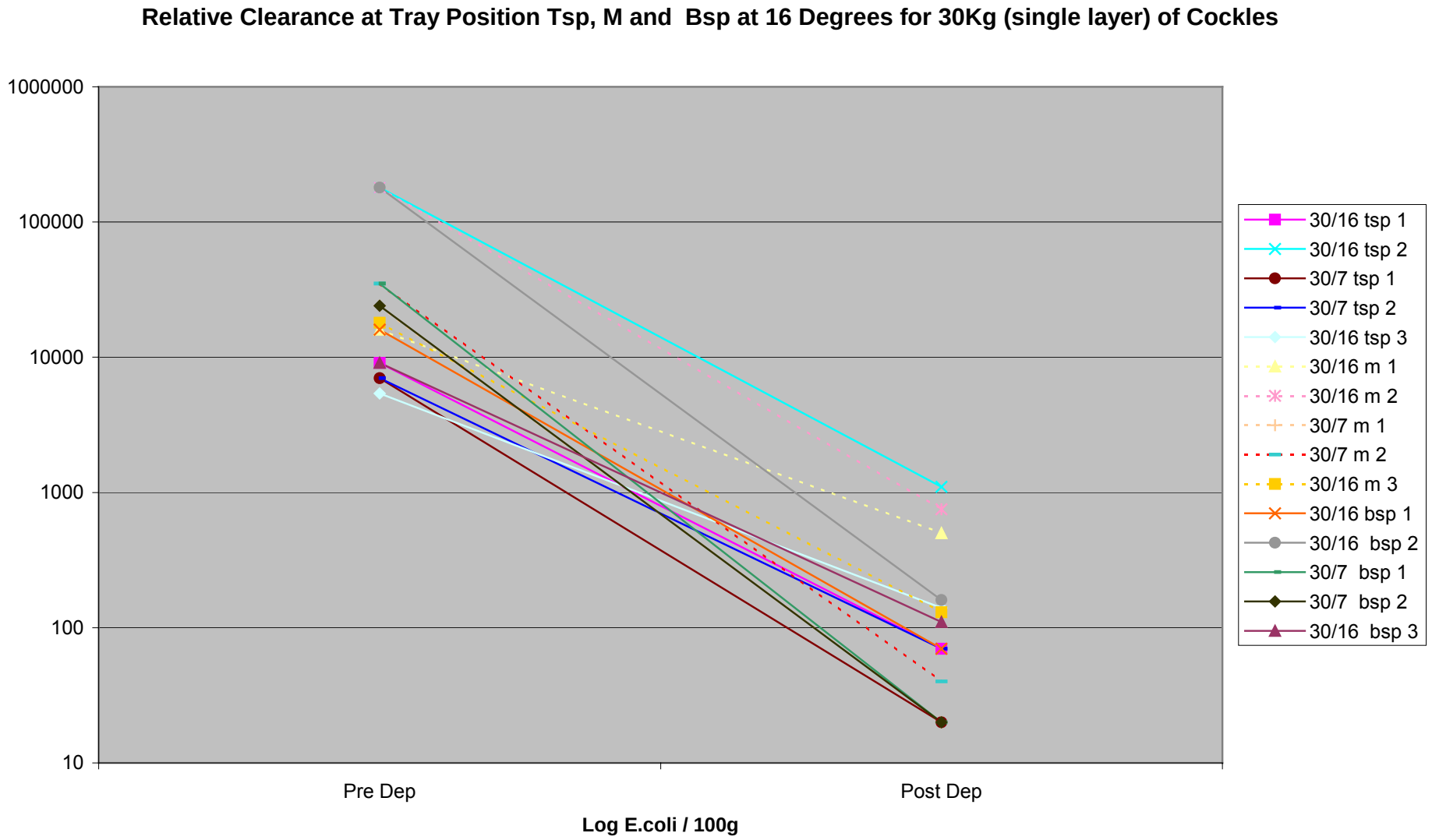
HMSO (1998). Food Safety (Fishery Products and Live Shellfish) (Hygiene) Regulations 1998. Statutory Instrument No 998. The Stationary Office. ISBN 0-11-065920-1.

7. APPENDIX .

Appendix 1: Post Depuration clearance following depuration in trays stacked in a multiple layer.



Appendix 1: Post Depuration clearance following depuration in trays stacked in a single layer.



Appendix 1: Post Depuration clearance following depuration in trays stacked in a single layer.