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Contract Reference C0725

Determination of Appropriate Conditions for the Depuration of Razor Clams

Funded by the Ministry of Agriculture Fisheries & Food

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Trials undertaken from November 1998 to May 1999

1. Summary

Trials carried out to determine appropriate conditions for the depuration of razor clams have found that, providing diver-gathered animals are banded and re-immersed soon after capture and kept banded during the depuration process, significant removal of *E.coli* can be achieved from shellfish artificially contaminated with primary sewage effluent.

Shellfish contaminated to class C levels of *E.coli* achieved significant clearance of *E.coli* after 42 hours at 10°C and a salinity of 30‰ and with a shellfish to water ratio of 1:4 (w/w).

Razor clams held in bundles of 10-12 animals restrained at each end by a rubber band were found to survive longer than loose individuals and were active under depuration conditions.

The best overall orientation of animals in trays when considering both trials data and commercial application was found to be for them to be held in horizontal bundles with body length perpendicular to the direction of flow of seawater within the system. Holding razor clams in vertically orientated bundles was found to be satisfactory in terms of the physiological response of the animals. However, vertical orientation presented problems such as trapping of the foot (which could lead to it being severed) and "escaping" of individuals from bundles, both of which were found to cause early death of the animal.

Insufficient data was obtained from these trials to be able to fully assess the ability of dredged razor clams to successfully undergo depuration and further work will be needed to investigate this.

The recommendations relating to the setting of Conditions of Approval are presented in section 6.

2. Introduction

The Food Safety (Fishery Products and Live Shellfish) (Hygiene) Regulations 1998 require that bivalve molluscan shellfish from class B harvesting areas must undergo depuration before they may go for human consumption.

All depuration systems used in England and Wales for this purpose must first be "approved" for use under these Regulations. The final approval is given by the local authority, however the specific approval conditions for the operation of the system itself are set by CEFAS on behalf of the Minister. These conditions are based on scientific work that has been undertaken historically by MAFF and the Sea Fish Industry Authority (SFIA). Such work has been carried out to determine the physiological requirements of the individual species undergoing depuration. It is only with such details e.g. minimum temperature, salinity and flow rate together with loading requirements, that such approval conditions can be set.

There are currently a number of newly identified species of commercial interest that have not been studied in this way and consequently approval of systems to depurate these particular species is not possible. It was intended that this project should specifically determine the physiological requirements for razor clams undergoing depuration so that approval conditions may be set by following on from work carried out by the Marine Laboratory Aberdeen (MLA) (Ref 1). Razor clams have been chosen in particular because of the level of interest shown by the industry in this particular group of shellfish and because of their marked morphological difference from traditionally approved species.

A number of new beds of razor clams have been identified and monitoring of these has so far indicated that the majority are of class B standard. The shellfish harvested from these beds will require either depuration or heat treatment (or relaying) before they may go for human consumption. The live trade provides the most benefit to the industry in economic terms and consequently depuration would be the preferred option.

Razor clams or razor shells are bivalve molluscs and are members of the family Solenidae. The shells of this group are so named due to their resemblance to the traditional barber's cut-throat razor. They have an elongated rectangular outline with their length being around 4 to 5 times greater than width. The species currently of commercial interest are *Ensis siliqua*, *E. arcuatus* and *E. ensis*. These trials concentrated on *E. siliqua* and *E. arcuatus* with the former of these species being of principle commercial interest in England and Wales. A further species *E. directus* is also found in some coastal waters such as the Wash although is currently thought to be of lesser commercial significance.

Razor clams inhabit the low intertidal zone down to depths of 40m and are found in all waters around the British Isles where there is a suitable substrate of fine to medium sand. They live in deep vertical burrows, feeding at the top of these by protruding short, fused siphons which are also used for respiration. Burrowing is facilitated by the use of a muscular foot which has also been observed to achieve a certain amount of horizontal movement of the animal along the surface of the seabed by a lateral "flicking" action (SFIA pers. comm.). The burrows themselves may be up to 1 metre in depth.

Ensis siliqua is the largest of the species and may grow up to 20cm long with such individuals weighing in excess of 100g each (see Figs. 3 & 4)

There are 3 recognised forms of harvesting of razor clams in England and Wales and these are beach collection (by hand), diver-gathering and harvesting by fluidised dredge. The former two methods may be achieved by pouring a quantity of crystaline granular salt onto the top of burrows which can be identified by characteristic depressions in the sand. After a matter of only a minute or two the animals will often emerge, either partially or entirely, from their burrows as a reaction to the salt, making harvesting then a simple matter of hand collection before they burrow back into the seabed.

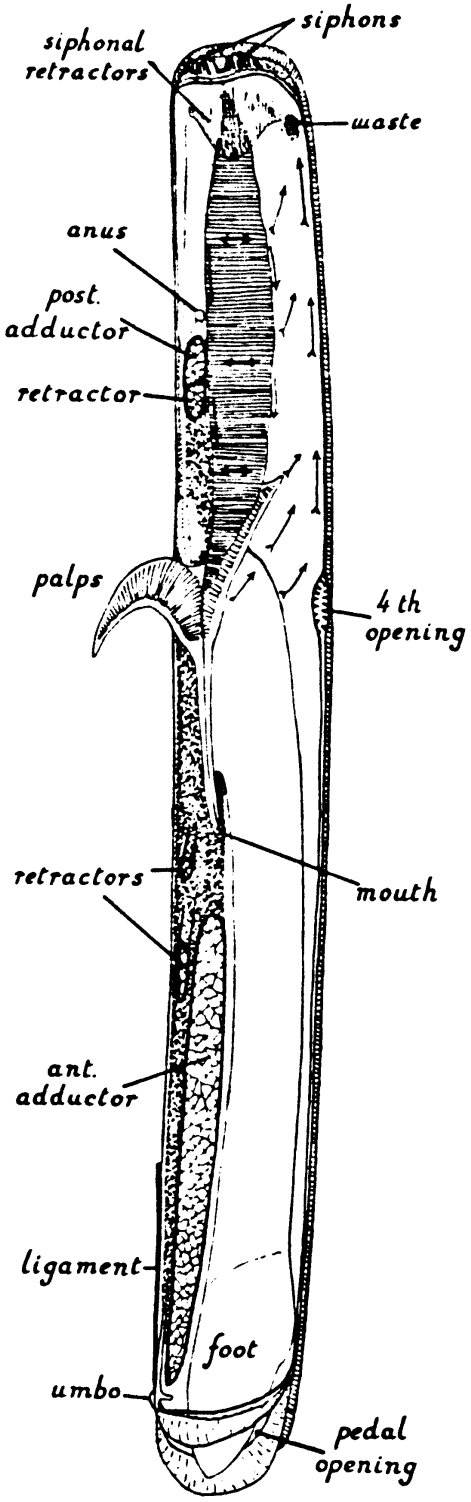


Diagram 1: *Ensis siliqua*

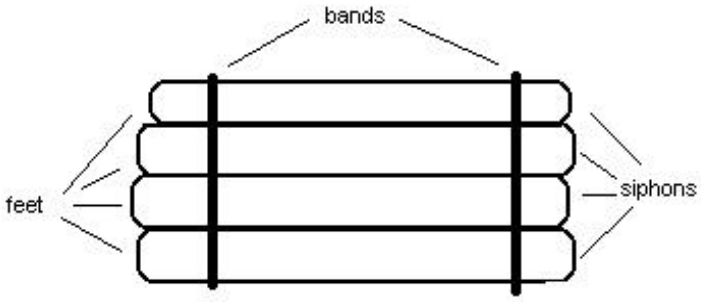
3. Methodology

Diver-gathered specimens were used in all trials (except trial 7) as this method of harvesting gave the most consistent source of high quality shellfish. Mechanised forms of harvesting such as dredging are known to cause damage to a significant proportion of the catch in other species such as cockles and such damage has been found to adversely affect depuration efficiency (ref 6). It would be reasonable to assume that the same situation might apply for dredged razor clams and the use of diver-gathered specimens was therefore intended to give the "best case" result against which razor clams harvested by other means could then be compared.

Razor clams were bundled and banded as soon as practicable (generally within 1 hour) after harvesting to maintain them in the best possible condition. Such banding was found to prevent splitting and/or gaping of the animal and thus extend its shelf-life. The same method of bundling was used in the depuration experiments (where applicable) but, in addition, all animals within a bundle under depuration conditions were orientated so as to face in the same direction (see Figs. 5 & 6 for guide to appearance of each end).

Individuals within a bundle were held sufficiently tightly to prevent splitting, gaping or "gripping" (where one individual splits and closes its shell on another) but not so tightly as to cause any crushing. As a guide, 10-12 adult *Ensis siliqua* (20cm long approx.) were held per bundle using 3 1/2 x 1/4 inch (89 x 6mm) rubber bands with 2 bands being used per bundle, one each end (see diagram 1).

Diagram 1



The Sea Fish Industry Authority (SFIA) undertook all trials work involving non-microbiological parameters and these were carried out either at Weymouth (trials 1 & 2b) or Ardtoe, Scotland (trials 2c-8). All microbiological trials using artificially contaminated razor clams were carried out at Weymouth.

Weymouth trials were carried out using diver-gathered shellfish from the class B razor clam bed in Weymouth Bay. Seawater salinity in the harvesting area was 35‰ with a temperature of around 11°C at the time of the trials. The trial tanks in Weymouth were located in the controlled environment of the tank rooms at the CEFAS laboratory with a 12 hour light/dark regime. The air temperature was constantly maintained at approximately 20°C in the tank rooms.

It became evident during early trials that a consistent and readily available supply of diver-gathered razor clams could not be achieved by using the shellfish from Weymouth Bay. An alternative source was investigated using an unclassified bed at Torbay, however, obtaining a sufficient number of specimens from this bed proved difficult. It was therefore decided after trial 2b that the rest of the trials should be carried out at the SFIA facility at Ardtoe, Highland, Scotland where a less weather-dependent supply of razor clams could be obtained. The trial tanks at Ardtoe were housed in a wet room subject to natural light conditions and ambient air temperatures.

As the trials at Ardtoe progressed it became clear that what were at first thought to be small *E. siliqua* were in fact *E. arcuatus.* Therefore a number of the trials originally carried out *with E. arcuatus* at Ardtoe were repeated with *E. siliqua*.

Trials 2c - 5 were carried out with *E. arcuatus*, trials 1, (2a), 2b and 6 were with *E. siliqua* and trials 7 and 8 used both species. Trials 4b and 6b at Ardtoe covered salinity and dissolved oxygen levels with *E. siliqua* and further shellfish : water ratio trials were also carried out with this species.

3.1 Experimental equipment

SFIA trials were carried out using 4 identical small scale model depuration systems measuring 965mm long, 560mm wide and 470mm deep (see figure 1).

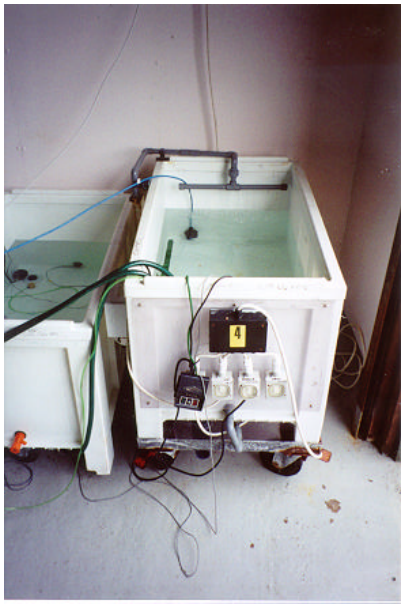


Figure 1

All trials (except those for the density experiments) used 150 litres of water which allowed a covering of approximately 10cm of water above mature adult *Ensis siliqua*. The same number of individuals per tank were used (minimum of 40-50) for each separate trial in 150 litres of water giving shellfish : water ratios of around 1:30.

The relevant features of the trials systems are as follows:

A closed circuit recirculation of seawater via 30W UV light unit with oxygenation being achieved primarily via a spray bar at one end of each tank. All experiments were carried out at a flow rate of 10 litres per minute. This was normally found to be able to achieve at least a minimum dissolved

oxygen saturation level of 75%, however, supplementary oxygenation, when necessary, was controlled/provided by a multichannel Oxyguard.

A temperature probe was placed in each tank (on the bottom in the middle) and 2 Oxyguard nodes. The Oxyguard controlled 4 Meiko UK Hi-Tech 1500 Mk 802 pumps which pumped air via air stones to each system as required.

A thermostatically controlled heater and a chiller coil were placed in each to allow temperature control. Temperature was monitored using a Grant 1200 Series Squirrel logger with one thermocouple per tank.

pH Measurement using a WTW pH91 meter.

Salinity Use of a WTW LF 320 conductivity meter with additional confirmatory checks using a refractometer and hydrometer.

Temperature Comark 9010 meter.

Ammonium (NH4) Measured as NH4. Initial trials involved the use of a Palintest however this was found to be lacking in sensitivity giving variable results and was replaced after trial 2b by a Dr Lange Lasa 20 Sensor Array Photometer.

Dissolved oxygen Conversion from ppm to % saturation was achieved using the formula:

(ppm/max ppm) x100

where:
max ppm at 35‰= $(0.0029 \times temp^2) - (0.2679 \times temp) + 12.011$
max ppm at 20‰ = $(0.0036 \times temp^2) - (0.3019 \times temp) + 12.627$
max ppm at 25‰ = $(0.0036 \times temp^2) - (0.309 \times temp) + 12.627$
max ppm at 30‰ = $(0.0035 \times temp^2) - (0.2884 \times temp) + 12.194$

3.2 Assessment Criteria

The 3 main criteria used for the assessment of depuration efficiency in the SFIA trials were:

1. Dissolved oxygen (d.o.) consumption

This was used to show the relative levels of activity of shellfish at the end of the 42 hour cycle and specifically over the last 2-3 hours. At the beginning of this 2-3 hour period all oxygenation of the water via either the spraybars or air pumps was stopped. No d.o. measurements were made for the first half hour after cessation of oxygenation to allow for the animals to resume normal functioning after having inevitably been disturbed by movement of the spray bars etc. The first half hour's worth of data after oxygenation had been stopped had earlier been found to be erratic and indicative of the animals having been disturbed and exhibiting intermittent activity. Any change beyond this "settling period", however, was measured with all tanks effectively starting from a zeroed d.o. point.

2. Visual observations of shellfish activity

These were made at intervals throughout the cycle and included assessment of siphon and foot activity, water clarity, foaming, faeces production and any other relevant features.

3. E.coli clearance

E.coli clearance was determined as a measure of depuration efficiency as part of the contamination trials and in the final microbiological verification trial.

A fourth measure was used to assess general survival characteristics under depuration conditions and this was the **Consolidated Quality Score (Post-depuration assessment)** which is defined as follows:

A subjective assessment of the post-depuration quality of the shellfish based on 4 main quality criteria and these are as follows:-

1 *Split* - Any splitting of the membrane from the pedal opening to the 4th opening (see diagram 1)- scored as 0 (no splitting) or 1 (splitting). See figure 2.

2 *Gaping* - Membrane totally split from pedal opening to 4th opening, foot can be observed inside sheath and animal has lost the ability to hold shell together - scored as 0 (no gaping) or 1 (gaping). See figure 2.

3 *Colour* - As assessed on foot, membrane around the pedal opening and the colour of the area around the siphons. During the course of the trials it was noted that the colour of the flesh changes from white through yellow to a brown colouration from the time of harvest and during depuration and subsequent storage - scored as 0 (white), 0.5 (yellow) or 1 (brown tinge). This change is believed to be associated with the physiological deterioration of the animal.

4 *Response* - Assessed by observing the movement of the foot through the bottom of the complete, split or gaping opening. The ability and speed at which the animal may retract its foot, and close or retract its siphons upon touching - scored as 0 (fast), 0.33 (slow), 0.66 (slight) or 1 (dead).

All of the above observations, although subjective to some extent, were made by the same worker and so the consistency of approach and assessment was maximised. This system of quality assessment has been developed by SFIA and adapted for the unique characteristics of razor clams

A set of consolidated quality scores (effectively being the average score) based on the post-depuration assessment scores was calculated for each trial variation. The lower the score, the better the quality of the individual.

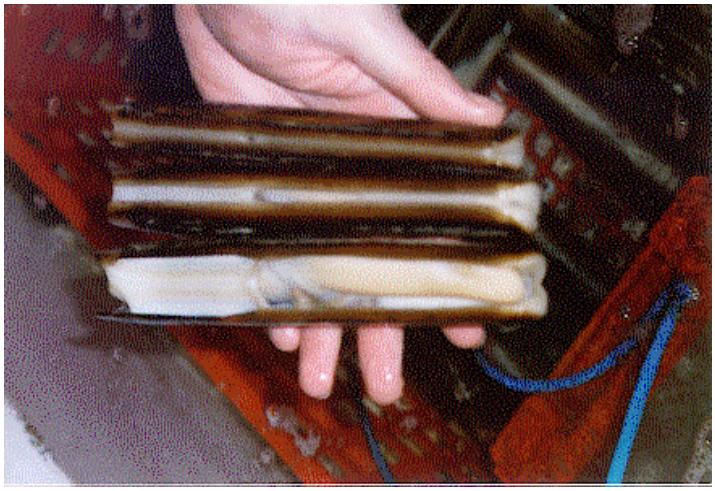


Figure 2 From top to bottom: healthy, split, gaping *E. siliqua*

Controls were kept at ambient temperature in bundles stored in their experimental orientations in a closed cool box.

A further determinand, **ammonium (NH4)** was measured and was chosen initially as its excretion is considered to be an indicator of overall metabolic activity. Its excretion by mussels has been shown to correlate with faecal deposition (Ref 4) and vary according to temperature and salinity (Ref 5). However, the ammonium results in these trials were disappointing and often contradictory and consequently, for the sake of clarity, they have not been included in this report.

3.3 Contamination procedure for razor clams used in microbiological trials

Unless stated otherwise the contamination procedure was as follows:

100ml of primary settled effluent (*E.coli* concentration of approx. 10^6 /100ml) was obtained mid treatment processing from Wessex Water's Loudsmill Sewage Treatment Works, Dorchester (predominantly domestic sewage – secondary treated final effluent). This was added to 170 litres of nominally clean seawater from Weymouth Bay in a small scale experimental depuration system (similar to that described above) housed in a microbiological isolator. Seawater was then recirculated for 1 hour $\pm$ 10 minutes (with the UV unit switched off). After this period of time the recirculation system was switched off and the system left for 10 minutes to allow any significant aerosols to disperse.

Shellfish were then added bundled with 10 individuals per bundle (2 bands per bundle) and packed vertically (all with siphons uppermost) in an Allibert 11037 tray and recirculation recommenced for 4 hours with water temperature being maintained at 12-13°C.

The system was then drained and, once drainage was complete, the shellfish were left for 10 minutes (or for as long as necessary) to allow for "spouting" to cease. As was observed in the MLA trials, adult *Ensis siliqua* can produce spouts of water in excess of 1m as a response to either being removed from water or to the water level falling below the siphons, as occurs on drainage of a system.

Shellfish were examined for *E.coli* using the standard modified method as reported by Donovan et al 1998 (Ref 2). Water/sewage samples were examined according to the standard potable water method as detailed in the Report on Public Health and Medical Subjects No 71 (Ref 3).

Artificially contaminated shellfish, when needed for subsequent depuration trials, were used within 2-3 hours.

4. Results

4.1 Determination of conditions for the artificial contamination of razor clams

Experiment 1
40 adult *Ensis siliqua* were contaminated as above but with the orientations described below. These razor clams were the remainder from the first trial investigating the effect of orientation and bundling (trial 1 - see below) and consequently had undergone 42 hours in the trial depuration tanks beforehand. Animals were kept in their trial orientations during contamination.

After 4 hours 4 samples were taken, each of 5 individuals. The results of these samples are as follows:

	***E.coli*/100g**
Vertical	5,400
Vertical	2,400
Horizontal	16,000
Horizontal	1,300

Experiment 2

90 *E. siliqua* were contaminated as above held in vertical bundles. 3 sets of 5 samples were taken randomly throughout the vertical bundles and tested for *E.coli* after 4 hours.

Results are as follows:

E.coli/100g
2,200
5,400
3,500

Samples were also taken for *E.coli* analysis after 24 hours (i.e. after being held in the same water as above with no additional effluent for 24 hours).

Results are as follows:

E.coli/100g
1300
2400
750

This appeared to demonstrate that no further uptake was taking place and that the razor clams were perhaps beginning to purge themselves of the *E.coli* - the 4 hour contamination cycle was therefore decided upon on the basis of this trial.

Initial feasibility assessment for the depuration of razor clams following artificial contamination

After the above sample had been taken, the remaining razor clams were rebundled and positioned vertically in an Allibert 11037 tray as before. String was tied across the tray to keep the bundles in position. The UV lamp was then switched on and water circulation recommenced. The temperature of the depuration tank water was 11°C. Three samples consisting of 5 individuals each were taken after 42 hours depuration. Results are as follows:

	E.coli
	20
	70
	20

The system was finally drained down and razor clams removed (6 days post harvesting at this stage). There were 28 razor clams alive and intact and 8 gaping or dead. All of these had remained bundled except 2 which had "escaped" during the depuration period both of which were dead/dying.

Experiment 3

250 *E.siliqua* were contaminated as above using 200mls of primary effluent. Three samples each of 5 specimens were then selected at random and tested for *E.coli*.

These gave the following results:

	***E.coli*/100g**
Sample 1	500
Sample 2	3500
Sample 3	5400

A further 5 specimens were measured, weighed and tested for *E.coli* with the following results:

	Length (cm)	**Total weight (g)**	**Shell weight (g)**	**E.coli**
Specimen 1	20	125.8	48.5	2400
Specimen 2	16.5	80.9	31.0	1700
Specimen 3	20	135.8	45.4	220
Specimen 4	16.5	74.6	29.5	16000
Specimen 5	20	137.5	48.5	2400

It can be seen that significant variation in *E.coli* content between individuals can occur after contamination and this may be indicative of differing levels of activity, differing percentage of body mass comprising the digestive tract or possibly some other factor(s).

As a further preliminary assessment of depuration ability, four SFIA tanks were then set up to operate at 90%+ dissolved oxygen with full strength seawater at 35‰ salinity and a starting temperature of 13°C. Problems of temperature control meant that the temperature increased to a little over 19°C during the 42 hour cycle. Each tank was loaded with *E. siliqua* in the orientations as described below. The results of these trials after 42 hours depuration are as follows (3 final samples were taken from each tank):

	***E.coli*/100g**
Tank 1 Banded horizontal	110
	20
	20
Tank 2 Loose horizontal	70
	40
	50
Tank 3 Banded vertical	20
	40
	110
Tank 4 Loose vertical	220
	90
	40

From these trials it was apparent that contamination of razor clams with primary sewage effluent to reasonably consistent levels of *E.coli* was possible using the procedure described above. Furthermore, the early signs were encouraging that microbiological removal could take place over a 42 hour period. The following trials were then undertaken to establish the conditions under which the activity of the animal could be optimised. It was assumed that the optimum conditions so determined would also maximise the removal of microbiological contamination.

4.2 SFIA trial results

Trial 1-Objective : to determine whether depuration of razor clams is viable

Trials commenced with a water temperature of 13°C and ended at a little over 20°C due to problems with temperature control. Dissolved oxygen levels were maintained above 75% throughout. Razor clams were held in 4 different arrangements, namely bundled horizontal, loose horizontal, bundled vertical and loose vertical. Individuals were orientated in the same direction within the bundles and vertical bundles were held with the siphons uppermost in keeping with the orientation of the animal in its natural environment.

Results are presented at appendix 2 (graphs 1 & 2)

Results of trial 1 indicate that *E. siliqua* are able to survive and apparently function in a system operating at a flow rate equivalent to 4 exchanges per hour with seawater at 35‰. The temperature for these trials increased from 13 to over 20°C which, whilst not intended, had the

effect of demonstrating that this species can withstand these sort of temperatures (under the conditions of this trial) without suffering excessive rates of mortality. The quality scores from this trial would appear to indicate that banding increased the effective shelf life of the individuals compared to animals held loose in the tank.

Trial 2 - Objective : To confirm optimum orientation in trays during depuration

Trial 2a carried out at Weymouth was abandoned due to problems with the shellfish supplied.

Trial 2b was carried out at Weymouth with *E.siliqua* and trial 2c at Ardtoe with *E.arcuatus.*

Trial 2b

Parallel trials were run with *E. siliqua* from Weymouth Bay orientated vertically and horizontally, both banded and loose. Dissolved oxygen levels were maintained at above 65%. The temperature at the start of the trials was just over 14° C and increased steadily during the trials to a little over 19°C, (again due to temperature control problems).

The dissolved oxygen consumption data show similar rates for the 4 orientation types, indicating similar rates of activity, although the vertical bundled orientation consumption rate was slightly higher overall. The post-depuration assessment scores in this trial do not show a significant difference between the orientation types although the horizontal banded orientation shows the best score overall.

Results are presented at appendix 2 (graphs 3 & 4)

Trial 2c

A further trial (trial 2c) on orientation was carried out at Ardtoe to investigate the effect of holding the razor clams in the vertical upside down (siphons lowermost) orientation to determine the effect that this would have on the activity of the animals. As noted above, this trial was carried out using *E. arcuatus.*

Results are presented at appendix 2 (graphs 5 & 6)

Trial 2c SFIA observations

20.00 17/4/99 – 15.00 19/4/99

Day	Time	Tank 2 Vertical	Tank 3 Horizontal	Tank 4 On Head
2	10.00	Siphons out all happy.	Feet out no movement	Feet slightly out some probing
2	17.00	Scum on surface. Siphons out all happy.	Clear water. Feet out no movement. Water clear.	Water clear. Lots of faeces on the tank bottom. Feet slightly out no movement.
3	09.00	Siphons pulsing. Plenty of scum on the surface.	Feet out ½way. No movement. Water clear.	Water clear. Feet slightly out. Some individuals probing.

The dissolved oxygen results of trial 2c (carried out at approximately 10°C) suggest very sporadic activity in the head down orientated animals and the post-depuration scores show greater deterioration in these animals than the bundled head-up vertically and horizontally orientated animals. Both these results and the quality score results would appear to suggest that the "head-down" orientation is not an appropriate way to hold these animals under depuration conditions. The results from this trial also suggest that there is little difference between the horizontal and vertical orientations. In terms of reducing the number of escapees (which tend to deteriorate quite quickly) and ease of loading trays the horizontal orientation is preferable. On the basis of this finding, the remainder of the trials were carried out using bundled animals held horizontally.

Trial 3 - Objective : To determine appropriate temperature range

Trials were set up to investigate physiological activity at 5, 10 and 15°C using *E. arcuatus.*

Results are presented at appendix 2 (graph 7)

The dissolved oxygen results for trial 3 show a marked difference in consumption between the 3 temperature regimes, with apparently very little activity taking place at 5°C and little difference between 10 and 15°C which show a significantly higher level of activity.

Trial 3 SFIA observations

Trial Started at 16.45 15/4/99 ended 11.10 17/4/99
Animals obtained from Kentra Bay.
All animals held horizontally.
Salinity 35‰

Day	Time	Tank 2 Ave 15.3c	Tank 3 Ave 9.7c	Tank4 5.2c
1	19.45	Only 11 feet slightly out no movement	Some rapid movement. Feet well out. Only 5 shells without feet extended.	21 feet extended slower movement.
1	21.30	No change	No change	No change
2	0900	One bundle not showing any feet at all some other animals with feet retracted	No change	Some feet slightly more extended. No movement.
2	17.15	All feet, bar one bundle well out. No movement	No movement. Feet well out	All feet extended slightly bar 2.
2	21.15	All feet out – no movement. No feet shown on one bundle	No movement- feet well out.	No movement feet well out.

Trial 4 - Objective : To determine appropriate salinity range

Trials were conducted at 35, 30, 25 and 20‰ using *E.arcuatus.*

Results are presented at appendix 2 (graph 8)
The salinity trials were carried out at an average temperature of 10.75°C. The dissolved oxygen consumption data indicates that there is a greater level of activity at 35‰ which is perhaps to be expected at this was the salinity prevailing in the harvesting area. It should also be noted that razor

clams generally occur in coastal areas where salinity fluctuation is minimal and at or approaching "full strength" i.e. 32-35‰.
The 3 lower salinity regimes yielded lower d.o. consumption rates with that at 20‰ being the lowest of all. Visual observation confirmed that little activity took place at 20 ‰.

Trial 4 SFIA observations

17.00 wed 21/4/99 – 11.00 Fri 23/4/99.

Day	Time	Tank 1 20‰	Tank 2 25‰	Tank 3 30‰	Tank 4 35‰
1	17.00	Start	Start	Start	Start
2	09.00	Siphons retracted Feet out	Siphons out. Feet out. Scum on surface.	Siphons out. Feet out Small amount of scum on surface. Lots of faeces.	Siphon out. Feet out. No Scum. Water clear. Some faeces.
3	11.00	Only at most ¼ have siphons open. Water clear.	All siphons open and tentacles up.	All siphons open. Small amount of scum on surface.	Most have siphons out & tentacles up. Water clear. Some faeces.

Trial Ardtoe 4b

This trial (as per trial 4 with *E. arcuatus*) investigated the effects of salinity (35, 30 and 20 ‰) on *E. siliqua* at 100% d.o. For comparison a tank was also run at around 50% d.o. at 35 ‰. As was found with *E. arcuatus*, the shellfish in the 20‰ salinity tank showed little activity.

The 35 and 30‰ salinity regimes at 100% d.o. showed similar d.o. consumption rates suggesting similar levels of activity. This assumption appears to be supported by the observation data (excluding the apparent spawning in tank 1).

The shellfish held at 35‰ and 50% oxygen appear to show erratic activity according to the d.o. consumption plot. This is similar to the plot obtained from the shellfish held at 20‰ and 100% d.o. (which in earlier trials was found to produce a poor response from the shellfish) and might suggest that the shellfish are not functioning well at 50% d.o. despite being in full strength seawater.

Results are presented at appendix 2 (graph 9)

Ardtoe 4b SFIA observations

All *E. siliqua.*

14.00 10/6/99 – 08.00 12/6/99

Day	Time	Tank 1 100% DO 35‰	Tank 2 100% DO 30‰	Tank 3 100% DO 20‰	Tank 4 50% DO 35‰
1	17.00	Feet out. Siphons out Faeces out. Clear water.	Feet out. Siphons out Faeces out. Clear water	Only 4 with siphons out. Very few feet out. No faecal deposition. Clear water.	Feet out. Siphons out. Clear water.
2	09.00	Feet out. Siphons out Faeces out. Slight slick	Feet out. Siphons out Faeces out. Clear water	Only 4 with siphons out. Very few feet out. Clear water. Some faeces on surface of water.	Feet out. Siphons out. Clear water
2	12.00	Tank gone Cloudy. Spawning ?? Drain ½tank down and refill with new seawater 13ºc	Clear	Clear	Clear
2	17.00	Clear. Feet & siphons out.	Clear. Feet & siphons out.	Clear. Some siphons out. Some feet out.	Spawned ??. Feet and siphons out.
3	08.00	Clear. Feet & siphons out	Clear. Feet & siphons out	Clear. feet & siphons out Small amount of faeces on bottom & water surface.	Clear. Feet & siphons out

<u>Trial 5 - Objective : To determine appropriate range of flow rates/dissolved oxygen levels</u>

Trials were set up to run at 100%, 75%, 50% and 25% d.o. saturations with *E. arcuatus.* Some difficulty was experienced in achieving the intended d.o. levels which were averaged out at approximately 95, 78, 71 and 65%. Results are presented at appendix 2 (graph 10) with the legend denoting the intended d.o. levels rather than actual.

The dissolved oxygen trials with *Ensis arcuatus* (average temperature 10.6°C) show a much greater consumption of dissolved oxygen in the 95% tank than the other 3 (average 78,71 and 65% respectively). This suggests a greater level of activity in the shellfish having been held at 95% saturation although may also be due to some extent to oxygen coming out of solution by physical processes not connected with the activity of the shellfish, however, no control data is available to verify this point. Observation of activity suggest little difference between oxygen regimes although

the production of some foam at 95% might indicate a higher overall level of activity at this level of d.o.

Trial 5 SFIA observations

18.30 29/4/99 – 12.030 1/5/99:

Day	Time	Tank 1 95% DO	Tank 2 78% DO	Tank 3 71% DO	Tank 4 65% DO
2	08.45	Siphons out. Water clear.	Siphons out. Water clear.	Siphons out. Water clear.	Siphons out. Water clear.
2	17.00	Siphons out. Slight slick on water	Siphons out. Water clear.	Siphons out. Water clear.	Siphons out. Water clear.
3	08.30	Siphons out. Slight slick on water. Slight foam	Siphons out. Water clear	Siphons out. Water clear	Siphons out. Water clear
3	12.30	Siphons out. Slight slick on water. Slight foam	Siphons out. Water clear	Siphons out. Water clear	Siphons out. Water clear

<u>Trial 6 - Objective : To determine appropriate shellfish:water ratio.</u>

Trials using *E.siliqua* were run at shellfish : water ratios of 1:2.2 and 1:9.3.

The shellfish:water ratio trials with *Ensis siliqua* carried out at an average temperature of 13.5°C show a predictable oxygen consumption pattern i.e. the regime with the higher shellfish loading showing a higher level of oxygen depletion. Interestingly, the plot for the 2.2:1 regime indicates a tailing off of activity below around 5ppm (50-55% saturation at 13.5°C) which might suggest that this is the threshold at which d.o. may be limiting activity. Observations of activity do indicate some difference with apparently some inhibition (fewer siphons out) taking place at the higher shellfish density. Inhibition of activity however is of course highly undesirable during depuration.

Results are presented at appendix 2 (graph 11)

Trial 6 SFIA observations

17.00 5/5/99 - 11.007.5.99:

Day	Time	Tank 1 2.2:1	Tank 2 9.3:1
2	0845	Foaming up nicely. Not all animals with siphons out. Faeces on top of foam.	Slight slick. Most siphons out.
2	17.00	Animals obscured by foam. Most siphons out. Faeces on floor and on top of foam.	Slight slick. All siphons out & most feet. Faeces on floor of tank and on surface of water.

3	09.00	Animals obscured by foam. Most siphons out. Faeces on floor and on top of foam.	Slight slick. All siphons out & most feet. Faeces on floor of tank and on surface of water.
3	11.15	Foam and faeces everywhere. Animals feet agitated	Slick, lots of faeces. Animals feet moving.

Trial Ardtoe 6b

This trial again using *E. siliqua* investigated the effect of 3 different shellfish:water ratios (1:4.6, 1:6 and 1:58). Little difference was detected in terms of visual observation between the shellfish at the trial ratios. Dissolved oxygen consumption rates follow a predictable pattern with the highest density of shellfish showing the highest consumption rate.
Results are presented at appendix 2 (graph 12)

Ardtoe 6b SFIA observations

15.15 12/6/99 – 14.0014/6/99

Day	Time	Tank 1 1:4.6	Tank 3 1:6	Tank 4 1:58
2	16.00	Water clear. Siphons out. Feet out.	Water clear. Siphons out. Feet out.	Siphons out. Feet out. Water a bit cloudy.
3	09.00	Siphons out. Feet out and moving. Water clear but foamy	Siphons out. Feet out and moving. Water clear but foamy	Siphons out. Feet out but still. Water a bit cloudy

Trial 7 - Objective : To determine the effect of different harvesting methods

This trial was intended primarily to compare the response of fluidised dredged razor clams with that of diver-gathered animals under depuration conditions. However, the work carried out on dredged animals was limited due to time and resource constraints and no data is presented. The work undertaken consisted of one day's worth of dredging covering both hard and soft ground off of the west coast of Scotland in a 36ft vessel equipped with fluidised bed dredging apparatus. A depuration trial was subsequently carried out with the dredged animals which included *E. siliqua* and *E. arcuatus* and the following preliminary observations were made:

Damage to the shell was found in a significantly higher number of dredged individuals than would normally be found in diver-gathered specimens. Given the higher incidence of visible damage it might be reasonable to assume that there would also be a higher incidence of “unseen” damage (i.e. to internal organs) in dredged animals compared with diver-gathered specimens which would have an adverse effect both on depuration efficiency and the subsequent shelf-life of the animal.

As an additional observation it was noted that animals harvested from soft ground were packed with sand and sediment to such an extent that subsequent depuration appeared to be significantly impeded.

Further work will be needed to investigate the relative damage and mortality rates of dredged animals compared with diver-gathered specimens and the subsequent depuration efficiency and shelf-life of the animals.

Trial 8 - Objective: To determine comparability between species

Trials were undertaken with *E. arcuatus* and *E. siliqua* at 2 temperatures averaging 15.6 and 7.3°C. The d.o. consumption results appeared to suggest a difference in temperature response between the 2 species at 15.6°C with *E. siliqua* perhaps being less active, however further work would be necessary to confirm this. At 7.3°C both species appear to be showing a similar level of activity. Visual observations appeared to show that *E. arcuatus* was the more active of the 2 species under the trial conditions.

Results are presented at Appendix 2 (graph 13)

Trial 8 SFIA observations:

17.00 10/5/9 – 09.00 12/5/99

Day	Time	Tank 1 Siliqua 7.3ºc	Tank 2 Arcuatus 7.3ºc	Tank 3 Siliqua 15.6ºc	Tank 4 Arcuatus 15.6ºc
2	09.00	Clear water. 50% siphons out.	Slick on top. All siphons out	All siphons out. Slight foam. More faeces than tk's 1 & 2.	All siphons out Slightly better foam than Tk 3. More faeces than 1 or 2.
2	17.00	Most siphons out. Slick on water.	Most siphons out. Slick and thin foam on water.	Clear water. Foam under spray bar only. Siphons out.	Good foam under spray bar. Siphons out.
3	09.00	Slick across tank. Slight foam ?.	Foam dense. Not as thick as tank 4. Siphons out.	Some foam ~¼Tank. Siphons out.	Foamed up all across tank. Thick & white. Siphons out.

4.3 Final microbiological verification trial

Following on from the SFIA work, a final microbiological trial was run at Weymouth using a set of conditions considered to be the minimum acceptable for depuration based on the results of the trials carried out.

208 razor clams were contaminated for 4 hours with primary sewage effluent as before at a temperature of 12-13°C. After this time 2 samples of 5 individuals were taken at random and tested for *E.coli* with results as follows:

	***E.coli*/100g**
Sample 1	**9100**
Sample 2	**5400**

The tank was then drained down, refilled with 80 litres of seawater and diluted with freshwater to a final salinity of 30‰. The remaining bundles were laid directly onto the bottom of the tank with body length lying perpendicular to the flow of water i.e. with siphons and feet facing the side walls. The trial commenced at 12.8°C and ended at 9.1°C with an average temperature over the 42 hour period of 9.9°C.

The flow rate was set at 310 litres per hour, which approximated to a little under 4 exchanges per hour with 80 litres of water. The shellfish to water ratio was 1:4. Dissolved oxygen at the start of the trial was 88% throughout the tank.

After 12 hours the water temperature was measured at 10.5°C, d.o. cascade 72%, middle 70%, suction 71%, salinity 30 ‰ and flow rate 300 litres per hour (reduced by foot of animal stuck in suction pipe). A thin layer of white foam was found to be covering approximately 20% of the water surface. Water clear and siphons extended and apparently functioning. Quick retraction of siphons when touched. Feet extending 2-4cms in most animals. Mucoid faecal deposits evident on base of tank. 2 samples of 5 individuals taken at random throughout tank and tested for *E.coli* with results as follows:

	***E.coli*/100g**
Sample 1	**9100**
Sample 2	**5400**

After 18 hours the water temperature was measured at 9.7°C, d.o. cascade 72%, middle, 70%, suction 70%, salinity 30 ‰ and flow rate 305 litres per hour (foot of animal having been removed from after suction pipe at 12 hours). Thin layer of white foam still covering approximately 20% of the water surface. Water clear and siphons extended and apparently functioning. Relatively quick retraction when touched. Feet extending 2-4cms in most animals. Mucoid faecal deposits evident on base of tank. Further 2 samples taken (5 individuals each) with results as follows:

	***E.coli*/100g**
Sample 1	**1300**
Sample 2	**1300**

After 36 hours the water temperature was measured at 9.1°C, d.o. cascade 82%, middle, 81%, suction 81%, salinity 30.5 ‰ and flow rate 310 litres per hour. Thin layer of white foam now covering approximately 50% of the water surface. Water clear and siphons extended and apparently functioning - relatively quick retraction when touched. Feet extending 2-4cms in most animals. Mucoid faecal deposits evident on base of tank. Samples were not taken for *E.coli* testing at this point.

After 42 hours the water temperature was measured at 9.1°C, d.o. cascade 80%, middle, 81%, bottom 81%, salinity 30.5 ‰ and flow rate 310 litres per hour. Thin layer of white foam now covering approximately 70% of the water surface. Water clear and most siphons still extended and apparently functioning - relatively quick retraction when touched. Feet extending 2-4cms in most animals. Mucoid faecal deposits evident on base of tank.

	***E.coli*/100g**
Sample 1 at cascade	**160**
Sample 2 in middle of tank	**220**
Sample 3 at suction end	**1300**
Sample 4 split/gaping individuals	**2400**

All animals sampled (except those in sample 4 after 42 hours) were apparently healthy with no sign of splitting or gaping. A total of 19 individuals out of 208 used in the trial were found to be split or gaping at the end of the 42 hour period.

The results of samples 1 and 2 are promising given the high initial *E.coli* loadings (class C range) of the shellfish and are particularly encouraging, as the shellfish were loaded directly onto the bottom of the tank and not raised clear of the bottom (away from settling faecal material as is the normal MAFF/CEFAS requirement). This was so as to achieve the required trial shellfish : water ratio of 1:4. In addition, the shellfish were disturbed at regular intervals for the taking of samples and determining speed of response to touch. The trial conditions can therefore be considered to be a worse case scenario. The result for sample 3 is disappointing but when taken in the context of the experimental conditions described above is perhaps more understandable. The considerable variation amongst individuals (shown in analysis of the 5 individuals from experiment 3 of the contamination trials) may also go some way towards explaining this higher result if a grossly contaminated individual (or individuals) was present in this sample - it is accepted of course that this argument could be also used to justify the opposite case. The sample of split and gaping individuals (sample 4) shows the worst result of all and it would be reasonable to assume that such individuals have an impaired level of functioning under depuration conditions. Given the consequences of this for final product safety and the fact that such split/gaping individuals have been found to have a much reduced shelf life anyway, it is a recommendation of this report that such individuals should be discarded.

5. Discussion and Conclusions

As was noted in the MLA report, razor clams are very different from any shellfish currently approved for depuration in England and Wales. The experience gained during these trials has demonstrated that razor clams are sensitive animals, susceptible to stress, that can deteriorate rapidly if left with their valves unrestrained i.e. out of their burrows or unbanded for any length of time and, in particular, for more than a few hours.

It was found that in stressed animals (e.g. those individuals that had been out of water for some time) the production of mucus can be significant. Such mucus production can cause problems during depuration, as was found in the MLA trials in which a reduction in flow rate was observed due to the blocking of the flow screens and pipework. It should be noted that the MLA trials used shellfish generally stored out of water for over 24 hours. Using freshly gathered specimens however e.g. those used within 6 hours of harvesting, mucus production was considered to be negligible and did not appear to cause problems during the various trial conditions presented above. Given the potential for mucus production, however, there may need to be an increased frequency of cleaning for pipework, UV lamp sleeves and tank walls in a commercial setting. In addition, the re-use of seawater would be inadvisable without further investigation.

The best way of holding razor clams under depuration conditions was found to be in bundles of approximately 10 animals restrained by a rubber band at each end. Banding was found to increase the life of the animal without adversely affecting the activity of the animals. Holding such bundles horizontally allowed for flexibility in the loading of trays and appeared to allow the same level of activity as banded animals held vertically.

Holding animals vertically, whilst seemingly preferable in terms of emulating the orientation of the animal in its natural environment, presented some problems in these trials. It was found that individuals within bundles held vertically (foot lowermost) could cause the entire bundle to move by flexing of the muscular foot. In some cases this caused the individual(s) to rise up out of the bundle and thus end up "free" (with valves unrestrained) in the bottom of the tray or tank. Such escapee individuals were invariably found to deteriorate more rapidly than those remaining within the bundle. Given the rapid deterioration of such individuals, it is a recommendation of this report that they should be discarded at the end of the purification cycle. Escapees were very rarely found from horizontally held bundles.

Ammonia results during the trials were disappointing and often inconclusive. It should be noted that ammonia production has been linked to the production of faeces (Ref 4), however, it is also believed to be produced under stress conditions (Ref 5) and its production in this case may or may not be linked to the production of faeces. Therefore, the use of ammonia production as a determinand in assessing depuration efficiency has the potential to be misleading.

Activity at 5°C was found to be limited and, until further work is carried out, a minimum temperature of 10°C is recommended for the depuration of these animals.

The activity level of razor clams at salinities below 30‰ was found to be significantly reduced compared with that at 35‰ and, given the natural environment of these animals being one of "full strength" seawater, this is perhaps not surprising. Consequently, it is recommended that the salinity

of water used for depuration should be maintained at the level prevailing in the harvesting area and, in any event, not less than 30 ‰.

The dissolved oxygen trials carried out showed no apparent inhibition of activity down to 65% saturation, however, there was some evidence of it becoming a limiting factor at 50-55% saturation (5ppm - see trial 6). Consequently, it is proposed that dissolved oxygen levels should be maintained at 65% or above.

The shellfish : water ratio trial observations with *E. siliqua* suggested some inhibition of activity at a ratio of 1:2.2. Subsequent trial observations made at a ratio 1:4.6 indicated that activity levels were similar to shellfish loaded at ratios of 1:6 and 1:58. However the microbiological results of the verification trial carried out at a ratio of 1:4 were disappointing and a ratio of 1:6 is recommended until further work is undertaken to clarify this point.

All trials carried out in this project were with banded bundles of razor clams held in a single layer within a tray (or tank). Furthermore, the effect of stacking trays was not investigated and so until further work proves otherwise it is recommended that purification should be carried out with a single layer of bundles held in a single layer of trays.

Given the reduced shelf-life of split/gaping individuals and the evidence presented in this report of their reduced depuration efficiency, it is recommended that they should be discarded as should individuals showing other signs of damage such as chipped or broken shells.

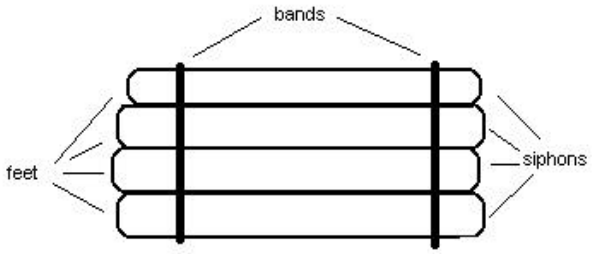
The limited trials carried out with dredged razor clams raised a number of questions that remain to be answered. Initial observations appeared to confirm that a significant level of damage can occur to razor clams when dredged (SFIA pers. comm.) and that this damage may lead to death or sub-lethal injury. Shell damage is obvious, however, internal damage to the soft body tissues is less apparent and may significantly impair the animal's subsequent ability to depurate. This effect has been noted in dredged cockles as discussed previously in section 3 (Methodology) (ref 6). Further investigation is therefore necessary to clarify the level and extent of damage caused by dredging and the effect that this may have in terms of the food safety and economic viability of the depurated product. The type of bed substrate may also have a significant effect on the subsequent depuration efficiency of the animals and may warrant further study.

As a final point for consideration, it was also found, and was reported by MLA that, upon draining down, razor clams can produce spouts of water to a height of 1 metre or more. Consequently, it would be sensible to take adequate precautions to ensure that electrical and other such sensitive devices are not placed in the immediate vicinity of purifying razor clams.

6. Summary of Recommendations

The recommendations below only apply to diver-gathered razor clams.

Post-harvesting handling: Razor clams intended for depuration should not show signs of damage to the shell or soft body tissues. They should be placed in bundles (double banded) immediately after harvesting (within 1 hour). As a guide, 10-12 adult *Ensis siliqua* should be held per bundle using 3 1/2 x 1/4 inch (89 x 6mm) rubber bands with, one band at each end.



Maximum time between harvesting and re-immersion: The maximum period between *harvesting and re-immersion* (in clean seawater or seawater of no worse quality than that found in the harvesting area) should be limited to 6 hours. In any event, the maximum period between *harvesting and purification* must be 24 hours.

Loading arrangement in trays: Bundles should be laid horizontally in trays with the length of the animal being positioned perpendicular to the direction of water flow within the tank. Bundles should only be loaded in a single layer within a tray. Trays should only be loaded in systems in a single layer.

Minimum temperature: 10°C

Minimum salinity: Seawater of the same salinity to that occurring in the harvesting area should be used and, in any event, should not be less than 30‰.

Minimum dissolved oxygen: 65% saturation

Maximum shellfish : water ratio: 1:6

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

Razor clams harvested by other methods

It is recommended that further work should be carried out to clarify the level and extent of damage caused to razor clams harvested by dredging and the effect that this may have in terms of their subsequent depuration ability.

8. References

1. Preliminary trials to assess the viability of purifying the razor fish *Ensis siliqua* (L.) by depuration using Ultra Violet sterilisation. F G Howard, D J Basford and J Graham.

2. Donovan T D, Gallacher S, Andrews N J, Greenwood M H, Graham J, Russell J E, Roberts D and Lee R (1998). Modification of the standard UK method for the enumeration of *Escherichia coli* in live bivalve molluscs. Communicable Disease and Public Health.

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4. Hawkins, Bayne and Clarke. Co-ordinated rhythms of digestion, absorption and excretion in *Mytilus edulis*. Marine Biology 74, pp 41-48. 1983.

5. Bayne, B L. Marine mussels: Their ecology and physiology (Internal Biological Program: 10). Cambridge University Press. ISBN0521 210585. 1976.

6. Boulter M et al. Trials to Assess the Viability of Purifying Cockles. Three Phases of Trials Conducted Between 1990 - 1992.

APPENDIX 1

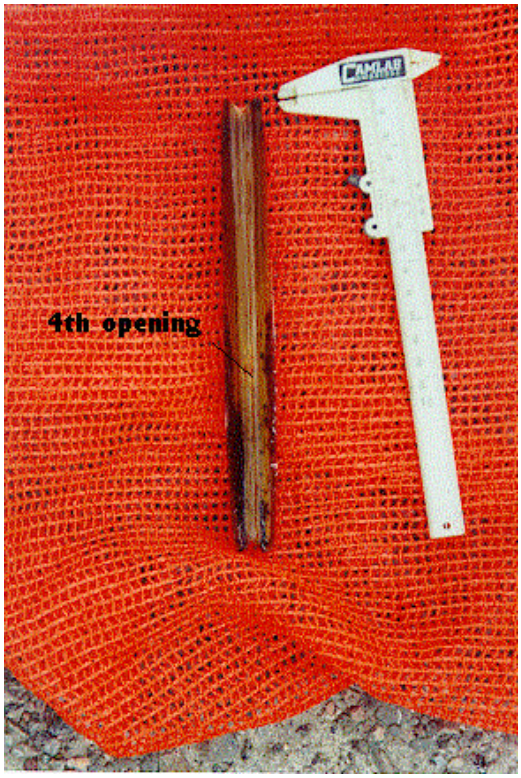


Figure 3 : *E. siliqua*: membrane (ventral) side 4th opening visible

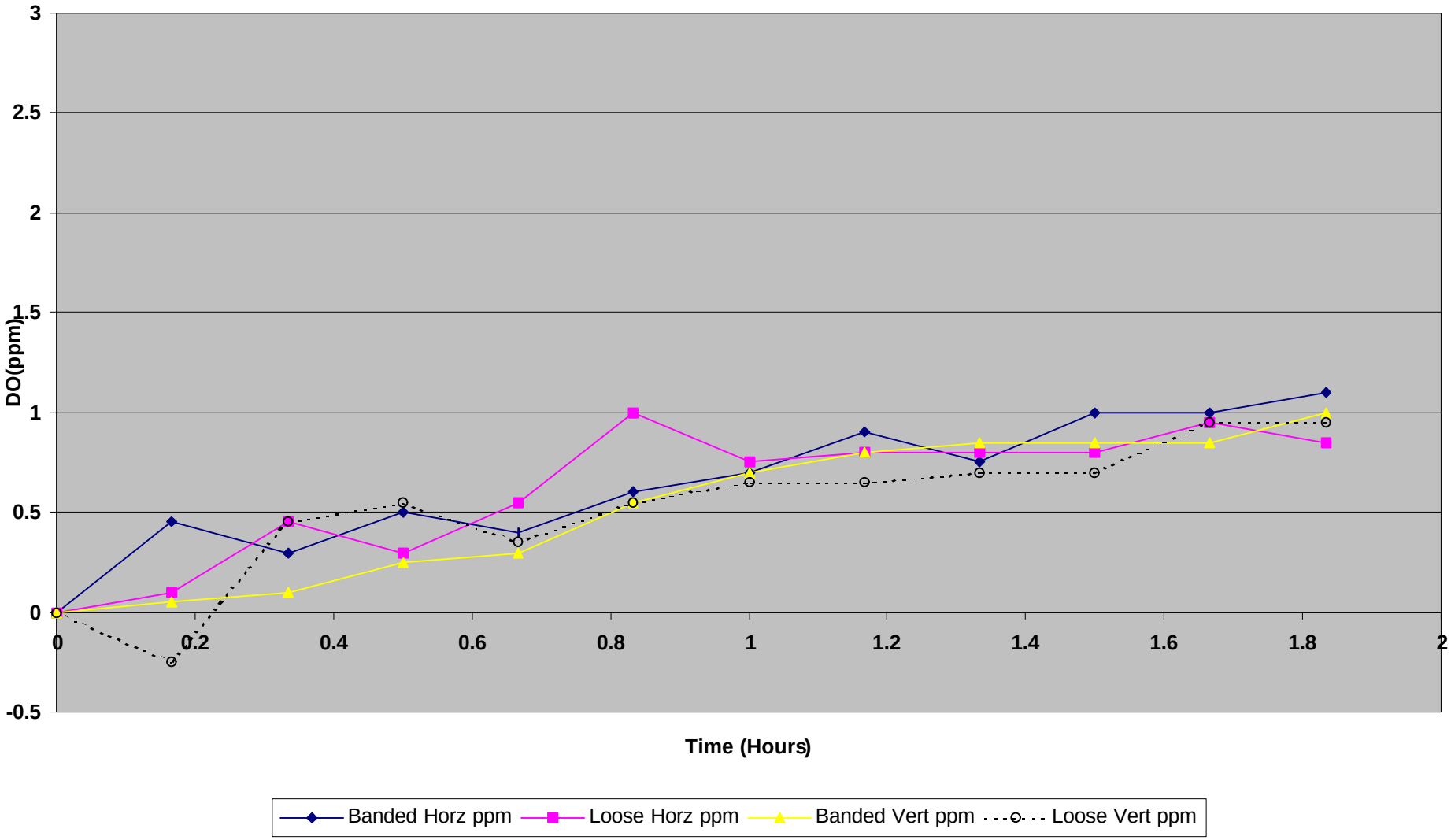
Figure 4 : *E. siliqua*: hinge (dorsal) side

Figure 5 : *E. siliqua*: foot (posterior) end

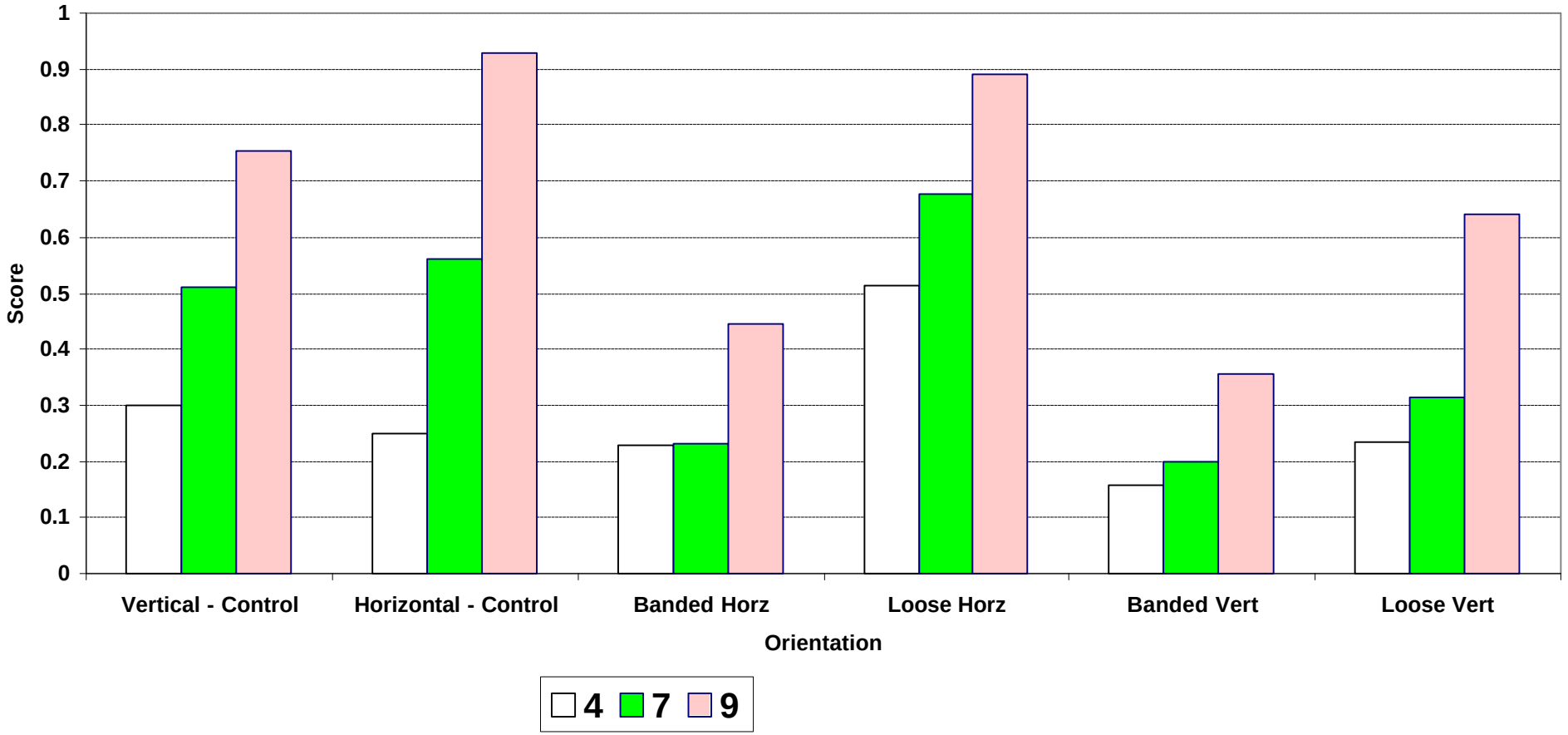
Figure 6 : *E. siliqua*: siphon (anterior) end

APPENDIX 2

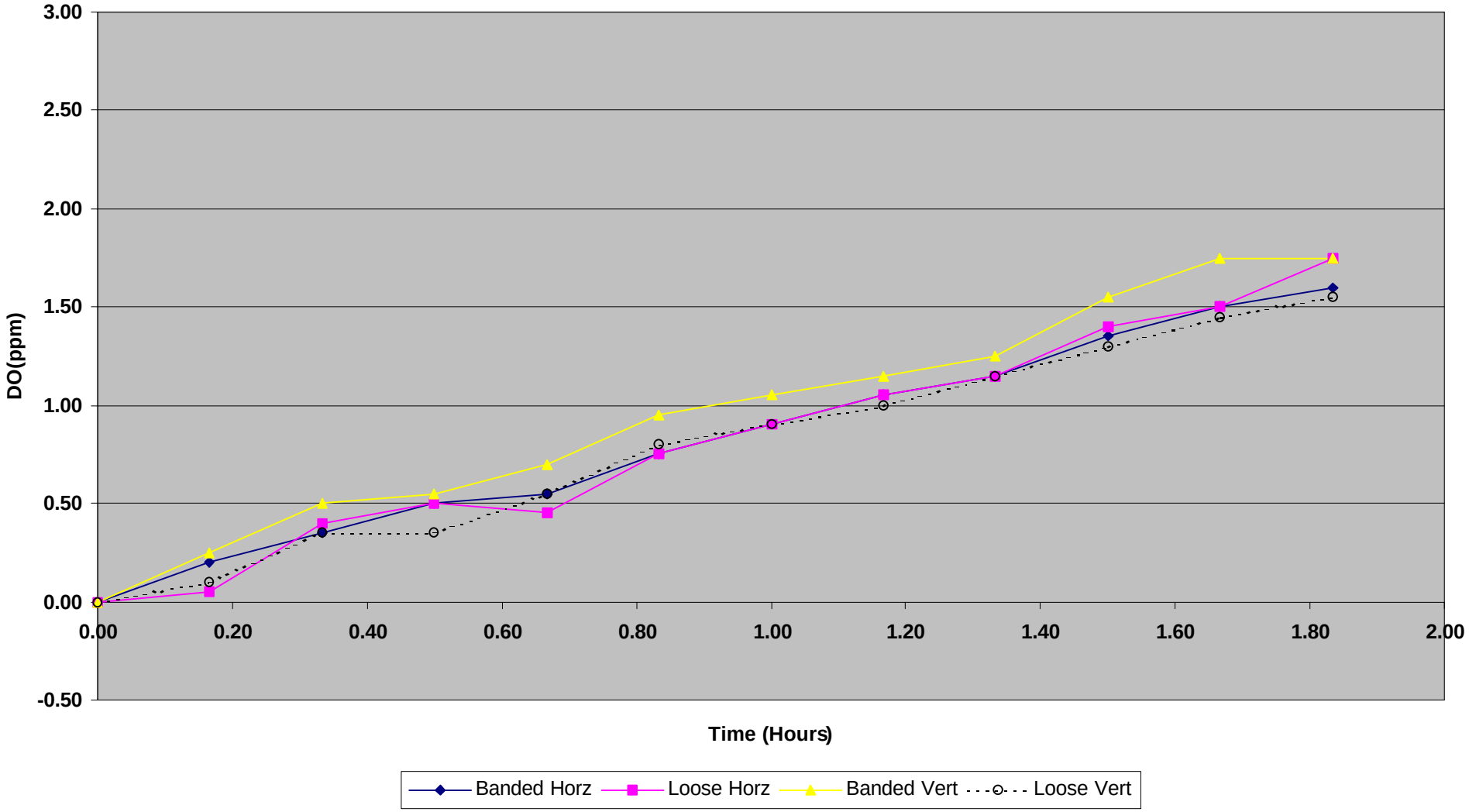
Graph 1 : DO Consumption - Trial 1 (Orientation)



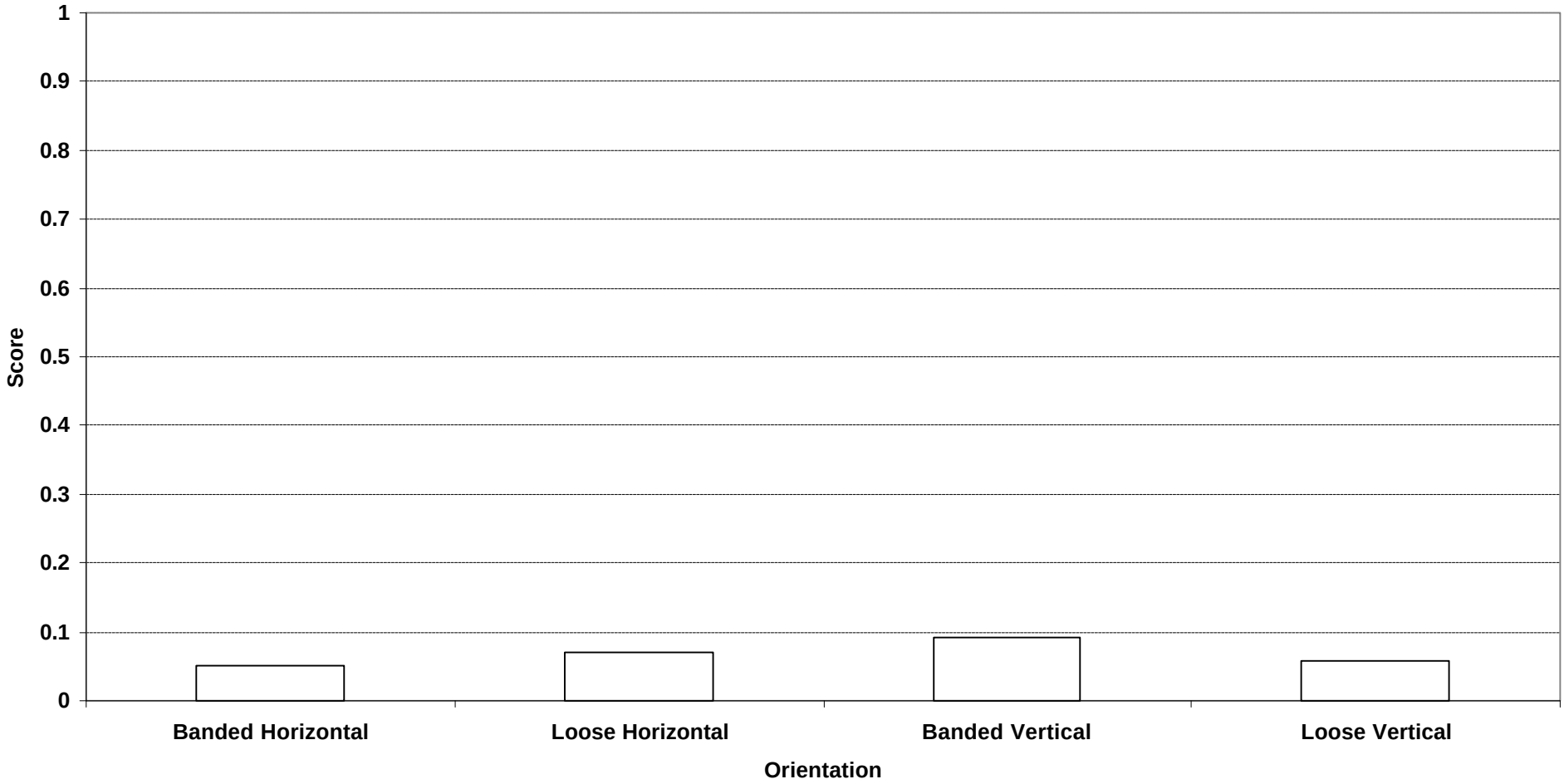
Graph 2 : Consolidated Quality Scores- Trial 1 (Orientation)



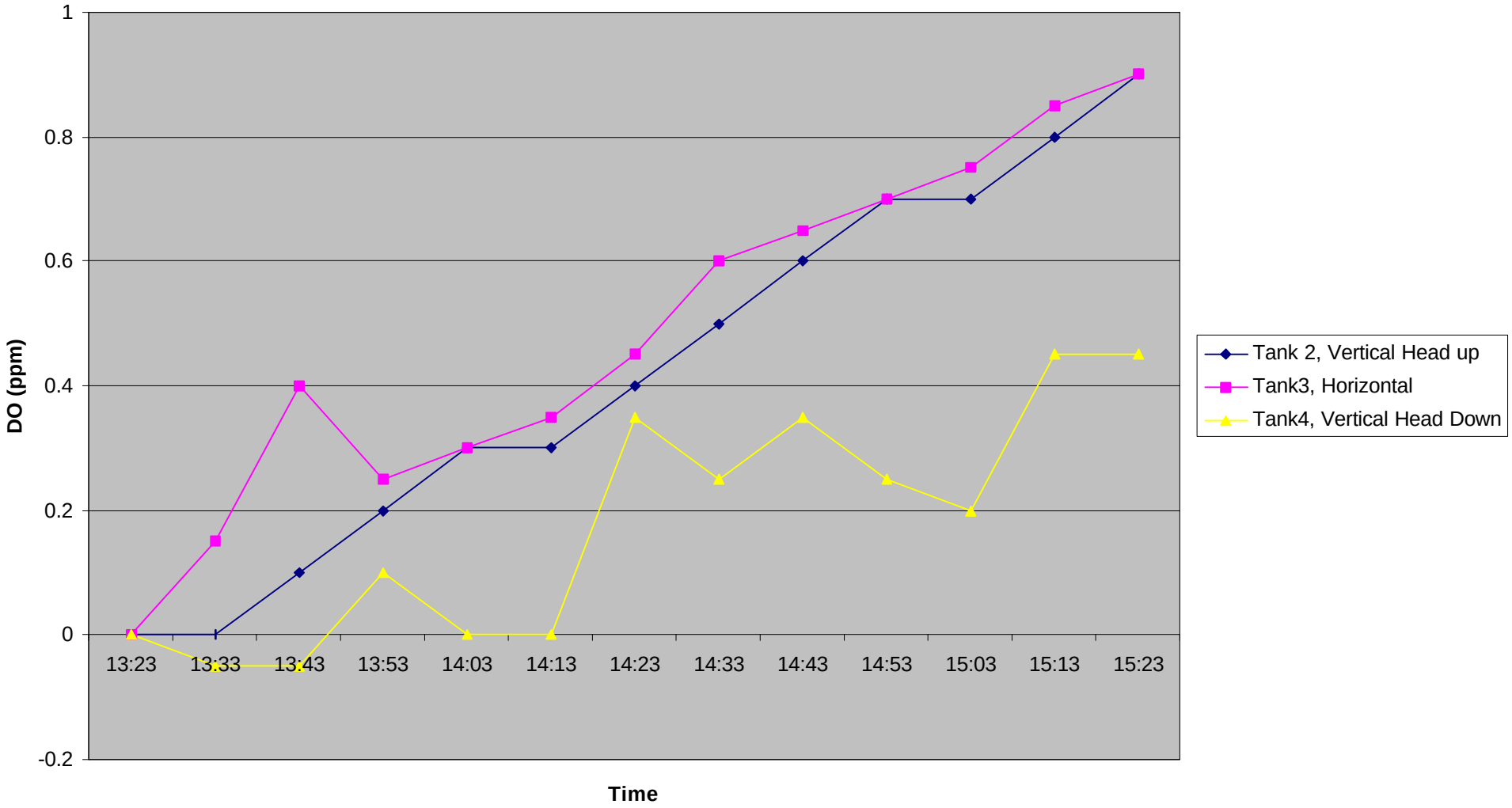
Graph 3 : DO Consumption - Trial 2b (Orientation)



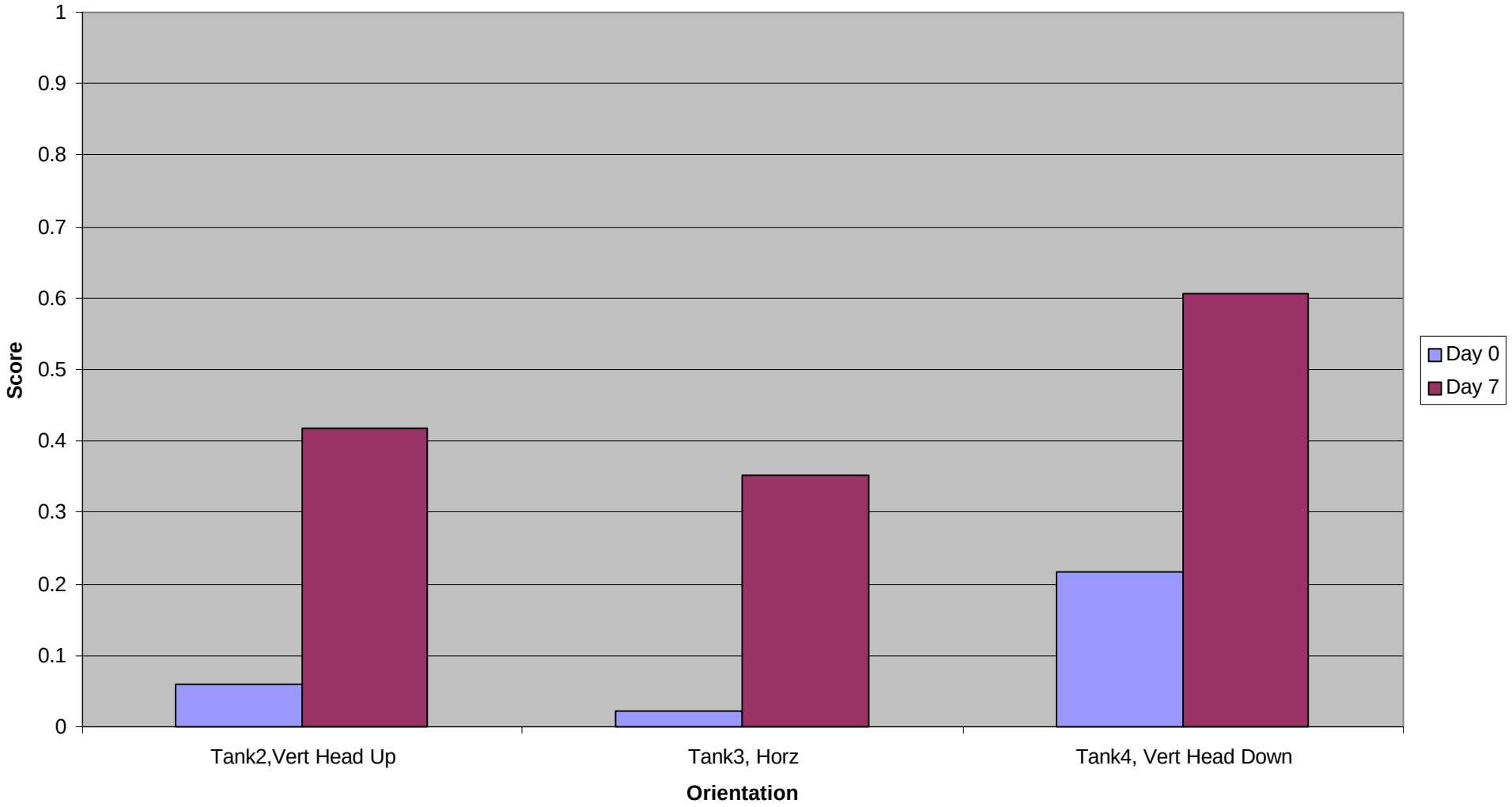
Graph 4 : Consolidated Quality Scores- Trial 2b (Orientation)



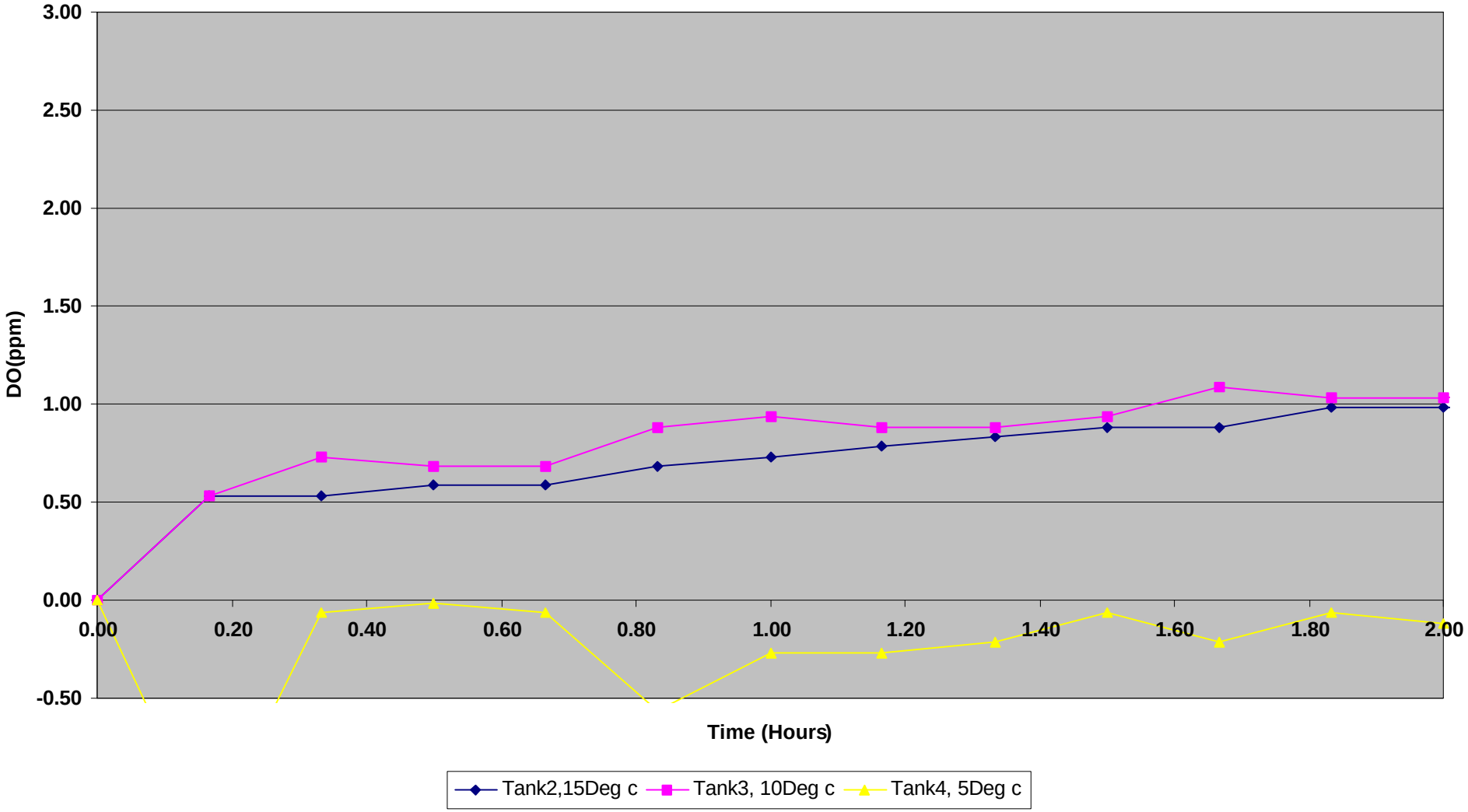
Graph 5 : DO Consumption - Trial 2c (Orientation)



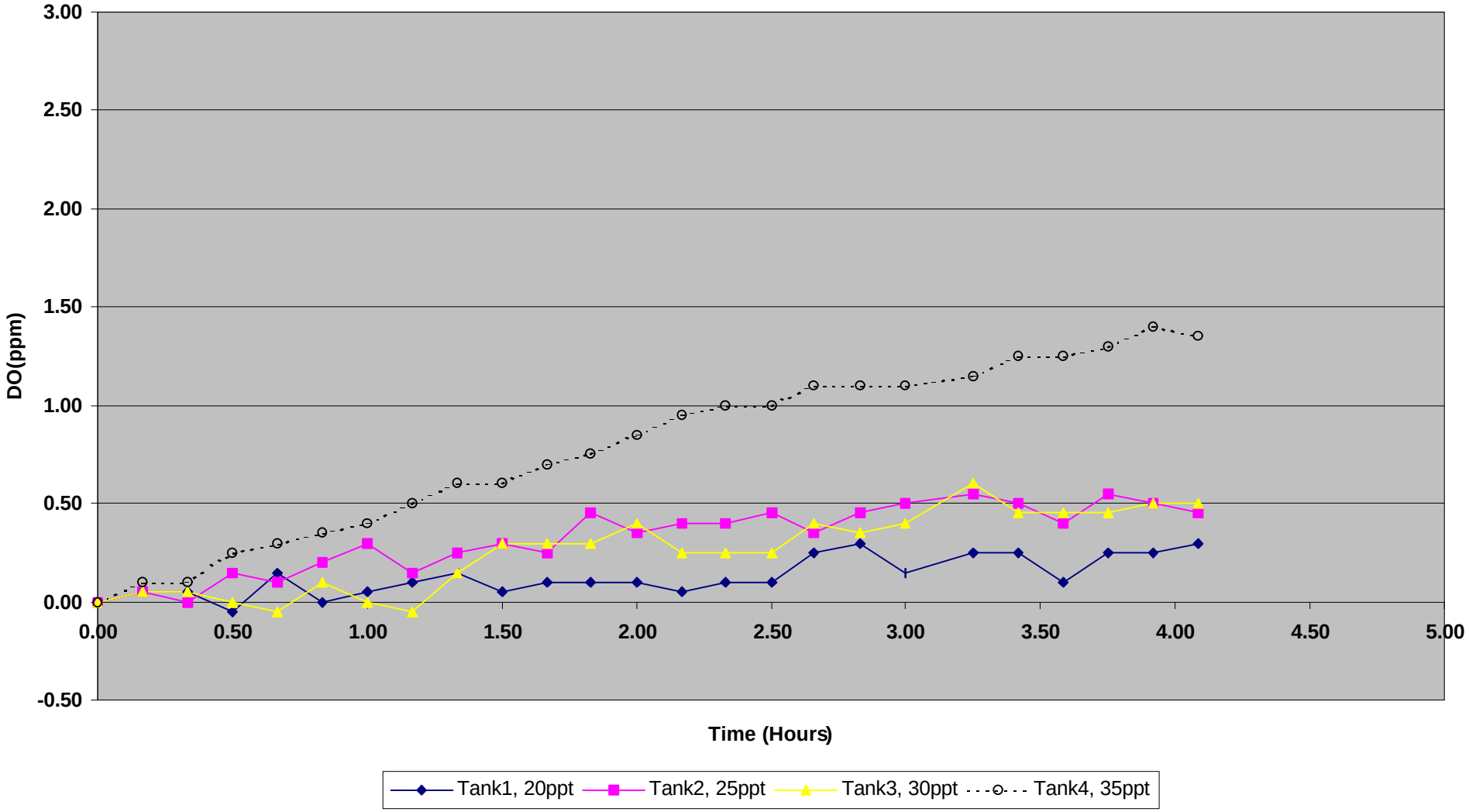
Graph 6 : Consolidated Quality Scores - Trial 2c (Orientation)



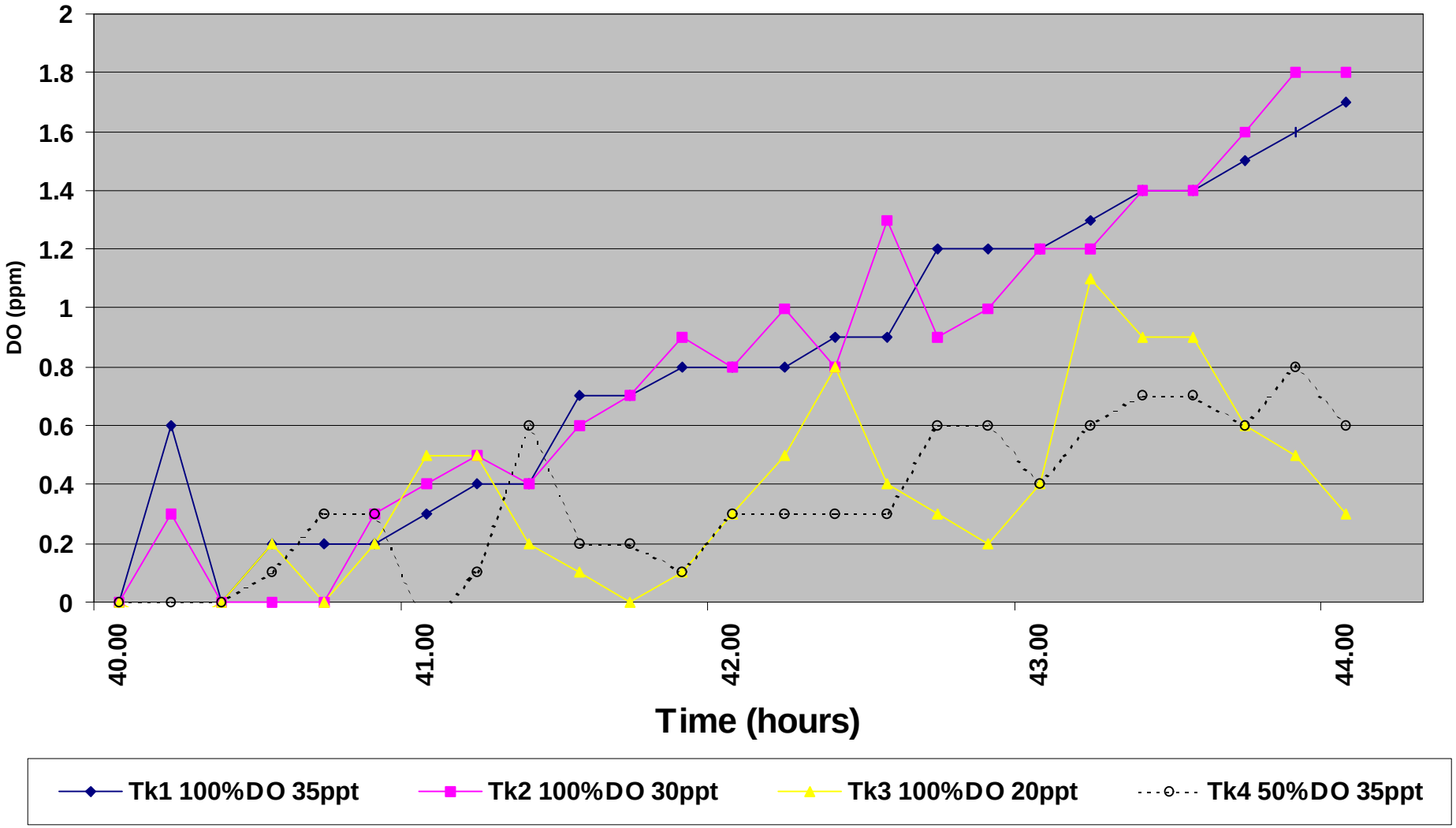
Graph 7 : DO Consumption - Trial 3 (Temperature)



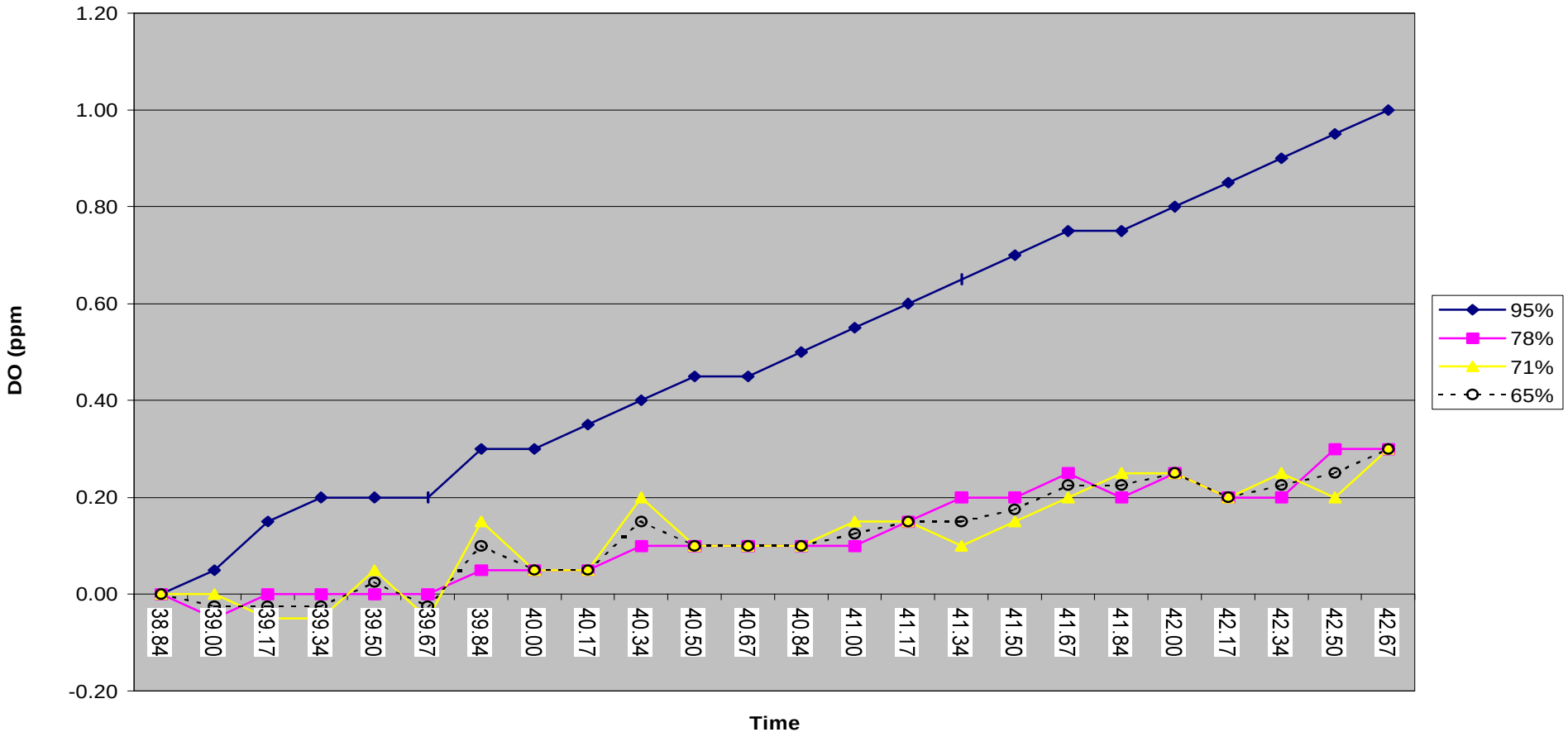
Graph 8 : DO Consumption - Trial 4 (Salinity)



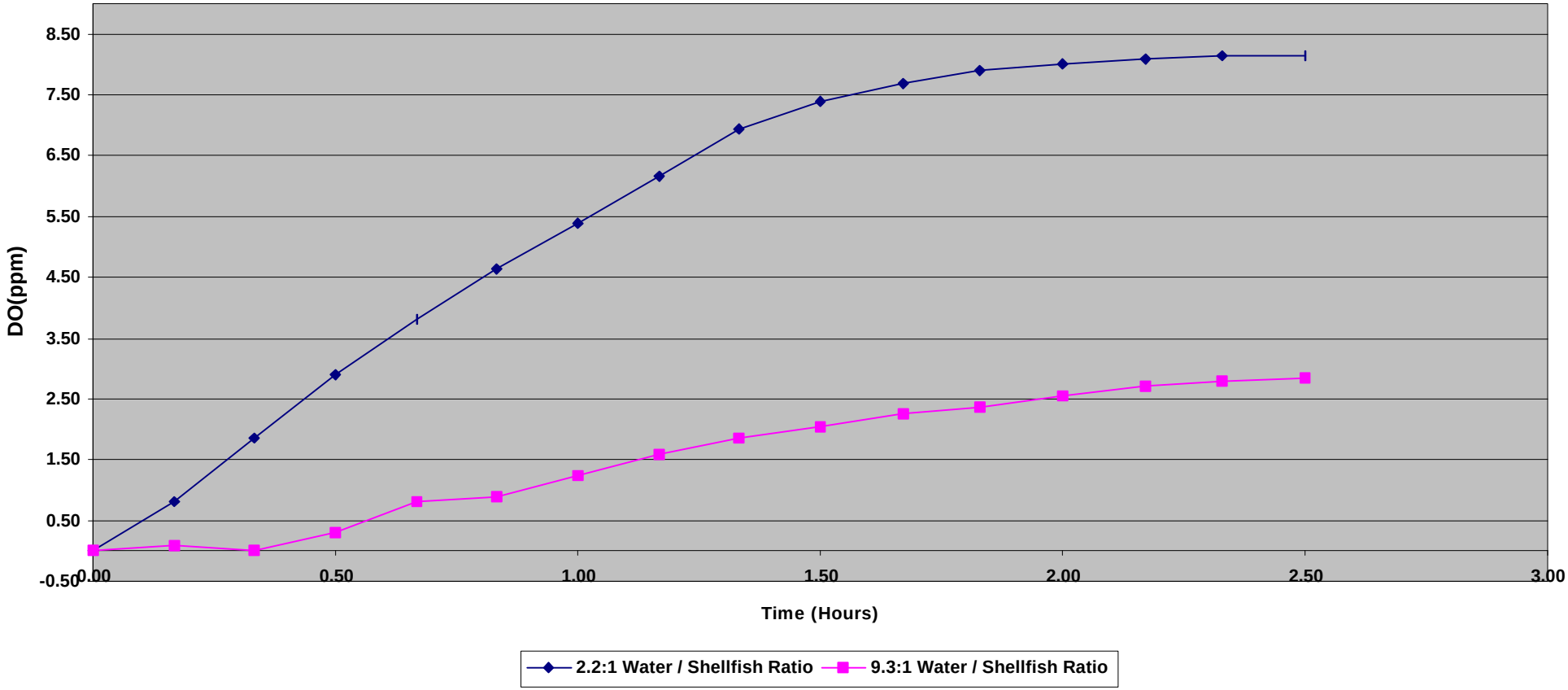
Graph 9 : DO Consumption - Ardtoe 4b (Salinity)



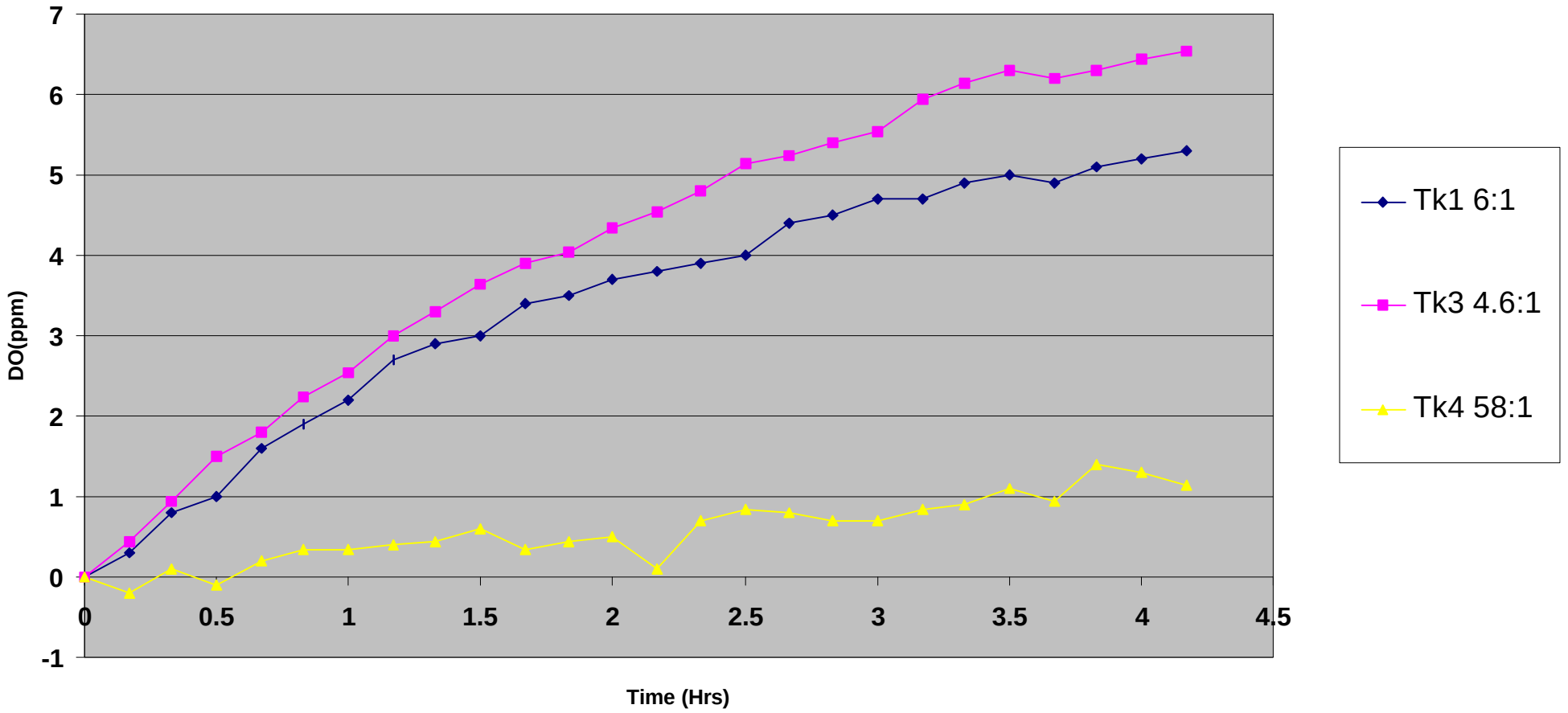
Graph 10 : DO Consumption - Trial 5 (Flow rate / d.o.)



Graph 11 : DO Consumption - Trial 6 (Water / shellfish ratio)



Graph 12 : DO Consumption - Ardtoe 6b (Water / shellfish ratio)



Graph 13 : DO Consumption - Trial 8 (Species comparison)