Final Report for The Food Standards Agency Scotland

Measurement of Domoic Acid in King Scallops processed in Scotland

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GLOSSARY

There are a wide variety of names associated with scallops. To reduce any uncertainty they are included in the following glossary of terms, as they relate to the work carried out in the study.

Adductor muscle: (white meat; muscle, roe-off). The most commonly consumed part of the scallop.

Domoic acid: (ASP), the chemical responsible for causing amnesic shellfish poisoning, sometimes incorrectly referred to as ASP.

End product testing: (EPT) testing of the processed product by the regulatory method to measure the domoic acid content.

Gonad: (roe; corals). The other commonly consumed part of the processed scallop; usually consumed together with the adductor muscle. The gonad contains both a male and female component (coloured cream and orange).

Half shell: a processed product where the adductor muscle is left attached to the lower (curved) shell with the gonad still attached but the rest of the tissue removed.

Hepatopancreas: (black, guts, offal). Strictly the digestive gland, although it is often used to indicate a larger part of the gut.

Mass: (weight), measured in grams and always (in this study) as a wet weight i.e. the associated water has not been removed.

Monitoring: the statutory regulatory process of measuring domoic acid in shellfish based around measuring a fixed number of samples from different, defined areas and at a defined rate of sampling. This is not the same as end-product testing.

Pooled: where a number of individual scallops or scallop parts are combined and homogenized, and the analysis undertaken on the combined material (opposite of individual analysis).

Rest of tissue: (offal), all of the scallop tissue left after the adductor muscle and gonad have been removed; consists of the mantle, the gills and digestive system.

Roe: (gonad) see gonad.

Roe-off: the adductor muscle after the gonad has been removed.

Roe-on: (meats; adductor muscle and gonad combined) the adductor muscle with the gonad still attached

Scallop: (clam, King Scallop, *Pecten maximus*). In this study the term scallop refers only to the King Scallop, *Pecten maximus*. The Mediterranean species is *P. jacobeus* and similar large scallop species occur elsewhere in the world.

Shucking: (dissecting) the process of removing the edible parts from the scallop i.e. usually the adductor muscle and roe combined.

Spawning: where all or some of the gametes within the gonad are released. This results in a loss of mass from the gonad followed by recovery (maturation of new gametes and a build up of gonad mass).

Virtual scallop: where the result is formed by calculating from individual parts (gonad, muscle, rest of tissue) what the whole animal would have had in it in relation to domoic acid.

Whole animal: the entire soft tissues of the scallop (i.e. adductor muscle, gonad and rest of tissue). Whole animal mass refers to the soft parts of the animal only i.e. not the shell.)

EXECUTIVE SUMMARY

Main findings, conclusions and recommendations:

- It may be possible to determine from which areas within a fisheries box scallops are most likely to have high levels of domoic acid, making it possible to accurately define scallop grounds where the levels of domoic acid in the scallop will be similar.
- Over 99% of the domoic acid associated with the scallops is consistently removed by shucking.
- Different processors produced significantly different results when shucking scallops from the same batch for both roe-off and roe-on product. Shucking standards are thus very important in determining the outcome of end product testing (EPT), particularly when the scallop gonads are small.
- Thorough washing was important in reducing the domoic acid levels in poorly shucked scallops. It is important to prevent the shucked scallop meats from coming in contact with the juices from the scallop offal.
- Implementation of full HACCP procedures to deal with domoic acid in scallops should not be difficult or overly expensive for scallop processors
- The analysis that resulted in the 4.6 mg/kg "trigger-level" assumed that the analytical methods used produced negligible variation in domoic acid and that the observed variation was natural. Shucking is in fact the greatest source of variation, making this assumption (and the calculation depending on it) invalid.
- The EPT should be based on the combined roe-on product (i.e. on both gonad and muscle simultaneously). If any product is formed from gonads or adductor muscle alone these should be separately tested. A batch should be all the scallops landed from a single box (or similarly defined area) to a single processor within one week regardless of the number or type of scallop gatherers.

Introduction:

Domoic acid is a neurotoxin that can cause amnesic shellfish poisoning (ASP). A recent European Commission Decision (2002/226/EC) allows for the harvesting of King Scallops while they are above the regulatory limit for domoic acid (20 milligrams per kilogram of shellfish flesh - mg/kg - up to a maximum limit of 250 mg/kg) providing they are processed and the resulting product contains less than 4.6 mg/kg in the marketable parts of the scallop as determined by end product testing (EPT). The main purpose of this study was to provide information for Food Standards Agency, Scotland (FSA Scotland). The study aimed to establish the acceptable and proportionate levels of end product testing for adductor muscle and gonad (roe-on) and adductor muscle only (roe-off) scallops. The information produced will be used to establish if different testing level requirements can be justified for the different scallop products. The study carried out sampling, domoic acid analysis, and assessment of processing practices to provide data, which will help determine the appropriate levels of End Product Testing for King Scallops in Scotland within a tiered testing regime. Additionally, the study also provided the opportunity to gather information on the natural variation of domoic acid in scallops.

The study was divided into three major components:

1: To collect and analyse data, which would allow the determination of the appropriate level of EPT to implement a tiered system for the harvesting and marketing of King Scallops, from waters affected by domoic acid**.**

2: To determine the possible levels of domoic acid contamination of Scallop gonad and adductor muscle (hepatopancreas fluid) during processing and handling and methods to reduce this.

3: To provide detailed descriptions of processing procedures and HACCP assessments leading to recommendations for each of the Scottish Scallop processors.

Results:
The study was based in box J5, chosen because the King Scallops in this area had high whole animal levels at the start of the study. The study used very large sample sizes and analysed domoic acid levels in individual scallops in order to accurately ascertain levels of variability within the scallops. Within this box (J5), domoic acid levels were found to vary between different sites and between the same sites sampled at two different times. The spatial variation in domoic acid levels between scallops in J5 was related to the depth at which the scallops were found. This may be due to differences in water movement at the different sites, resulting in different feeding conditions for the scallops. Such information may be useful in future to help define scallop grounds that will be more manageable than the existing fishing boxes currently in use.

The amount of domoic acid increased in gonads from samples taken in December 2001 to those in February 2002. This was true for all the sites sampled. However, there was no obvious increase in domoic acid in the whole animal over the same period.. The most probable reason for this is that as the gonad increases in size, it encompasses more of the gut within its structure.

The ratio between amount of domoic acid in the combined adductor muscle and gonad to the amount in the rest of the scallop tissue never exceeded 1:99. Therefore, over 99 % of the toxin is always associated with the animal's offal. Levels of domoic acid found in the offal were often very high so that eating the offal from only a few scallops could potentially result in injury. There was evidence that at very high domoic acid concentrations the procedures used to clean-up samples prior to analysis could lead to an underestimation of the actual domoic acid content. This can be overcome by diluting the samples. The extent to which this extraction problem may have affected previous studies is unknown.

Significant differences were found in domoic acid levels in both adductor muscles and in gonads of scallops shucked by different processors. This appeared to be due to differences in the effectiveness in removing small pieces of offal tissues, particularly the hepatopancreas ("black") from around the adductor muscle and gonads. When adductor muscle only was produced, all of the processors managed to produce a product with very low levels of domoic acid associated with it. For the roe-on product (gonad attached to the adductor muscle – the gonad was then removed and analysed separately), the differences observed between the processors were more marked. The least effective processors had difficulties with shucking scallops that had very small gonads. This resulted in higher concentrations of domoic acid

detected after shucking in small gonads, though the actual amount in each individual gonad was either similar or even less than was found in larger gonads.

The experiments in this study showed that shucking was more important in determining the level that would be achieved in the EPT than natural variation. Therefore, it is impossible to accurately predict the EPT domoic acid results for any given processor solely from gonad monitoring data. Good shucking is therefore paramount in producing "clean" product.

Immersing the roe-on product in hepatopancreas fluid containing a very high domoic acid concentration resulted in high levels of domoic acid contaminating the adductor muscle and gonads. This could be partly removed by washing but a significant amount of domoic acid remained bound to the tissues and was not removed by washing. Deliberately produced, poorly shucked, roe-on product was compared to well-shucked product and also half-shell product. Subsequent washing had little effect on the well-shucked and half shell product but did result in a major reduction in domoic acid levels in the poorly shucked product. Soaking the scallops resulted in a loss of domoic acid from the scallop and this domoic acid could be detected in the soak waters. The half shell product had similar and in some cases lower levels of domoic acid than the traditional roe-on, fully shucked product so this can be an acceptable equivalent for the traditional roe-on product within the tiered system.

A total of 22 different processing plants in Scotland indicated that they currently shuck scallops. Seventeen of these were visited in order to conduct HACCP assessments. Examples of best practice were identified and used to form the basis of practical recommendations to processors to aid in the adoption of best practice throughout the industry. HACCP flowcharts were prepared for each of the processors. Emphasis was placed on the definition and adherence to good shucking practice and the importance of reducing the possibility of contamination. Discarding broken scallops before processing and avoiding contamination of shucked meats by fluids coming from the offal were identified as important steps to help prevent such contamination. It is recommended that training qualifications for shuckers be drawn up and introduced.

It is recommended that a batch of scallops is all the scallops landed to a single processor from any number of boats or divers working the same box (or area) within one week. It is also recommended that the EPT be based on the roe-on product, both because this is the industry end product and because the combined product may have a different level of risk than would be indicated by the gonad alone. It is further recommended that processed scallops carry a label advising that a gonad only product should not be produced as this may result in a product that exceeds EU limits for domoic acid.

The adoption of best practice should be relatively straightforward and inexpensive for industry.

1.0 BACKGROUND

In 1987, many Canadians were inflicted by a severe and unprecedented form of shellfish poisoning[1&2]. Three died, some had severe after effects and all were acutely ill. The symptoms were most severe in elderly people. All the affected Canadians had consumed mussels but these did not contain any of the usual shellfish toxins. Further bioassay analysis singled out a surprising cause – domoic acid. This compound had originally been found in seaweeds and was not suspected to be a potential threat to human health as it was regularly used as an anti-helminthic in Japan [2]. However, it was shown that domoic acid acts as a powerful glutamate agonist with both gastrotoxic and neurotoxic activity [2], and at high doses capable of producing permanent damage to the victim's central nervous system [1&2]. Epidemiological analysis of the outbreak coupled with human exposure data suggested that harmful symptoms began to emerge in sensitive individuals at amounts over 1 mg/kg body weight with fatal doses associated with amounts of 5 mg/kg body weight [2]. The most severe symptoms were seen in elderly victims with renal impairment[1]. A severe impairment of short term memory seen in some of the patients led to domoic acid poisoning toxin being termed Amnesic Shellfish Poisoning (ASP).

The source of the domoic acid was also unusual. Rather than the more normally associated dinoflagellates, this phycotoxin was originating from a diatom (*Pseudonitzschia*) [1&2]. Surveys soon detected domoic acid in other shellfish from North America[2]. Domoic acid has not been firmly implicated in any further shellfish poisoning incidents since the initial incident in Canada, although fatalities of wildlife consuming filter feeding fish (such as anchovies) have been linked to domoic acid [3]. Risk assessments carried out by the Canadians led to a tolerable daily dose being established as 5 mg for a 60 kg adult from the most sensitive groups. Assuming a standard portion size of 250 g, this gave a concentration of 20 mg/kg of shellfish flesh. This was adopted as the interim safety limit for domoic acid in shellfish. Recent reviews of shellfish toxin limits have concluded that 20 mg/kg for a 250 g portion is a robust limit and no recent scientific evidence has emerged to suggest that this limit should be changed [4&5].

Domoic acid was added to the EU Shellfish Directive (91/492/EC) in 1997 with a limit of 20 mg/kg of shellfish flesh imposed on the whole or individual parts of a bivalve mollusc consumed (Council Directive 97/61/EC). As soon as testing for domoic acid within Europe commenced, bivalves containing domoic acid were detected, leading to closures in both Spain and Denmark. Testing in Scotland began in 1997 and in the following years domoic acid has been detected in scallops over a very wide area of Scotland leading to extensive closures. In 2001 the incidence of domoic acid in scallops was less severe than previous years but still resulted in extensive fishery closures. The FSA Scotland under advice from the UK National Reference Laboratory (NRL, Fisheries Research Services, Marine Laboratory, Aberdeen) and in light of practice elsewhere in the EU (notably Ireland) operated a pragmatic approach to the problem, focusing on the parts of the scallops consumed rather than on whole animal levels. This allowed the introduction of shucking boxes where animals over 20 mg/kg in the whole animal could still be harvested provided they were shucked and the gonad level in the shucked animals did not exceed 20 mg/kg. Suggestion from industry, led to the development of a proposal by FSA Scotland to introduce a tiered system where animals with gonads over 20 mg/kg could still be harvested for adductor muscle only. The tiered system was considered by the EU Commission in 2001 and a proposal for a Decision was tabled involving these basic elements. The committee's deliberations included two additions: a total ban on scallop harvesting when whole animal levels exceeded 250 mg/kg of domoic acid and

a proposal to limit harvesting to adductor muscle only when the gonad level exceeded 4.6 mg/kg of domoic acid. These figures derived from a statistical study[5] undertaken by the UK NRL on behalf of FSA Scotland and related to a calculated level that would prevent no more than one in a thousand scallops, with a gonad level over 20 mg/kg of domoic acid, being harvested. The EU Decision (2002/226) does allow for revision of these levels when further scientific data is forthcoming.

As the competent authority for implementation of this system, the FSA Scotland was left with a number of important questions that required urgent answers to enable the tiered system to be implemented in 2002. Principal amongst these was to determine the appropriate level for end product testing (EPT). EPT is mandated in both the original EU Directive (91/492/EC) and underlined in the Decision (2002/226/EC). However, the EU Decision does not define a batch. FSA Scotland therefore commissioned a study to rapidly look at trying to define what was the appropriate level of EPT for both the roe-on (adductor muscle and gonad) and roe-off (adductor muscle only – sometimes also referred to as white meat) product and thus define a batch.

An additional concern was whether or not the processing industry could produce shucked scallops that would be below the EU decision (2002/226/EC) regulatory limits. Virtually all the scientific data available had been derived from the monitoring programme or from laboratory experiments and not processors. A single small experiment [6] comparing the UK NRL with a single processor was the only data available and this showed no significant differences between pooled samples based on scallops shucked by UK NRL or the processor. The tiered testing system limits were based on laboratory derived data and the variation encountered in this data[5]. Therefore more extensive scientific data on the shucking differences by different processors on domoic acid concentration in end products was required.

2.0 INTRODUCTION

Integrin was successful in winning the contract in open competition to conduct this study and proposed a series of experiments.. The approach was to use simple experimental designs using large sample sizes and to base the studies around individual variation. While the implementation of the tiered system would rely on pooled samples, data on individual variation was seen as a mechanism to provide a powerful insight into the underlying variation of domoic acid in scallops. Individual variation data backed by large sample sizes also meant that confidence limits would be very precise and the statistical interpretation both straightforward (because of the central limit theorem) and discriminatory. Previous studies had suggested (using Power analysis) that differences of as large as 4 mg/kg could not be resolved until the sample size exceeded 20 individual scallops (Dirk Campbell pers. comm.). Using high n values, statistical significance at $p < 0.05$ is easily obtained but may not be of any practical importance. To reflect the high n values used in this study, a higher level of significance was chosen ($p < 0.01$). The study was limited to box J5. It was chosen as there was existing data available for this box and the sea area was thought to be representative, comprised as it does of an offshore area with a single large island in the middle of it. Additionally, the Jura boxes have some of the highest recorded levels of domoic acid[7]. During the study period J5 was closed by FEPA order as the scallop gonad level was over 20 mg/kg (as indicated by the monitoring programme data). Under the new tiered system J5 would have been closed for at least the start of the period as whole animal levels were over 250 mg/kg. As a representative example of a box with falling high domoic acid levels the

scientific evidence gathered was considered appropriate to adequately inform decisions on protecting public safety. The temporal limitations of the study are a more pertinent problem but this was dictated by the need to produce results quickly. It would be prudent to repeat some of this work at a time of increasing domoic acid levels.

The industry study to examine differences in procedures used five processors, representing approximately 25 % of the industry in terms of volume and chosen to represent a spread of industry practice and geography. A commercial fishing vessel was used to collect single batches of scallops. As some of the samples derived from boxes that were controlled under FEPA orders, the scallops had to be handled under restrictive conditions to ensure they did not enter the food chain. This made it impossible to do the sort of blind sampling that would have been optimal for the study but the processors were given no instructions other than to shuck the supplied sample to their usual standards, thus where possible shucking practice was as close to normal conditions as possible. Witnessing the shucking gave no suggestion that the scallops were shucked to anything other than usual industrial standards.

The scale of the study not only produced definitive data on the effect of shucking practice; it also produced the best data to date on domoic acid in industrially processed scallops. This data was further analysed to shed more light on some of the causes of underlying variation in shucking practice. This can be used to examine the assumptions that underpin the existing EU Decision (2002/226/EC) and whether or not these are justified.

The study also identified all the seafood processors in Scotland that state that they are active in processing scallops. As many processors as possible were visited in an effort to understand the different techniques used and identify best shucking practice that could then be disseminated throughout the industry. This HACCP study was complemented with a study where scallops were experimentally contaminated with domoic acid and the effects of different decontamination practices on domoic acid reduction were studied.

3.0 GENERAL METHODOLOGY

3.1 SAMPLING

Sampling was carried out using the *Christy M* chartered by Integrin. C. Bavington of Integrin was present to supervise all sampling. Batch codes were assigned in accordance with the study plan (Annex 1). Batch codes for the shucking and contamination study (objective 2, section 5.0) are listed in Annex 4. Batches were stored and transported at ambient temperature.

All material received at Integrin was handled in accordance with the study plan (Annex 1).

3.2 ANALYTICAL PROCEDURES

Scallop tissue was analyzed for the presence of Domoic acid, following the method of Wright & Quilliam [8] using HPLC with diode-array detection, Integrin method IM001 (Annex 2). Briefly:

Samples were analysed in groups of 30 or 40 per day. All sample masses (wet weights always) were recorded and the sample was homogenised until completely homogenous. Between 0.5 and 4.0 g (4.0 g was used wherever possible) of tissue homogenate was weighed out and homogenised with 4 volumes of extraction solvent (50 % aqueous methanol). The homogenate was centrifuged for 30 min at 8,000 g, and the resulting supernatant was decanted and diluted 1:5 with extraction solvent. Where samples were known to contain high levels of domoic acid, out of the range of the test, additional dilutions were included at this point.

The diluted supernatant was filtered through a 0.45 μm nylon filter, and a glass-fibre pre-filter if necessary. Five ml of the filtered, diluted extract was loaded onto a pre-conditioned solid phase extraction (SPE) strong anion exchange (SAX) cartridge, washed with 10 % aqueous Acetonitrile, and eluted with 2 ml of citrate buffer.

An aliquot of the final sample was analysed by HPLC, separated by reverse-phase chromatography on a C18 column, eluted isocratically with 10 % aqueous Acetonitrile with 0.1 % TFA, monitored using a photodiode array detector (PDA).

Domoic acid standards, prepared to give an analytical range of 1 – 250 μg/g, were run together with and used to quantify samples. A negative control of scallop tissue, shown by exhaustive extraction to contain no domoic acid, was prepared in the same way as the samples. A positive control of scallop tissue, containing a predetermined concentration of domoic acid (Laboratory Reference Material, LRM), was prepared in the same way as the samples. Certified Reference material (CRM) for domoic acid (MUS 1B, obtained from NRC Canada) was used as the positive control for some sets of analyses.

For the purposes of this study results are not corrected for recovery. Recovery is calculated using the result obtained from the positive control expressed as a percentage of its known concentration (LRM or CRM). Integrin's internal quality control data has shown that recovery is the largest source of uncertainty arising from the analytical method (Annex 2). Therefore, when running large numbers of samples for statistical analysis, as has been done in this study, applying a recovery correction to each group of samples analysed would

introduce apparent differences between groups which arose merely from the uncertainty of the recovery value applied. Recovery values obtained from positive controls were used for quality control purposes, to determine conformance with the specified limits for recovery (80 - 120 %). Groups of analyses were repeated where the associated recovery did not meet these limits.

3.3 QUALITY CONTROL.

The method for determination of domoic acid in shellfish (IM001, Annex 2) is accredited by UKAS (UKAS Testing Laboratory number 2384). All job-sheets, associated records and data have been checked by QA to ensure correct recording of procedures and data, and to assess compliance of internal quality control (IQC) parameters with Integrin's specifications.

Where IQC specifications have not been met retests have been carried out, or where the non-conformances has been judged not to affect test results the data has been released, with full justification detailed on a concession form.

3.4 STATISTICS

Statistics

Statistical analyses were made using a PC computer running the standard statistical software module of *Statistica 6* (Statsoft, Tulsa). The data were tested for normality and then appropriate parametric or non-parametric tests applied. Details of the individual tests used are given in Annex 3.

3.5 RESULTS AND RECORDS

Results database

All results have been entered into the FSA01 database, which has been checked to ensure correct data entry. Where retests have been performed the database has been updated to contain the correct data (Raw data in Annex 6).

Records

References to all records arising form this study can be found in Annex 4.

4.0 OBJECTIVE 1:

To collect and analyse data, which would allow the determination of the appropriate level of End Product Testing (EPT) to implement a tiered system for the harvesting and marketing of King Scallops, from waters affected by domoic acid.

4.1 INTRODUCTION:

The experiments detailed in this section of the study were designed to produce a definition of batch through the analysis of trawl variation within a box. The experiments also aimed to define the appropriate level of end product testing (EPT) required for the different products resulting from scallop batches with different toxin levels. This part of the study aimed to address the question of whether different processors end up with significantly different results when processing the same batches of scallops. The box sampling at each level of domoic acid was done on a single day using a commercial fishing vessel.

This study aimed to considerably enhance our knowledge of domoic acid in scallops beyond the existing state of the art. At the beginning of this work there were few studies on the necessary level of EPT. There is limited data on variability within a box[7] but the current study aimed to provide a more comprehensive data set. The study also aimed to provide robust statistical analysis through the analysis of individual variation in tissues (rather than the use of pooled samples) and by using large sample sizes aimed to give the data high statistical confidence. There have been no comparative studies of differences in efficacy of toxic part removal between processors so the study will be particularly valuable in helping identifying best practise for the industry.

Shucking experiments:

The adductor muscle is the tissue most frequently consumed from scallops. In North America only the adductor muscle is routinely eaten but in Europe scallops are most commonly marketed as roe-on; i.e. the gonad is left attached to the adductor muscle. In practice the roe is often removed at the point of use, either by chefs or the consumer themselves though the product is probably most commonly consumed as a serving of between 5 and 10 meats consisting of the roe-on adductor muscle. Meat yields (based on mass) are used to determine the value of scallops, both to pay the boats or divers landing the harvest and then to the processors. The gonad can sometimes contribute up to 50 % of the edible meats (more usually 25 %) therefore the presence or removal of the gonad significantly affects the price of the product .

The adductor muscle should in principle contain no domoic acid at all since there are no ramifications of the digestive system within its structure[9]. Any domoic acid present is therefore contamination, deriving either from a failure to remove all the gut tissue or because of contamination of the adductor muscle by fluids emanating from the offal[5].

The gonad does have domoic acid associated with it because a loop of the gut runs through the gonad. Previous studies had shown that the gonad is the most variable tissue within the scallop with relation to domoic acid concentration[5&7]. Regulation within the UK has mostly relied upon gonad testing, with levels above 20 mg/kg leading to closures regulated by FEPA orders imposed by FSA Scotland. A partial tiered system was introduced last year to test whole animals in addition to the gonad. If the whole animal level is above 20 mg/kg and the gonad is below 20 mg/kg then the area is declared a “shucking box” and all scallops

harvested from the area are required to be shucked. Once gonad levels exceed 20 mg/kg the box is closed.

Most of the available data on domoic acid variation in scallops has come from animals taken as part of monitoring programmes or in specific experiments[5&7]. Scallop shucking for the monitoring programme has been carried out within laboratories and by scientifically trained staff. This situation is far removed from the realities of industrial scallop processing where the scallops are shucked and processed at great speed. There may be differences between scallops shucked in processing factories and within laboratories. In a small study[6] comparing the UK NRL and a Scottish North-Eastern processor, no significant differences were found in gonad concentrations between samples from the laboratory and processor. However, the study used pooled gonads and was too small scale to reveal any genuine statistical differences. Discovering differences between processors may also help point towards best practice. The experiments described in this part of the study were to investigate the effects of shucking done within processing factories for both roe-on and roe-off product. As far as possible the procedures followed were those normally applied by the different processors. No instructions were given to the processors as to how they should undertake the shucking.

4.2 METHODOLOGY

First sampling

A batch of 870 scallops was collected in two hauls taken over the same ground (site 9) in box J5 at a domoic acid concentration of 20 - 30 mg/kg gonad (scoping samples taken on 29th October 2001 showed gonad concentrations of 19.9 mg/kg, 23.0 mg/kg, and 31.0 mg/kg), on 1st November 2001, 04:00 – 06:30. The haul was taken between positions 56º 04.41 N, 06º 04.53 W and 56º 04.77 N, 06º 05.88 W. The batch was randomly divided into 6 bags of approximately 140 scallops, which were sealed and labeled with the sub-batch identifiers. The batch was stored on deck at ambient temperature until landing (1st November 2001, 08:30).

On landing, sub-batches were distributed by Integrin personnel to the five commercial processors within a maximum of 24 hours. Sub-batches were transported at ambient temperature. On delivery to the processors sub-batches were more or less immediately shucked in the presence of an environmental health officer (EHO), to white meat only. Where possible Integrin personnel were also present to witness shucking. All white meat was placed in sealed containers, labeled with the sub-batch identifier and passed to Integrin personnel for return to Integrin for analysis. All sub-batches were returned to Integrin by 2nd November 2001.

In addition to sending scallops to processors, a sub-batch of 117 king scallops were received at Integrin on 1st November 2001. Fifty scallops were shucked to adductor muscle, gonad, and rest-of-tissue, in accordance with the study plan (Annex 1). Each part from individual scallops was given an identifier to allow correlation with the other parts from the same animal. Fifty scallops were shucked to whole tissue. All shucked tissue was stored frozen (-20 °C) until analysis. Aliquots of tissue homogenate from all samples have been retained in storage at – 20 ºC.

Second sampling

On 18th December 2001 (06:15 – 19:30) 10 batches were collected from box J5. Monitoring results for J5 samples of 8th December 2001 showed domoic acid concentrations of 13 & 37 mg/kg gonad. Each site was sampled using a single tow, with the exception of site 04, in which 605 scallops were sampled from two tows over the same ground. Site sampling details are listed in Table 1 of Annex 4. The batch collected at site 04 was randomly divided into 6 bags of approximately 100 scallops, and bags were sealed and labeled with the sub-batch identifiers. All batches were stored on deck at ambient temperature until landing (18th December 2001, 20:30).

On landing processor sub-batches were distributed by Integrin personnel to the 5 processors and to Integrin. All batches were transported at ambient temperature. On delivery to processors sub-batches were shucked, in the presence of an EHO adductor muscle and gonad (roe-on). Where possible Integrin personnel were also present to witness shucking. All white meat was placed in sealed containers, labeled with the correct identifier and passed to Integrin personnel for return to Integrin for analysis. All processor sub-batches were returned to Integrin by 20th December 2001.

A sub-batch of 100 king scallops, from site 04, was received at Integrin on 19th December 2001. Fifty scallops were shucked to adductor muscle and gonad i.e. roe-on product, in accordance with the study plan (Annex 1). Forty scallops were shucked to whole tissue.

All other batches were received at Integrin on 19th December 2001. Fifty scallops, from each batch, were shucked to adductor muscle and gonad, in accordance with the study plan (Annex 1). Where the batch size allowed, 10 scallops were shucked to whole tissue.

All shucked tissue was stored frozen (-20 °C) until analysis. Aliquots of tissue homogenate from all samples have been retained in storage at – 20 ºC.

Third sampling

On 13th February 2002 (08:40 – 19:30) 10 batches were collected from box J5. Monitoring results for J5 samples of 12th February 2002 showed domoic acid concentrations of 15 & 23 mg/kg gonad. Each site was sampled from a single tow. Site sampling details are listed below in Table 2 in Annex 4. Bags were sealed and labeled with batch identifiers. All batches were stored on deck at ambient temperature until landing (13th February 2002, 20:30).

On landing sub-batches were transferred to Integrin. All batches were transported at ambient temperature and received at Integrin on 14th February 2002. Fifty scallops, from each batch were shucked to adductor muscle and gonad, in accordance with the study plan (Annex 1). Where the batch size allowed, 10 scallops were shucked to whole tissue.

All shucked tissue was stored frozen (-20 °C) until analysis. Aliquots of tissue homogenate from all samples have been retained in storage at – 20 ºC.

4.3 RESULTS

Natural variation

***Whole animals and "rest of tissue" samples*:**
In the samples taken in early November 2001 from the Sound of Colonsay (site 9 – Fig. 1) high levels of domoic acid were detected in the whole animal and "rest of tissue" samples. Whole animals had a mean domoic acid concentration of 248 mg/kg (n= 50; SD = 66.2). These ranged from a minimum of 136 up to a maximum of 381 mg/kg. The mean amount of domoic acid in each scallop was 17.1 mg. Rest of tissue samples had a mean domoic acid concentration of 731.5 mg/kg (n = 50, SD = 141.1), ranging from a minimum of 525.4 to a maximum of 1001.9 mg/kg. The mean amount of domoic acid in each "rest of tissue" sample was 24 mg. Approximately seven and a half scallops would produce enough "rest of tissue" material to produce a 250 g "serving". This would result in a total dose of 180 mg, which is equivalent to 3 mg/kg body weight (in a 60 kg human) – a potentially serious dose to vulnerable people[1&2]. The worst-case dose from the data set would result in a total dose of 5.6 mg/kg body weight – a potentially fatal dose[1&2]. The mean results were broadly in line with rest of tissue samples taken in December 1999[7] although the very high levels encountered in that study in a very few scallops (up to a maximum of 3600 mg/kg) were not observed during the current study.

An interesting feature of the data was that when "virtual" whole scallops were produced by adding the three tissue components together (adductor muscle, gonad and rest of tissue), the mean concentrations were significantly higher than whole scallops from the same batch (328 mg/kg versus 248 mg/kg: Fig. 2). There is no obvious explanation for this. It may be that the differences represent genuine individual variation between the scallops studied – they are after all not the same scallops but the differences are larger than would be expected purely as the result of natural variation. Both samples passed their quality assurance checks but a methodological explanation for the differences cannot be ruled out as a possibility.

50 scallops collected in November from site 9 were dissected into their respective gonad, adductor muscle and rest of tissue (gut, mantle, gills etc). When analysed, these showed the usual pattern, with over 99% of the domoic acid being found in the rest of the tissue component (Fig. 3). The gonad had on average the next highest amount but in some individual cases the adductor muscle amount was higher than the gonad because of its greater mass. Gonad concentration was positively correlated with gonad amount ($r^2 = 0.74$, $P < 0.01$).

Comparisons between different sites within J5 (Figs. 1 and 4) showed that whole animal domoic acid levels were variable in different parts of the box. Unfortunately no data was available from site 8 but the highest values were associated with the Sound of Colonsay (Sites 1, 9 and 10) rather than the Ross of Mull area (Sites 3-7) to the north of J5. Whole animal domoic acid concentration and amount were both significantly negatively correlated against depth though the correlation was relatively weak (r^2 was only around 0.4); whole animal mass only showed a negative correlation for the December sample (Fig. 5).

The ten sites sampled within J5 focussed on the two main scallop grounds within the box: the Ross of Mull scallop ground (which also includes part of box J2) and the Sound of Colonsay scallop ground. Significant differences were found between the sites both in the state of the gonads of scallops from the different sites and in their associated amounts of domoic acid. Given the significant differences in domoic acid levels in scallops from the same batch found

between different processors (section 4.4) in shucking the same batch of scallops there is obvious concern that temporal or spatial differences may be merely the result of different shucking standards. However, it is encouraging that the pattern of variation between the sites is very similar for both of the two major sampling occasions (December and February), i.e. the sites that are high in December are also those ones that are high in February. We can thus conclude that shucking practice used at Integrin was sufficiently homogeneous over the study period so as not to obscure natural underlying variation.

Sampling was planned to take at least sixty scallops from each site and separate these into 30 that were individually analysed for gonad domoic acid content, twenty that were further separated into two groups of ten to form two pooled samples that were analysed for gonad content and the remaining ten were used to form a whole animal pooled sample. At some sites, insufficient scallops were collected to do all of these tests and the priority was to do the individual samples, followed by the gonad pools and finally the whole animal pool. This resulted in missing data for some sites for the whole animal pool and in one case a missing gonad pool sample.

The gonad pools were to be used to look for differences between genuinely pooled samples and virtual pools formed by combining the results of ten individual gonad results. While in many cases there were no differences between the real and virtual pools, there were some samples where the concentrations of domoic acid were clearly different from the virtual pools (Fig.6). Closer inspection revealed that these pooled samples were formed from scallop gonads that were significantly lighter than the sample population used for the individual results. Quizzing the analysts involved in producing the results revealed that they were biasing the results by rejecting smaller gonads for individual testing and instead using these to form the pooled samples. The logic was that the very smallest gonads had only sufficient mass for one extraction. For the very small gonads there would thus be insufficient tissue to replicate the analysis if this was required. Ironically, the analysts had not been briefed on the possible inverse correlation between gonad concentration and gonad size in an effort to prevent unintentional bias in the selection of gonads for analysis. This bias only affects the site results as for all the other experiments all the scallops were analysed regardless of their size. However, for the comparison of sites and between sample dates we have to consider the possible effects of this bias on the results. Fig. 6a-c shows gonad mass, gonad domoic acid concentration and gonad domoic acid amount for all ten sites and for both sample dates. The results for the means of the individually analysed gonads are shown in red while the green bars represent the corrected figure if the pooled samples are included. The effect of the analyst bias has been to slightly inflate the mean gonad size in the population of individually analysed gonads and has no effect on the mean amount of domoic acid per gonad. Gonad concentration was more obviously affected with a general elevation in gonad concentration in the corrected means (Fig. 6c). This shows that gonad concentration is not independent of gonad mass and that small gonads have elevated concentrations of domoic acid. While the bias has produced an effect on the values obtained, it has had no effect on the comparisons between sites and between sample dates. The bias is therefore ignored for the rest of this chapter.

There are clear differences between the different sites both spatially and temporally. Gonads in February 2002 are almost all heavier, contain more domoic acid and have higher concentrations of domoic acid than ones sampled in December 2001 (Figs.7, 8, 9). In many cases these differences were statistically significant, summarised in table 3.

Site	Gonad Mass	Gonad Amount	Gonad concentration
1	P<0.01	NS (P<0.05)	NS
2	P<0.01	P<0.01	P<0.01
3	NS (P<0.05)	P<0.01	P<0.01
4	P<0.01	P<0.01	P<0.01
5	P<0.01	P<0.01	P<0.01
6	P<0.01	P<0.01	NS
7	NS	NS	NS
8	P<0.01	P<0.01	NS
9	NS	NS	NS
10	P<0.01	NS	NS

Table 3. Probability of statistical significance in differences between sample dates for each site and for gonad mass, domoic acid amount and domoic acid concentration. Mann-Whitney "U" test; n=30 in all cases. P: probability, NS: not significant

The data for domoic acid concentration and amount shows the skewness that has been typically found in other studies [5]. The skewness meant that the average median value was only 65% of the mean (n = 600). While the majority of gonads within a site were found to have a restricted distribution for amount and concentration of domoic acid, outliers and extreme values were almost all associated with the upper whisker of the distribution (Figs.8, 9) with very few outliers below the mean. Variance was also proportional to the mean so the data cannot be considered normal. Gonad mass was, however, normally distributed, There was significant variation between the different sites for both sample dates for gonad mass, domoic acid concentration and domoic acid amount (Kruskal-Wallis and median test, $p < 0.01$).

Gonad concentration and amount both showed positive and significant ($p < 0.01$) correlations with whole animal amount and concentrations: i.e. sites where there was a high domoic acid level in the whole scallop also had high levels in the gonad (Fig.10). Gonad concentration showed a positive and significant ($p < 0.01$) correlation with gonad amount (Fig.11), with a wide scatter at higher values.

The differences in gonad domoic acid concentration and domoic acid amount between the different sites can be related to two different possible causes. Virtually all the scallop components measured showed negative and significant correlations ($p < 0.01$) to depth (Fig.12 and table 4), suggesting that scallops in shallow water are more prone to having higher levels of domoic acid in them than ones from deeper water. However, the shallow sites were also generally all in the Sound of Colonsay (Sites 1, 8, 9,10) so the variation could be related to geographical position rather than water depth. Post-hoc analyses of the site data for both sample months (using *Newman-Keuls* and *Tukey*) suggest that only sites 8 and 9 are truly discrete from the other sample sites as they are both significantly ($p < 0.01$) different from all the other sites but are not significantly different from each other. These are also the two shallowest sites and the scallops from these sites had the largest gonads. This suggests that the gonad domoic acid content is associated with the depth of the site rather than its geographic location but this requires further investigation.

	Depth	*wcd*	*wcf*	*wad*	*waf*	*wmd*	*wmf*	*gcd*	*gcf*	*gad*	*gaf*
Depth	1.00										
wcd	-0.68	1.00									
wcf	-0.84	0.77	1.00								
wad	-0.66	0.98	0.79	1.00							
waf	-0.75	0.75	0.94	0.79	1.00						
wmd	-0.64	0.76	0.71	0.88	0.75	1.00					
wmf	0.07	-0.01	0.11	0.08	0.44	0.21	1.00				
gcd	-0.79	0.57	0.75	0.61	0.70	0.77	-0.06	1.00			
gcf	-0.77	0.46	0.86	0.47	0.79	0.62	0.03	0.82	1.00		
gad	-0.80	0.70	0.88	0.80	0.90	0.93	0.24	0.80	0.76	1.00	
gaf	-0.78	0.69	0.83	0.78	0.80	0.94	0.11	0.69	0.65	0.95	1.00

Table 4: Correlation matrix for different scallop parameters.
*Depth is site depth; wcd: whole animal domoic acid concentration, December sample; wcf: whole animal domoic acid concentration, February sample; wad: whole animal domoic acid amount, December sample; waf: whole animal domoic acid amount, February sample; wmd: whole animal mass, December sample; wmf: whole animal mass, February sample; gcd: gonad domoic acid concentration, December sample; gcf: gonad domoic acid concentration, February sample; gad: gonad domoic acid amount, December sample; gaf: gonad domoic acid amount, February sample. Values are *r*.

Site	Whole animal concentration (mg/kg) (Dec)	Whole animal concentration (mg/kg) (Feb)	Gonad amount as % whole animal amount (Dec)	Gonad amount as % whole animal amount (Feb)
1	112.4	174.9	0.50	0.45
2	No Sample	81.3	No Sample	0.48
3	130.7	80.8	0.12	0.67
4	127.6	91.7	0.05	0.39
5	78.5	98.6	0.39	0.64
6	197.6	166.8	0.15	0.30
7	No Sample	106.7	No Sample	0.24
9	234.1	243.5	0.52	0.85
10	No Sample	128.9	No Sample	0.38

Table 5: Mean domoic acid amount as a percentage of whole animal amount for each site and date.
***Site 8 is missing because no whole animal data was available for either date.**

When the mean amount of domoic acid in each scallop gonad is calculated as a percentage of the whole animal amount (taken from the whole animal pooled sample), no mean value for gonad amount exceeded 1 % of the whole animal amount, and most were considerably lower (Table 5). There was no obvious correlation between gonad domoic acid concentration and gonad mass (Fig.13). In Fig. 14 gonad mass and domoic acid concentration and domoic acid amount are related to depth in surface plot. The graph (14b) clearly shows that the amount of domoic acid is highest in gonads of intermediate size (8-18 g) from shallow water (25 – 30 m). While concentration (Fig. 14a) is highest in the smallest gonads (4-8 g) from the shallowest water (25-35 m), high concentrations were also detected from the deepest sites and linked to medium sized gonads (10-18 g). This shows how gonad domoic acid concentration can be decoupled from amount and produces more noise when trying to interpret it than with domoic acid amount.

Processor variation

Adductor muscle (Roe –off):

Comparisons of the adductor muscle mass distributions (Fig. 15) showed that the processors all returned adductor muscles of very similar mass distributions. No significant differences (at the chosen level of significance $p < 0.01$) were seen between them (*chi sq*. $p = 0.03$).

Much of the data relating to domoic acid amounts and concentration were below either the limit of quantification (LOQ, < 1 mg/kg) or below the limit of detection (LOD, < 0.5 mg/kg). While the HPLC software did produce values between 0 and 1.0 mg/kg, the actual numerical values are of course imprecise because of the low resolution of the method at levels below the limit of quantification. This required the use of non-parametric rank methods to test for significance between the processors. First the data was explored to detect any anomalous values. The most obvious extreme value was in the data set from processor P4 where a sample concentration of 6.4 mg/kg and a total amount of 206.8 μg of domoic acid was recorded. It was, however, noted when this adductor muscle sample was received that it still had the gonad attached. This sample was therefore not considered further in the analyses. Four other outliers (one from P1, two from P2 and one from P5) were found to retest at less than 50% of the original values and these samples were also removed from further analysis. Two outliers in Integrin's dataset were reanalysed but were within 50 % of the original values and the original values were retained. Only the five processors were compared with each other for the statistical analyses, as the numbers of adductor muscles shucked by Integrin was half that ($n = 50$) of the sample numbers for each processor ($n \leq 100$).

Fig. 16 a and b show categorized histograms for domoic acid amount and concentration in the adductor muscles originating from each processor respectively. The differences were highly significant (*Chi sq* = 147.5, df = 4 $p < 0.001$ for concentration; and *chi sq* = 150.5, df = 4, $p < 0.001$ for amount). Fig. 17a shows a box and whisker plot of the five processors and Integrin for mean amount of domoic acid in the adductor muscle and Fig 17b shows mean concentrations for domoic acid in the same. As indicated above, the actual figures should be treated with caution because of the large numbers of adductor muscles where the domoic acid concentration is below the LOD or LOQ but the graph indicates that some of the processors were able to produce adductor muscles with similar or lower amounts of domoic acid to adductor muscles shucked by Integrin.

The mean whole animal concentrations ($n = 50$) measured by Integrin from the same batch was 247 mg/kg and reconstructed whole animal values (taken by adding the adductor muscle, gonad and rest of tissue results together) were even higher (382.2 mg/kg: Fig. 2). There were no correlations between adductor muscle mass and domoic acid concentrations. There were no significant or even evident correlations between adductor muscle concentrations (or amounts) and rest of tissue domoic acid concentrations (or amounts), indicating that the amount of domoic acid in the whole animal had little relevance to the amount in the adductor muscles.

Adductor muscle and gonads (Roe-on):

In December 2001, J5 was re-sampled for the roe-on experiment but in a different part of J5 than had been sampled for the roe-off experiment. This site (site 4) was chosen on pragmatic grounds: the ten sites in J5 that were being sampled were done in turn and this site was the first that yielded sufficient scallops to undertake the experiment.

The whole animal concentrations from this batch showed a mean of 127.6 mg/kg (n = 30, SD = 42.8). Gonad concentrations from scallops shucked by Integrin showed a mean of only 0.76 mg/kg (n = 30, 95 % confidence limits ± 0.92). Though the maximum value was 11.7 mg/kg, only three samples were above the LOQ. The sample producing the extreme, maximum value was retested but the retest value was well within 50 % of the original value (10.5 mg/kg, as opposed to the original value of 11.7 mg/kg) so this value was retained in the analysis.

The two pooled gonad samples produced by Integrin showed much higher concentrations of 2.3 and 1.1 mg/kg compared to the mean individual result. The gonad mass showed a mean value of 5.18 g (n = 30, SD = 1.35, though the mean mass of the two pooled samples was lower (3.86 and 4.04 g). This variation in gonad mass and concentration between the pooled and mean individual results indicates a probable bias in the sampling as previously discussed. If the pooled and individual results were combined this would indicate a mean concentration of 1.14 mg/kg domoic acid per gonad and a mean gonad mass of 4.69 g. The mean amount of domoic acid per gonad for the Integrin samples (pooled and individual combined) was calculated at 4.33 µg.

Gonad mass for the processors' samples (Fig. 18a, b) ranged between 0.34 g to a maximum of 10.74 g. The mean value was 4.00 g. The gonads produced by the different processors did not depart from normality in relation to their mass (*Kolmogorov-Smirvov,* $p < 0.1$, Fig. 18a). The mean gonad masses did, however, differ significantly between processors (*ANOVA*: $p = 0.002$). Processor 3 produced roe-on scallops with the lowest mean gonad mass (mean = 3.53 g) while roe-on scallops from Processor 2 had the highest gonad mass (mean = 4.53 g).

Individual gonad concentrations of domoic acid varied from below the limit of detection to a maximum of 47.2 mg/kg. Six sample results were retested: one extreme value for Processor 1, which was accepted; two for Processor 3 one of which was an extreme value and was rejected, one a retest of a very low value (for quality control purposes) which was retained; two extreme values for Processor 4 (one retained, one rejected) and one extreme value for Processor 5 (which was the highest value recorded) and which was rejected after retest. After retests, there was only a single gonad sample that had a concentration of over 20 mg/kg left in the data set.

Gonad concentrations and gonad amounts were not distributed normally for all of the processors (Fig.19a, b). High degrees of skewness and kurtosis were encountered. The high sample numbers used were sufficient to overcome this and allow the use of parametric tests but variance was also found to correlate with the means thus invalidating the use of *ANOVA*. Applying instead the non-parametric *median test* and *Kruskal-Wallis test* showed that the differences between processors were significant ($p < 0.001$ for both tests, for both concentrations and amount of domoic acid in the shucked gonads). Post-hoc tests (*Newman-Keuls*) showed that for concentration Processor 1 and Processor 3 both differed significantly ($p < 0.01$) from all the other processors (including each other), while Processor 2, 4 and 5 did not differ significantly from each other. For comparisons of amounts of domoic acid per gonad, the results differed slightly in that Processor 1 was no longer significantly different from Processor 4.

Studying the data in more detail reveals that the processors differ not only in terms of the variances of the data but also in their distribution (Figs. 20a, b). The highest concentration in a gonad is seen for Processor 4 while the highest amount in a single gonad is recorded for Processor 1. Fig. 21 shows concentration plotted against amount both for the total data set

(Fig. 21a) and for each processor (Fig. 21b). While there is a significant ($p < 0.01$) positive correlation between concentration and amount, the data becomes quite scattered at high concentrations.

Gonad domoic acid concentration shows a negative correlation with gonad mass when the results from all of the processors is combined and this correlation is also present in the graphs of individual processor results (Fig. 22) except for Processor 1 where the outliers and extreme values have a disproportionate effect and no correlation is obvious. However, none of these correlations approached statistical significance. The graphs also illustrate that small gonads are more variable in relation to gonad domoic acid concentration than larger ones, particularly for gonads less than 3 g. Processor 1 is again an exception to this.

Gonad amount shows a weak, non-significant, positive correlation to mass (Fig. 23) for all of the processors except Processor 3 where no correlation is evident. Fitting a polynomial curve (Fig. 23b) to the data suggests that the data are distributed in bell curves except for Processor 1 where again the extreme value produces a different distribution. As would be expected, gonad concentration shows a positive correlation with gonad amount (Fig. 24a, b) but there was considerable scatter in the relationship, particularly at higher concentrations. This reflects the disproportionate influence of small gonads on concentration.

	Gonad mass %				
	P1	**P2**	**P3**	**P4**	**P5**
P1	100	91	117	102	110
P2	110	100	128	112	120
P3	85	78	100	88	94
P4	98	89	114	100	107
P5	91	83	106	93	100
	Gonad amount %				
	P1	**P2**	**P3**	**P4**	**P5**
P1	100	55	489	66	59
P2	182	100	889	120	108
P3	20	11	100	13	12
P4	152	83	742	100	90
P5	167	93	826	111	100

Table 6, % pairwise comparisons of gonad mass and gonad domoic acid amount between processors (P1, P2 etc)

Comparing all the gonad parameters between the different processors, it appears that Processor 3 produced such a low amount of residual domoic acid through removal of virtually all of the associated gut tissue, resulting in a lower gonad mass than the other processors. This is not simply the result of removing gonad mass. As can be seen in table 6, the removal of a small percentage of tissue by Processor 3 results in a much larger percentage removal of domoic acid. Processor 1 achieved a better domoic acid amount than either Processors 4 or 5 despite having a mean gonad mass higher than these two processors. This suggests that the shuckers are using different cutting techniques with Processor 3 achieving the best reduction though with a possibly greater than necessary loss of actual gonad tissue Processor 1 is achieving good results with a better gonad yield but is being let down by a few less well shucked gonads, with results independent of the size of the gonad. The remaining three processors are having particular difficulties with small gonads resulting in more associated tissue being left on.

It is instructive to examine the data as if it had been produced from pooled samples. Table 7 shows the equivalent gonad domoic acid concentrations of ten pooled scallops. The order that the scallops were processed should be random so the pools are taken as the mean of each consecutive ten scallops in the results database.

Sample	P1	P2	P3	P4	P5
1	2.41	3.41	0.32	4.8	2.14
2	4.33	3.74	0.1	2.78	2.45
3	2.03	3.24	0.67	3.35	7.03
4	1.39	3.27	0.07	1.24	4.86
5	2.95	5.71	1.55	2.58	7.08
6	1.76	4.37	0.15	3.86	4.11
7	2.92	5.34	0.45	4.33	6.44
8	2.07	5.92	2.31	4.7	6.78
9	1.68	3.98	0.71	5.89	2.19

Table 7. Theoretical domoic acid concentrations (mg/kg) in pooled (n=10) gonad samples for each processor (P1, P2 etc).

All of these pooled results would have been well-within 20 mg/kg. Processors 1 and 3 would have found that the scallops were below the 4.6 mg/kg gonad trigger level as laid out in the new legislation in all cases, whereas 2 and 4 would have found that the scallops were above the trigger level in over a third of cases and 5 would have incorrectly found that the scallops were over the trigger level in over 50 % of cases. Using pools of 20 scallops rather than 10 did not produce any marked differences to these results.

These pooled results do not take into account differences in the masses of the different gonads used to form the pools. As the gonads vary quite widely in their masses some gonads may have a disproportionate effect on the pool. This is an unbalanced pool but because this study used individual gonad results we can correct this to produce a balanced pool to see if this has any effect on the outcome. The pools can be balanced by considering the mass of each gonad as a percentage of the mass of the pool then applying this percentage to the amount of domoic acid in each gonad and recalculating the domoic acid concentration for the balanced pool. Balancing the pool consistently lowered the calculated concentration. This was on average a 14 % reduction in domoic acid concentration (between the five processors and Integrin), with percentage reductions ranging from only 3 % to a high of 29 %. However, the highest reductions were associated with the lowest domoic acid gonad concentrations so that the differences in terms of concentration between the balanced and unbalanced pools for each processor were always small (ranging from 0.09 mg/kg to a maximum of 0.61 mg/kg). This effect is too small for it to be a major concern compared with other aspects of the analyses.

Turning to the worst-case scenarios, Table 8 shows the worst theoretical pooled concentrations and amounts for each processor. These were calculated by selecting the ten highest individual gonad values for each processor and taking a mean of the results.

	P1	P2	P3	P4	P5
Mean gonad size of pool	4.72	3.92	2.1	3.26	2.76
Concentration	8.49	11.28	4.75	14.26	16.52
Amount	40.1	44.28	10.0	46.6	45.6

Table 8 Theoretical worst domoic acid concentrations (mg/kg) and amounts (µg) in pooled (n=10) scallops for each processor (P1, P2 etc).

An interesting feature is that while the worst concentrations vary considerably, the worst amounts are much more similar for all processors except Processor 3. From the table the effect of gonad size is again obvious. For all the processors except Processor 1, the mean gonad mass of the theoretical worst pool is noticeably lower than the overall mean gonad mass. This shows the disproportionate effects of small gonads on concentration measurements which is smoothed out when amounts are looked at instead.

4.4 CONCLUSIONS:

Natural variation

Studying the variation in domoic acid content between different sites and at different times has revealed much valuable information that will help in our understanding of the underlying reasons for the variability of domoic acid in scallops. The use of large sample sizes, both in terms of sites examined and individual scallops analyzed, has allowed a greater degree of precision than has been achieved in previous studies. It not only allows significant differences to be identified but where there is a lack of significant differences the sample sizes used allows us to conclude that this is the result of homogeneity rather than weaknesses in the data.

The scallop gonad is a complex and dynamic organ, subject to a rapid change in size and mass at spawning[10]. Domoic acid in the gonad appears to be only truly associated with the gut loop travelling through it though poor shucking can (and probably frequently does) result in pieces of hepatopancreas being left on which distorts the true level. This was evident in this study from the effect of bias in producing the pooled samples. Domoic acid concentration should be independent of the gonad mass but it is clearly higher in smaller gonads from within a population (Figs. 13, 14). This is discussed in more detail in the next section but the results here show the importance of ensuring that any scallops used in a pooled analysis are of the same size as the general population otherwise gross misinterpretation can occur.

Whole animal data will not be as sensitive to seasonal differences in body mass as the gonad, as the proportional difference in mass due to spawning will be much lower in the whole animal[10]. Nevertheless, concentrations will fall when body mass increases due to the increased mass of the gonad prior to spawning[10]. If a box is being monitored then the decreasing domoic acid concentration caused by the dilution effect of the increasing gonad mass could be interpreted as depuration of domoic acid from the scallop if concentration is the only measure used. Conversely, after spawning the gonad shrinks markedly[5] so that the dilution factor produced by the gametes is lost and concentration will rise (pers. observ.). This may lead to erroneous interpretation that there is a phytoplankton episode going on whereas all that is being witnessed are changes in the dilution factor caused by gametogenesis. For these reasons, it is important to look at the amounts of domoic acid for

both whole animal and gonad when looking for evidence of bloom activity and for making comparisons between different sites and at different times. Concentration on its own is a very misleading comparator.

A surprising result from this study was that, as the gonad grew in mass, the amount of domoic acid per gonad generally increased (Fig. 8), and concentration also increased (Fig. 9). It would have been expected that the amount of domoic acid in the gonad would remain stable as the gonad increases in size while the concentration fell. The natural conclusion would be that there was a phytoplankton event was occurring leading to these increased levels of domoic acid. However, the whole animal data showed no increase in domoic acid concentration or amount. We cannot rule out the possibility that there was a phytoplankton event occurring even though the samples were taken in the winter – it would be possible that winter storms were re-suspending resting but toxic diatoms. The whole animal data is incomplete and only derived from pooled data so we cannot be sure that there was no actual increase in whole animal domoic acid levels that is just being masked by the inadequacies of the whole animal data. However, it is more likely that what is being witnessed is either translocation of domoic acid into the gonad gut loop from elsewhere in the gut or the increase in domoic acid level in the gonad is being produced by the gonad encompassing more of the gut within its structure, sufficient to offset the dilution effect of additional gonad mass. This requires further study.

This has two practical consequences. From a monitoring viewpoint, the dynamic nature of the gonad makes it very difficult to interpret changes in domoic acid levels in the gonad unless there is additional whole animal and gonad mass information also available. With this information a judgement can be made as to whether any differences in domoic acid concentrations in the whole animal or in the gonad are the result of changes in domoic acid amounts or just changes in the dilution factors caused (mostly) by changes in gonad mass as a consequence of gametogenesis. From a processors viewpoint, there is no "off" season for domoic acid in the gonad - the gonad levels can alter at any time of the year due to spawning or growth.

Fishing Box J5 is not spatially homogeneous with regards to domoic acid levels and this raises questions as to the benefit of fishing boxes for defining scallop areas. It also raises many points about how the monitoring data should be obtained. However, an encouraging feature of the data is that the variation is quite restricted in its geographical location. All of the sites that are associated with the Ross of Mull area (3, 4, 5, 6 & 7) are effectively the same, especially when domoic acid amount in the gonad is compared rather than concentration. Site 2 is also similar to the Ross of Mull ground sites and could be included in this ground rather than the Sound of Colonsay ground despite its proximity to the latter. The Sound of Colonsay ground is much less homogeneous with relation to the domoic acid content of the scallops but most of the variation is associated with sites 8 & 9. These are at either end of a shallow ridge extending out from Colonsay. It thus seems that sites that have similar physical characteristics within a fishing box are likely to have similar domoic acid levels regardless of their location within the box. The fact that the two sampling dates produced virtually identical patterns of variation in domoic acid levels while recording increases shows that the observed spatial variation in domoic acid levels is not a random artefact of the data.

This is important because it demonstrates the need to base monitoring around specific areas within a box to avoid apparently random fluctuations in domoic acid levels that are in fact

caused by spatial differences. The data in the current study are limited to ten sites and only two sample dates. Nevertheless, the results of the current study suggest that it may be possible to predict where in a box the highest levels of domoic acid are likely to occur in scallops based on the physical features within the box. Whilst there was an inverse correlation between domoic acid levels and site depth, we suspect that the hydrodynamics of the site are actually the important factor rather than simply depth. Sites 8 & 9 may have strong currents passing over them, resulting in excellent feeding conditions for the scallops. The large gonad size of scallops from these sites appears to confirm this. However, a more intensive and planned study of the relationship of domoic acid levels in scallops and the physical characteristics of their environment is necessary before any hard conclusions can be drawn from this observation. If it can be established that it is possible to accurately predict which sites within a box will have the highest amounts of domoic acid then these sites should be used for regular monitoring of the box since scallops taken from elsewhere in the box will have lower amounts. With further studies it may also be possible to define specific areas within grounds that are permanently closed because of high domoic acid levels associated with these sites while leaving the surrounding area free for harvesting. Using this approach, within J5, would lead to sites 8 & 9 being under a constant ban and the monitoring sites being chosen as sites 1 and 6 because of their high whole animal levels and their locations within the box.

Another important result is the consistent finding that the mean amount of domoic acid associated with the gonad is always below 1 % of the whole animal amount. Therefore, while it may be difficult to predict the actual levels of domoic acid in a scallop gonad, this < 1 % "rule" does mean that we can confidently predict what the upper limits of domoic acid amount in a gonad are going to be armed only with the information of the whole animal amount.

Processor variation

Caveats: The results from both the processor experiments are very revealing and important to discussions on how best to monitor and manage domoic acid in processed scallops. There are, however, several caveats. The first is that while the experiments benefited from the large sample sizes used, they were very restricted in terms of the geographical origins of the scallops and the range of domoic acid loadings in the whole scallops. The former may not be much of an issue, at least within a Scottish context but it is prudent to be cautious in extrapolating from the current results to the situation in scallops throughout the year. The adductor muscle only (roe-off) shucking study was done on scallops that came from a population where the whole animal concentration was at the upper limit that will be allowed under the tiered system (250 mg/kg). While not the worst possible case, the experiment should reflect the highest whole animal levels that processors will encounter when the tiered system is implemented. The adductor muscle and gonad (roe-on) shucking experiment used scallops from a population where the whole animal concentration was only approximately half of this (127.6 mg/kg). It is possible that at the upper limits of whole animal concentration the importance of the shucking process becomes less influential as natural variation exerts a greater effect. The same may be true at very low whole animal domoic acid concentrations but this is of no importance from a food safety viewpoint. It would be instructive to repeat this experiment at the upper limits of whole animal concentration (250 mg/kg) and at higher levels.

The second major caveat is that although we portray the results as comparisons between different processors, we have really compared the results of different, individual shucking staff. Their training and skills will reflect the processors within which they work but we are fundamentally comparing the skills of individuals. The different processors did not adopt identical approaches to producing the experimental samples. For example, Processor 3 used their most senior shucker who worked alone (and at impressive speed) to produce both the roe-on and roe-off products while Processor 4 used two shuckers to produce their roe-on product, one experienced and one who was completely new to the job. For these reasons, the rank order between the processors will not necessarily remain the same if the experiment is repeated or on a day-to-day basis – it will depend crucially on who is doing the shucking within each processor. The purpose of the experiments was not, however, to compare individuals, but rather to see if different practice could produce different results from the same batch of scallops.

***Adductor muscle only (Roe-off)*:**
That significant differences were found in adductor muscle only domoic acid concentrations and amounts between the processors was perhaps surprising given the low levels of domoic acid in all of the shucked adductor muscles. However, it was evident to the study analysts that the adductor muscles varied in the quality of shucking in that the amount of non-adductor muscle tissue associated with the shucked meats varied visibly between the processors. This was reflected in the eventual results. The processors were not used to producing a roe-off product so their competence would no doubt increase if they were regularly producing shucked meats. They all, however, managed to produce adductor muscles that had similar and in some cases lower mean concentrations than those shucked by Integrin staff under laboratory conditions. All the processors managed to produce shucked adductor muscles that were well within the regulatory concentrations and even at the highest concentrations they would have imparted a dose of only 0.75 mg of domoic acid in a 250 g serving and the mean amount in all cases would have been less than 0.25 mg compared with the regulatory amount of 5 mg [1,2,5]. The domoic acid recorded in the adductor muscles probably all derived from leaving small pieces of non-adductor muscle tissue on (mostly hepatopancreas or gut tissues but in at least one case the gonad) and the experiment suggests that with good shucking practice there should be very little or no domoic acid present in the adductor muscle. This does not preclude the possibility of gross contamination from hepatopancreas fluids resulting in much higher concentrations being present but there was no evidence of any such contamination during the processor shucking experiments.

The study therefore confirmed previous observations[5] that shucked adductor muscles contain only low levels of domoic acid and that all the processors in the study were able to produce a good standard of shucking. Improvements in shucking are possible but, even without these in effect, adductor muscles contain very negligible levels of domoic acid. There was no evidence that the resulting adductor muscle amounts of domoic acid related to the domoic acid concentration in the whole animal and this reflects the random nature of leaving small pieces of non-adductor muscle tissue in place. Therefore it is not possible to predict what the adductor muscle level of domoic acid will be from the whole animal level result.

The amounts of domoic acid in well-shucked adductor muscles are so low that it is improbable that they would ever breach the regulatory concentrations for domoic acid. However, gross contamination through really poor shucking or soaking from hepatopancreas fluids (HP) could potentially raise concentrations of domoic acid sufficiently to push the finished product above the regulatory limit. This is best controlled through good visual

inspection of the product and the imposition and enforcement of quality standards within the processors. The HACCP procedures laid out in section 6.0 should prevent most contamination through contact with HP fluids and it is unlikely that all ten adductor muscles in an EPT would be contaminated to a significant level.

Given that the levels of domoic acid in the processed adductor muscle is totally unrelated to the domoic acid amount in the whole animal, there is little point in relating the frequency of EPT for the adductor muscle to the levels of domoic acid in the whole animal or the gonad. Instead, routine but infrequent testing for the purposes of quality control is probably the most appropriate control measure, reinforcing the HACCP procedures. This could be supplemented by random inspections by EHO's inspecting both the visible quality of the product and having select samples tested.

Adductor muscle and gonad (Roe-on):

Processor 3 produced a product where there was virtually no domoic acid left after processing. This shows that virtually all the observed variation between the different processors in domoic acid levels in the roe-on product is down to differences in shucking quality. In consequence, monitoring gonad concentrations cannot predict the EPT result, as this will depend on the shucking process more than natural variation within the batch. It is necessary to repeat the caveat that this may not hold at higher whole animal domoic acid concentrations and this needs to be investigated further. The bulk of the observed variation is effectively an artefact of the shucking process. This also calls into question previous comparisons of scallop gonad variation where the data was obtained by different laboratories[5]. The statistical study underpinning the trigger level value of 4.6 mg/kg presumed negligible analytical variation[5]. The roe-on experiment completely reverses this and shows negligible natural variation coupled with considerable analytical variation, which is likely to be associated with the shucking process and with variability of subsequent sample preparation and clean up (see section 7.3). Shucking practice by monitoring laboratories is not subject to the same stringencies of other parts of the analytical process – indeed it is difficult to see how this can be measured except by doing large scale experiments of the type used here as the same scallop cannot be shucked twice.

The other salient feature of the experimental result is the emphasis on the importance of gonad size in producing high concentration results. This is understandable when gonad gametogenesis and spawning are considered: the gametes are effectively diluters of any domoic acid in the gut loop. Any changes through gametogenesis will produce changes in domoic acid concentrations that are not directly linked to amounts of domoic acid. Thus, when a gonad spawns, the domoic acid concentration will rise and since spawning can result in an order of magnitude lowering of gonad mass, domoic acid concentration can also increase by an order of magnitude. However, the processor roe-on experiment showed that the situation is even more extreme than this. Most of the observed variation caused by shucking is associated with small gonad mass. This is likely to be because smaller gonads are more difficult to completely free of associated gut tissue (other than the gut loop) and any remaining material will have a proportionally greater effect on small gonads than on larger ones. Very small gonads (< 2 g) were, however, less common than larger ones at the time of year this study was undertaken.

In conclusion, gonad concentrations obtained as part of a monitoring programme may have little relevance to the end product result produced by any specific processor (or individual shucker).

Whole animal concentrations were again a good predictor of the maximum domoic acid amounts associated with gonads and thus concentration if the gonad masses are taken into account. As with Integrin's results on the same batch, all the processors produced individual gonads that contained less than the predicted 1 % of the total whole animal domoic acid amounts, and in most cases much less than 1 %. Thus the additional levels of domoic acid in the gonads produced by the shucking process are encompassed within the 1 % limit for this batch. However, the differences between the processors mean that EPT data obtained by one processor cannot be used as a proxy or predictor of the EPT result of another processor. EPT therefore must be based on each processor's individual results – they cannot share test results from the same batch.

Close comparison of the experiment's outcome in terms of mean gonad mass, domoic acid amount and concentration suggests that different shucking techniques may produce payoffs in the end result. Processor 3 produced the "cleanest" gonads but at the cost of also producing the lowest mass yields. Processor 1 produced a much better mass yield while still producing low domoic acid amounts and concentrations. They did, however, also produce some of the worst extremes in terms of amounts seen in the data set. Integrin's analysts were trained in shucking by Processor 1 and Integrin's results for this batch do most resemble those of Processor 1. The differences in sample size and the bias in the Integrin data caused by analysts choosing to pool small gonads cautions against over-interpreting similarities between Integrin and the Processor 1 but both produced generally very low levels of domoic acid associated with the shucked gonads though with occasional extreme or outlying values that appeared to be unconnected with gonad size. Processors 2, 4 and 5 all had problems with shucking small gonads and if these were removed from their datasets they would have achieved similar results to Processors 1 and 3. Clearly, different shucking practices produce different results and a challenge for the industry will be to identify more precisely the optimal technique for producing the lowest possible contamination of the shucked meats by associated gut tissue whilst maximising meat yields.

From a practical viewpoint the experiment also questions the merits of leaving very small, spent gonads on the shucked product. Some processors generally remove these as a matter of course as they are very unappetising in appearance. Removing all gonads that are less than 2 g would drastically reduce the mean concentrations observed and much of the observed variation.

Encouragingly, all the processors produced shucked gonads that would have passed an EPT based solely on the gonad – even in the worst-case scenarios. Only a single gonad in the dataset was over 20 mg/kg and this was again a relatively small gonad (2.4 g). It would be prudent to replicate this experiment with a batch having a mean whole animal domoic acid concentration of 250 mg/kg. However, if their shucking efficiency holds good at these higher domoic acid concentrations, the processors should be able to produce shucked meats where the mean gonad concentration would pass EPT right up to the regulatory ceiling based on whole animal concentrations (though gonads tested immediately after spawning may give difficulties).

The EPT result will be influenced both by natural variation but more importantly by the shucking standard. The latter is effectively independent of the natural level of domoic acid so must be checked, regardless of the "real" value. As natural variation is related to both temporal and spatial factors, the EPT frequency must take these into account. An appropriate

level of testing is therefore suggested as being one per batch, the batch being defined as all the scallops from a single box landed to a single processor within one week. The number of vessels making the landings within this time is immaterial.

Better definition of scallop grounds would further enhance the confidence that this frequency of EPT is appropriate. Basing the monitoring stations in areas of a box where the highest levels of domoic acid are likely to occur will help ensure that all scallops taken from a box within a week are likely to pass the EPT providing good processing standards are adhered to.

5.0 OBJECTIVE 2:

To determine the possible levels and methods to reduce domoic acid contamination of Scallop gonad and adductor muscle with hepatopancreas fluid during processing and handling.

5.1 INTRODUCTION:

Processing is not necessarily a neutral process. Most of the domoic acid in scallops is associated with the gut, especially the hepatopancreas. The hepatopancreas is intimately associated with the gonad and if shucking fails to remove this adequately then the domoic acid concentration associated with the product can be much higher than would be anticipated from the status of the box from which the scallops were fished. In the main, this problem can be removed by ensuring good shucking practise but other elements of the process such as washing, soaking, half-shell shucking, and contamination of work surfaces and tools between batches could also be influential in determining end product concentrations of domoic acid.

The experiments associated with this part of work aimed to look at both normal industry practise and their possible contaminating effects. The experiments were also designed to investigate worst case scenarios and investigate specific practises (such as washing) to define best practise that could be applied in industry. All sample sizes were set at 30 scallops in order to allow the significance of small differences in domoic acid concentrations between treatments to be determined. While it is recognised that each type of treatment may lead to only a small improvement in domoic acid contamination, these improvements may be incremental and in combination they may make a considerable difference to end product levels of domoic acid.

Again, there are very few definitive studies of these processes therefore the experiments have considerably enhanced the state of the art and are invaluable in helping to inform the definition of best practise and the HACCP assessments.

5.2 EXPERIMENTAL DESIGN:

For the purposes of these experiment one batch of scallops was purchased from Processor 1 and shucked at their premises. The study used scallops from box J3 at a domoic acid concentration of 72 mg/kg whole scallop.

The batch was processed by the shucking team at Processor 1 under the supervision of C. Bavington from Integrin.

The shucking team consisted of the following personnel:

'Opener' –	responsible for opening scallops for the other shuckers, and for the entire half-shell shucking process.
'Normal' shucker -	responsible for the preparation of shucked meats to the normal standard maintained at Processor 1
'Poor' shucker -	responsible for preparation of poorly shucked meats i.e. with black left on the end product.
'Washer'	responsible for washing the shucked meats

Each shucker worked on a separate table using separate knives and containers.

Two experiments were conducted. In the first (see HP contamination experiment below), shucked adductor and gonad (meats) were soaked in hepatopancreas (HP) fluid for one hour then subjected to washings with water of varying durations. This experiment was to simulate the effects of HP fluid contamination caused by rupturing guts on capture and during transport or of shucked adductor and gonad (meats) being contaminated during the shucking process through immersion in HP fluids. A preparation of hepatopancreas homogenate had been previously prepared, from scallops received at Integrin as a scoping sample for box J5 at domoic acid concentration of 20 - 30 mg/kg gonad, and analyzed at Integrin for use as a contaminant (shown to > 3000 mg domoic acid / kg).

HP contamination experiment	Samples
120 meats soaked in hepatopancreas for 1 hour at room temperature, and subsequently split into batches of 30:	30 Unwashed 30 Washed for 1 minute 30 Washed for 5 minutes 30 Washed for 10 minutes

In the second experiment (see shucking experiment below), a large batch of scallops was divided into three groups: a normal group; a half shell group (Fig. 28) and a poorly shucked group (Fig. 29). Poorly shucked scallops were prepared so that black was visibly left on the end product. The team was not used to preparing a poor product, but the 'poor shucker' managed to prepare a product comparable to that seen in some retailers. All three groups were then again subdivided and subjected to different treatments (unwashed, washed, chilled dry, and soaked).

Shucking experiment	Samples
120 normally shucked meats, subsequently split into batches of 30	30 unwashed 30 washed 30 washed, and chilled for 24 hours 30 washed, and soaked for 24 hours
120 poorly shucked meats with, subsequently split into batches of 30	30 unwashed 30 washed 30 washed, and chilled for 24 hours 30 washed, and soaked for 24 hours
120 half-shell scallops, subsequently split into batches of 30	30 unwashed 30 washed 30 washed, and chilled for 24 hours 30 washed, and soaked for 24 hours

Washing was carried out using the standard washing procedure employed by Processor 1. Briefly, shucked meats were placed in plastic colanders and rinsed under running water with periodic agitation. 30 meats were washed for approximately 30 seconds, using approximately 12 liters of water. Half-shell products were placed between metal racks and sprayed using a hose fitted with a diffuser nozzle. 30 half-shells were washed for approximately 50 seconds using 25 liters of water. Contaminated meats were washed in the same way as other meats but for the extended periods defined above.

Soaking and chilling of the shucked products was carried-out at Integrin, on the same day as the shucking. After weighing, meats and half-shells were chilled at 3 ºC (± 2 ºC) for 24 hours in plastic bags, with no added water. After weighing, meats and half-shells were soaked for 24 hours at 3 ºC (± 2 ºC) in plastic boxes with filled with tap water (equivalent to approximately 50 % of the unsoaked weight).

All samples were placed in separate bags, sealed, and indelibly labelled with sample identifiers (see Annex 4 for list of batch codes). Samples were transported at ambient temperature to Integrin on the day of shucking. All shucked tissue was stored frozen (-20 °C) until analysis. Aliquots of tissue homogenate from all samples have been retained in storage at – 20 ºC.

Samples of wash waters were collected and swabs were taken from shucking tables using standard microbiological swabs (see water samples and swabs below). Swabs were drawn over each shucker's table until saturated with the liquid present. Each shucker used an area of about 1 m^2, but only a small part of this was required to saturate the swabs used.

Water samples & Swabs

Knife wash 01	Water from knife-rinse bowl used by the poor shucker.
Knife wash 02	Water from knife-rinse bowls used by normal shucker.
Knife wash 03	Water from knife-rinse bowls used by opener
Bucket wash 01	Wash water from half-shell washing.
Bucket wash 02	Wash water from muscle plus gonad washing.
HPwash01	1 minute wash water from 60 HP contaminated scallops.
HPwash02	2.5 minute wash water from 60 contaminated scallops.
HPwash03	5 minute wash water from 60 contaminated scallops.
Hpwash04	10 minute wash water from 60 contaminated scallops.
Swab01	Poor shucker's table
Swab02	Normal shucker's table
Swab03	Opener's table

5.3 RESULTS:

The HP fluid used in the experiment had a concentration of 3647.5 mg/kg and approximately 600 g was used to soak the scallops. The HP fluid contamination experiment (Fig. 25) produced high concentrations of domoic acid in the unwashed meats (mean = 128.4 mg/kg). A one-minute wash removed approximately 75 % of this domoic acid, reducing the mean concentration to 34.9 mg/kg. However, extending the wash period to 5 minutes made no difference to this level (34.75 mg/kg) and extending this to 10 minutes resulted in only a small further drop (26.47 mg/kg). Washing produced a highly significant difference over the unwashed group ($p < 0.001$) but no significant differences were found between the washing groups ($p > 0.01$ – *Newman-Keuls* post hoc test). The 10-minute wash did produce a difference that was significant to the two shorter washes if a lower standard of significance is applied (*Newman-Keuls* $p = 0.03$).

The scallops used in the process experiment had mean whole animal concentrations of 72.6 mg/kg (Fig. 26) with a SD of 38.5 (n = 10). The gonads had mean concentrations of 3.7 mg/kg (SD = 2.55, n =10). The gut-loop and crystalline style had a domoic acid concentration of 26.2 mg/kg. The process experiment used very poorly shucked meats with a mean concentration of domoic acid of 19.3 mg/kg before washing. The half shell and normally shucked meats had lower concentrations (1.56 mg/kg and 1.34 mg/kg respectively). The initial wash had no significant effects on the normal or half shell products (Fig. 26) but produced a dramatic fall in the domoic acid content of the poorly shucked meats. This was the only part of the dataset that showed a significant difference when all the treatments and products were considered together (*Newman-Keuls* $p< 0.01$). Separating the different products from each other showed that for the poorly shucked product the initial wash produced a significant reduction in domoic acid concentrations and amounts. Although soaking the product did result in a reduction in both domoic acid concentration and amounts, this was statistically insignificant. None of the treatments resulted in a significant difference for the normally shucked roe-on product, indeed there were no obvious differences between the treatments at all. A surprising result was that the treatments produced a highly significant effect for the half shell product (Fig. 27). Merely leaving the half shell product for 24 hours greatly reduced the domoic acid concentration and amount (*Newman-Keuls,* $p < 0.001$). Soaking did not produce a significant increase in reduction over this.

Soaking produced an increase in the mass of the products (table 9)

Product	**Unsoaked mass (g)**	**Soaked mass (g)**
Normal	26.62	31.43
Half shell	26.54	33.23
Poorly shucked	28.35	35.68

Table 9. Effect of soaking for 24 hours on meat mass (gonad and adductor muscle combined)

This increase in mass explains some of the reduction in concentration but cannot account for all of it indicating that some of the domoic acid is being lost to the soak water. This was most evident for the half shell product but the poorly shucked product also lost a proportionally high amount of domoic acid and as the starting position was much higher for this product, the amount of domoic acid being lost was much higher and would have resulted in higher concentrations in the soak water than for the other products.

The different washes of the HP contamination experiment produced high concentrations of domoic acid in the wash water (137.1 mg/kg for 1 minute; 110.1 mg/kg for five minutes and 14.9 mg/kg for ten minutes). Swabs from the shucking tables detected low concentrations of domoic acid where the poorly shucked scallops were prepared (2.7 mg/kg) but no domoic acid was found on the tables for the other two products. Knives and wash water taken from the shucked scallops produced only trace amounts but a concentration of 3.7 mg/kg was detected in the soak water for the poorly shucked products.

5.4 CONCLUSIONS:

The HP fluid contamination study has shown that significant contamination of the adductor muscle and gonad can occur if these are allowed to sit in HP fluids. The NRL found a similar result with contamination of the adductor muscle in a smaller experiment[5]. One of the striking things about the data is that, although washing considerably reduced the amount of domoic acid associated with the contaminated tissue, the length of washing time had little effect. This indicates that some domoic acid is difficult to remove and is presumably bound but that most of the contamination is unbound. The experiment clearly shows that HP leakage can be a serious source of excess domoic acid in the shucked product and unlike residual tissues left on, it is much more difficult to detect through visual inspection. Therefore, effort has to be put into ensuring that HP leakage does not occur during scallop processing.

It is no surprise that poorly shucked meats have higher domoic acid concentrations than well shucked ones. The resulting scallops were not exceptional and scallops of similar appearance have been witnessed in shops. The purpose of the second experiment was, however, to look at the effects of post shucking practices to help determine which of these are important. Interestingly, the results suggest that post-shucking practice has little impact for the normal (i.e. well shucked scallops). This is in stark contrast to the poorly shucked scallops where post-shucking practice, particularly washing, leads to significant reduction in domoic acid levels. This means that although washing is only of marginal importance when the scallops are well shucked it is crucial as a secondary safety measure to the shucking practice. The significant reductions in domoic acid levels associated with leaving the half shell product for 24 hours with or without soaking was unexpected. The half shell product was predicted to behave similarly to the normal product (and hence would be able to be classified the same as normally shucked material). There was no expectation that this product would have superior performance. Repetition of the experiment would be advisable before placing too much emphasis on this result, however, it may be that the physiological “health” of the half shell product was sufficiently different from the fully shucked product to alter the result (e.g. by allowing muscular activity to continue for longer which resulted in flushing of the gut loop).

The environmental samples show that domoic acid does spread beyond the scallops themselves during shucking. The most serious contamination is of the table itself and measures should be put in place to minimise this and to reduce contact between the shucked meats and the table. The other area of concern is soak water. These had measurable amounts of domoic acid in them and represent a possible mechanism for concentrating the toxins. Such waters have to be renewed frequently and should not be used for other purposes.

These experiments have shown both the dangers of contamination and the importance of good shucking. It should be remembered that the concentration figures given here relate to adductor muscle and gonad combined. For the adductor muscle and gonad combined to breach the statutory limit of 20 mg/kg is much more serious than for the gonad alone to fail

because the concentration does relate very well to the amount ingested (whereas it frequently does not in the case of the gonad alone).

4.0 OBJECTIVE 3:

To provide detailed descriptions of processing procedures and HACCP assessments leading to recommendations for each of the Scottish Scallop processors.

6.1 INTRODUCTION

To ensure that the tiered system is effective in delivering benefits both in terms of consumer safety and industry viability it has to be underpinned by effective management policies within the processing industry. There is no UK HACCP system available to industry relating to shellfish toxins at present though HACCP is used for this purpose in the USA[11]. The survey and HACCP recommendations will do much to identify and disseminate best practise. The HACCP survey was carried out in the second half of the project and was informed by the results from the first two study objectives on EPT definition and the shucking experiments.

FSA Scotland sent out a circular letter to all seafood processors in Scotland seeking to determine processors who were involved in scallop shucking and to ask for additional information on the tonnage of scallops processed per annum. Processors were also asked if they would agree to a confidential visit from Integrin to conduct a HACCP assessment of their premises. Where possible, the visits coincided with actual scallop processing. However, this was not always achieved, as scallop landings are sporadic.

The primary purpose of the visits was to identify best processing practice and highlight any poor practices, particularly in light of the contamination experiments detailed in objective 2. It was envisaged that the information gathered could then be used to make general recommendations that could be incorporated into HACCP systems for each of the processors. The HACCP visits were conducted by Mrs Napier, Dr McKenzie or Dr Bavington. A number of processors were visited by all three to ensure homogeneity in the study approach.

6.2 RESULTS:

22 active processing sites were identified during the study and a total of 21 companies were involved (Fig. 30).

Processing plants visited were:
West Coast Sea Products (Kirkcudbright),
Taste of the Sea (Castle Douglas)
Scotprime (Ayr)
MacMillan Seafoods (Campeltown)
Loch Fyne Seafarms (Tarbert, Loch Fyne)
Islay Crab (Islay)
West Coast Shellfish (Lochgilphead)
Ardmore Fish (Mull)
Barra Atlantic (Barra)
Kallin Shellfish (North Uist)
Dawnfresh (Bellshill)
Iona Shell (Dunfermline)
Scobere (Aberdeen)

MacDuff Shellfish (Mintlaw)
Speyfish (Buckie)
Shetland Norse (Shetland)
D. Watt (Shetland)

Processors indicating that they are involved in shucking but who were not visited:
Hebridean Seafare (two plants: Stornaway and Invergordon)
Pecten Max (Macduff)
Fraserburgh Seafoods (Fraserburgh)
Keltic Seafare (Dingwall)

A great diversity of practice was found and some near universal concerns of processors were uncovered during the processor visits:

The system of Movement Order Documents (MOD's) was clearly a source of confusion to boats, processors and issuing Environmental Health Officers. In some cases perfect MOD records were in place but this information was not used subsequently. In other cases there were no MOD's and every scenario in between these two extremes was observed. This was due to fishing boats being reluctant to use MOD's, or confused as to who should have the document. It was pointed out that most or all boats do not have photocopiers and therefore retaining copies of MOD's was difficult for the boats. Some EHO's were issuing boats with multiple MOD's. In other cases the processor was issued with MOD's which were then used for all landings. There was a lot of comment from processors that the MOD's were confusing and unsuitable for the purpose intended. There appeared to be no standard format therefore boats that work in different authority areas had to use several different types of forms and systems.

A second common concern was recruiting and retaining shuckers within processors. This made it difficult to maintain standards of shucking within the processors.

While there was concern over the costs of end product testing (EPT), more concern was expressed by the processors over the time taken for testing. The majority of the scallop processors in Scotland sold their shucked scallops as a predominantly fresh product and there is usually less than 72 hours between shucking and consumption. The high cost of product recalls was highlighted: a product recall involving a supermarket would result in a £25K charge to the processor. Some of the processors did not have access to e-mail or the internet and a surprising number were unaware that they could receive the weekly toxin results from the FSA Scotland monitoring programme. In many cases the boats making the landings were small inshore boats with small landings (up to 1000 scallops). There was comment from some processors that the tiered system will favour large vessels which stay at sea for some days depending on how batches are determined.

However, batch separation and subsequent batch traceability is either already well developed or will be easy to implement within processors on the advent of the tiered system. All the boats and dive boats are paid on yield of adductor muscle and gonad therefore, the processors need to keep each landing separate right up until the washing phase to allow them to calculate how much money the boats will be paid. Batch separation before and during shucking is thus already controlled within the processors. An excellent and widely observed practice was for the bags that the boats used have the boat's name on them. There was some concern amongst processors that they may not be given the correct information concerning the origins of the

scallops. Additionally, it was pointed out by processors that this may become more difficult once the tiered system is in place as boats will be only legitimately able to fish in certain boxes. It was perceived by the processors that it would be easy to mix scallops from different boxes and pass them on to a processor as having originated from one box.

Post shucking batch traceability was also generally good, as the scallop products have to carry the shucking date (to calculate shelf life), and the processor name or identifier. The scallop product is generally shipped in relatively small consignments packed in two kilogram "ice cream" tubs and sent out as eight kilogram packages in most cases. Therefore, it should be possible to have a unique identifier that follows the batch of scallops throughout its life. One good practice identified, that was surprisingly not universal, was the use of computer-generated labels for the shipped product. These are easy to manipulate to allow the unique identifier to be attached to the consignment of scallops.

There was a diversity of shucking methods between processors and it is difficult to comment on which method is likely to be the best in terms of productivity or domoic acid reduction. However, the foundations of good practice were observed in most processors: complete removal of the black line (anal tube) and close trimming of the hepatopancreas (gut) to the gonad followed by rigorous washing. Post shucking inspection and re-trimming were observed as quality control measures in some processors. However, a common problem identified in many of the processors was allowing the shucked adductor muscle and gonad meats to come into contact with the juices emanating from the shucked scallops. This is an obvious route for contamination of the end product from hepatopancreas fluid and the contamination experiments carried out under objective 2 (section 5.0) of this study suggest that this is a serious problem which requires to be addressed by processors.

Disposal of the hepatopancreas (gut) after shucking seemed to be well handled. In all bar two cases it was going to landfill, often bagged up to prevent gulls and other vermin accessing it. Interestingly no processor mentioned gulls as a problem even when the waste was stored outside. There was concern expressed by processors over increasing landfill charges and at least one processor expressed an interest in drying the waste to reduce its weight. There was also an interest in composting the offal. However, such practices should be subject to close scrutiny before they are permitted for routine treatment of wastes to ensure that there is no possibility that domoic acid ends up concentrated in another food product through such practices (proposed legislation concerning the composting of animal products will probably make this illegal)

The Quality Assurance (QA) / Quality Control (QC) systems in processors ranged from non-existent to fully implemented HACCP systems. Additionally, access to training was highlighted as an issue for some of the processors based in the Hebrides, as even basic hygiene courses were difficult to access for them. Computer aided distance learning or roving hygiene trainers may be of assistance here.

A frequent comment from processors was that the tiered system seemed likely to encourage a poorer quality product (frozen or soaked product derived from scallops landed after some days at sea from larger vessels that may produce greater environmental impact). The smaller processors generally rely on small local vessels and produce a dry or semi-soaked product that depend on rapid delivery to market. These small processors are also within the most socially vulnerable areas and are also more likely to be scallop specialists.

6.3 HACCP RECOMMENDATIONS AND CONCLUSIONS:

The act of shucking will remove most (99 %) of the domoic acid in the scallop, hence the rationale for the tiered system. However, shucking is not a neutral activity: the study observed good and bad practice but with minimal increase in effort all of the processors visited would be able to operate to best practice.

The greatest risk associated with the processed product lies within the gonad however, the adductor muscle is also capable of being contaminated. As the adductor muscle is usually the heavier of the two components in the shucked product, the adductor muscle can sometimes contain more domoic acid than the gonad even if its domoic acid concentration is lower. Therefore, both adductor muscle and gonad have to be considered to reduce the levels of domoic acid found in the shucked product and therefore the end product testing should consider both components (see section 4.0).

Domoic acid on the shucked product derives from pieces of the digestive system-hepatropancreas ("black") retained on the shucked product; the content of the digestive loop in the gonad and external contamination of the shucked product. The latter occurs either before the product is shucked or during shucking. Good shucking practice will remove all the "black" and prevent contamination. Experiments carried out in this study under objective 2 (section 5.0), suggest that different washing practices may also reduce the level of domoic acid associated with the gonad loop. However, this may not be consistent as it will depend on the state of the scallops and whether the domoic acid is contamination or genuinely associated with the gonad loop. The domoic acid associated with the gut loop in the gonad may effectively be an irreducible level associated with the shucked product.

Given the serious consequences to the processor if the EPT fails, there is a major incentive for the processor to ensure that their shucking standards are of a high quality. An accurate knowledge of the domoic acid that is associated with the gut loop in the gonads from each batch could allow the processors to use their knowledge of their shucking standards and any seasonal factors to estimate the probability that the product will fail the EPT. There are two pieces of information that are vital to the processor: that the monitoring data is accurate and that the scallops delivered to them come from the correct box associated with that result.

Recommendation 1: Monitoring laboratories involved in routine testing for domoic acid should use best shucking practice. (*This is to ensure that the processors have comparable data upon which to base their own risk assessments*). A database should be maintained so that it shows all boxes/areas with latest data and date of data.

Recommendation 2: Shells cracked or crushed on dredging or in subsequent transfer should always be discarded. (*This is to prevent contamination of the adductor muscle and gonad through hepatopancreas fluids leaking from ruptured guts; it also reduces the possibility of slivers of shell being in the shucked product*).

Recommendation 3: Development of a shucking training standard that will be required by any processor shucking scallops. This should consist of briefing on algal toxins, the location of toxins in scallops, training in shucking technique, hygiene and safe disposal of wastes. This standard should also include a statement of shucking quality that all processors will adhere to (e.g. all hepatopancreas ("black") must be removed including the black line).

Recommendation 4: That shuckers work where possible in teams with an opener / pre-trimmer separate from the final shucker. (*This will reduce the contamination of the product and provide additional internal quality checks*).

Recommendation 5: Shucked product must be kept separate from hepatopancreas fluid (juices) emanating from shucked scallops and separate from shucked hepatopancreas (gut). The shucking technique should be adequate to ensure this. (*This is an important measure to prevent contamination of the shucked meats from hepatopancreas fluids*)

Recommendation 6: Washing should use a vigorous technique in running water. After washing the product should be examined to ensure that it meets the shucking visual standard. Any product that does not should be re-trimmed.

Recommendation 7: Batch separation must be maintained at all times, with wash down of tables and knifes between batches (and preferably as often as possible).

Recommendation 8: Waste soft parts of the scallop (e.g. hepatopancreas and trimmings) should be shucked into separate bins immediately and not allowed on the shucking table. Waste soft parts of the scallop must go to landfill or incineration.

Recommendation 9: Wash waters and water used for soaking the scallops must be discarded after use and not reused. (*Soak waters can contain relatively high levels of domoic acid that have leached out of the product; re-use might lead to a concentration of domoic acid in the water contaminating the scallops*)

A HACCP process flow and control charts for each processor are shown in Annex 5.

7.0 EXTERNAL QUALITY CONTROL

7.1 METHODOLOGY

In order to provide external assessment of the accuracy of Integrin's results, samples were sent to three external laboratories, these were:

CEFAS, Weymouth, England.
Chemical surveillance department, DARDNI, Belfast, Northern Ireland.
National Biotoxin Reference Laboratory, Marine Institute, Dublin, Eire.

The samples were selected to represent the full range of tissues and domoic acid concentrations tested in this study. External laboratories were given no specific instructions but were advised of samples containing more than 100 mg/kg (to allow them to incorporate any necessary dilutions to fit with the range of their assays). All laboratories carried out analyses by HPLC with photodiode array detection. The selected samples were prepared from frozen tissue homogenates. They were weighed out into aliquots of 8 – 10 grams and refrozen. The samples were shipped frozen with ice-packs by next-day courier delivery. The first set of samples sent to laboratory D was not frozen on receipt and therefore was spoiled. There was insufficient material available to resend all 10 samples so only the remaining 6 samples were tested by lab D.

7.2 RESULTS

Results are shown in table 10.

		Integrin	Lab B	Lab C	Lab D	Mean	SD	CV
Sample	**Tissue**	**Domoic acid (µg/g)**	**Domoic acid (µg/g)**	**Domoic acid (µg/g)**	**Domoic acid (µg/g)**	**Domoic acid (µg/g)**		**%**
1	Adductor muscle	1.7	LOQ	0.42	Not done	NA	NA	NA
2	Adductor muscle	1.5	LOQ	<0.33	Not done	NA	NA	NA
3	Whole	219.8	233	236.76	297	246.64	34.352	13.928
4	Pooled Gonad	2.3	2.24	3.18	3.24	2.74	0.5438	19.847
5	Pooled Gonad	13.1	7.71	10.82	10.6	10.56	2.2093	20.927
6	Pooled Whole	174.9	120	166.54	155	154.11	24.159	15.677
7	Pooled Gonad	11.5	4.16	6.22	5	6.72	3.297	49.062
8	Muscle + gonad	29.6	22.9	35.17	Not done	29.22	6.1437	21.023
9	Muscle + gonad	20	7.19	11.94	7.78	11.73	5.9062	50.362
10	Muscle + gonad	45.1	32.2	48.35	Not done	41.88	8.542	20.395

Table 10. Domoic acid concentrations determined at Integrin and three external laboratories.
LOQ = Limit of quantification

Results for Integrin, Lab B and Lab C are for domoic acid only. The results for Lab D are for domoic acid plus epidomoic acid. Results for Lab C and Lab D are corrected for recovery, using certified reference material (MUS1B, NRC Canada) as the positive control.

A high level of variation is seen for all the samples (mean coefficient of variation (CV) = 23.95 %), especially samples 7 & 9 (CV = 49.1 % and 50.4 % respectively). The results obtained by Integrin from these two samples were the only ones to differ to any meaningful extent from the external laboratories. For the remaining samples there is no consistent difference between Integrin and the other laboratories. Laboratory B was consistently lower (for the 8 samples that returned a numerical value) than Integrin and all the other labs (mean % difference = -30 %). There was no pattern seen with regard to tissue type tested or the level of toxin being tested.

7.3 DISCUSSION AND CONCLUSIONS

Despite the variation seen there are a number of encouraging features in the data:

1.Samples for which Integrin determined low values, near the limit of quantification (LOQ), were retested by the external labs as being either LOQ or near to Integrin's result. Variation seen at these low concentrations is not important given the uncertainty of the test method.

2. The retest results for samples for which Integrin determined high values (>150 µg/g) were confirmed by the external labs with the lowest level of variation seen for any of the samples (CVs of 13.7 % and 14.6 %).

3. Eight of Integrin's results were confirmed within the level of variation seen between the external labs (average CV of 21.89 %).

For samples 7 and 9 Integrin's results were consistently higher than the external labs (55 % higher in both cases). It is not possible to rule out analytical error but all the quality control parameters for these analyses met Integrin's specifications and there were no recovery

problems. The differences could be arising from inhomogeneity. Sample 7 was a pooled gonad sample and sample 9 was an individual adductor muscle plus gonad from a batch which had been deliberately poorly shucked. Therefore, both of these samples would be susceptible to inhomogeneity.

It is important not to draw too many conclusions from a small number of samples and incomplete data set, but the level of variation seen between all the labs raises a number of important issues which are relevant to the operation of the tiered testing system. Clearly analytical variation at the level seen (about 20 %) will critically affect the status of fishing areas affected by domoic acid. There is no reason to believe that the variation is due in any way to failure of the laboratories involved, but that it reflects the variation that will be seen between and within labs, especially when they are testing large numbers of samples at speed (which will be necessary for the implementation of the tiered testing system). There are two likely sources for the variation seen: sample inhomogeneity, and analytical variation (particularly the SPE extraction step). It was not in the remit this study to carry out full homogeneity studies (as would be done for a ring trial) before sending samples to external labs, and in most cases there was insufficient material to do this. It is unsurprising therefore, that a level of variation was seen, especially as seven of the samples contained adductor muscle which is particularly difficult to work with. The main source of analytical variation is probably the operation of the SPE clean-up stage (as discussed in section 4.3, Natural Variation, paragraph 6). Analytical details were not provided by all of the labs, but it likely that they all use slightly varying techniques and Lab C used an automated SPE system. Method development work should be a matter of urgency in order to improve the precision of the existing test method in all analytical laboratories prior to the introduction of the tiered testing system. Differences between the shucking efficiency of different laboratories have not been studied and this is likely to further increase analytical variation between the laboratories.

8.0 CONCLUSIONS AND RECOMMENDATIONS:

The EU Commission Decision (2002/226/EC) on the tiered system calls for end product batch testing of shucked scallops. The EU Decision definition of a batch is not defined therefore the primary purpose of the current study was to help produce a workable definition of what a batch should be. The guiding principles are that the batch testing must provide satisfactory public protection while not placing an unnecessarily onerous burden on industry. The different elements of this study were designed to generate information that could be used to determine a suitable level of batch testing.

The most important and relevant result from the survey of 10 separate trawls within box J5 is that there is genuine differences in domoic acid levels between scallops from different areas (spatial variation) and between samples taken from the same areas at different times (temporal variation). This has implications for both monitoring programmes and for EPT. Domoic acid levels in the gonad were shown to be capable of increase even when there was no apparent additional domoic acid coming into the whole animal via the environment (water sampling was not carried out in J5 during this study so the evidence for this statement comes from the apparent lack of any rise in whole animal concentrations during the study period). Another important finding was that domoic acid levels associated with the rest of tissue component (i.e. everything except the gonad and adductor muscle) consisted of > 99 % of the total domoic acid load. This requires to be confirmed for scallops where the whole animal concentrations lie out with the levels found in the current study.

The shucking experiments showed that processors (or shuckers) processing scallops from the same batch gave significantly different results. This means that the EPT result cannot be predicted (by monitoring activity) and it will depend on the shucking standard implemented by the individual processors. This was true for both adductor muscle only (roe-off) and adductor muscle and gonad (roe-on) products. There is, however, a genuine best-practice procedure that if fully implemented between different processors would reduce the amount of domoic acid associated with the end product.

The contamination and processing experiments showed that significant contamination of the adductor muscle and gonad (roe-on) product could occur if it came into contact with the hepatopancreas (gut) fluid and that this contamination could be difficult to remove. The washing and soaking experiments showed that the half shell product could be considered as a fully processed product. These experiments also showed that soaking could reduce domoic acid levels and that the removed domoic acid would move into soaking and washing solutions, resulting in lower domoic acid amounts in the end product and a risk of considerable domoic acid concentrations in the wash water.

Lastly, the HACCP study found examples of both good and bad practice within Scottish scallop processors, however, adjusting industry practice to improve the domoic acid loading of the end product should neither be difficult nor too expensive for processors to implement.

Modelling domoic acid content in shucked scallops

From the information generated during this study and incorporating previous findings [5-7], it is possible to produce a model of domoic acid in King Scallops that may have considerable utility in helping make judgements about EPT.

The consistent finding during this study that the “rest of tissue” is the location of more than 99 % of the total amount of domoic acid in a scallop allows the upper limits of domoic acid that can be found in the scallop processed products to be calculated. This figure makes allowances for variation caused by shucking practice unless there is a gross failure of the shucking process (i.e. large amounts of hepatopancreas ("black") being left on or heavy contamination from hepatopancreas (gut) fluids.

From this, the maximum amount of domoic acid in any product can be predicted from the amount in the whole animal. The model can be used to predict the maximum amount in any particular shucked product; to predict maximum results from monitoring programmes and to predict the amount (and concentration) of domoic acid in a portion of scallops to be consumed.

The latter is the easiest to calculate. We assume that the shucking efficiency does not fall below 99 %; that the average portion size for roe-on scallops is 250 g and that the roe-on portion forms at least 50 % of the whole animal flesh mass. Thus, to achieve a portion of 250 g of roe-on product, a total of 500 g of whole animal (tissue mass) requires to be processed. Presuming the scallops were taken from a box at the new maximum limit (250 mg/kg domoic acid whole animal), then the total amount of domoic acid in the 500 g (0.5 kg) of whole animal will be:

0.5 (mass of scallops) x 250 (domoic acid concentration) = <u>125 mg</u>

As only 1 % of domoic acid is left in the roe-on product, the maximum amount left will be:

125 mg x 0.01 (shucking efficiency)

= 1.25 mg of domoic acid

In a 250 g serving of roe-on product, the maximum concentration of domoic acid should not exceed:

1.25 mg ÷ 0.25 (mass)

= 5 mg/kg

To repeat the assumptions: the shucking efficiency does not fall below 99 % (i.e. less than 1 % of the domoic acid is left in the shucked product; the roe-on product is at least 50 % of the total whole animal tissue mass; that the scallops do not exceed 250 mg/kg whole animal concentration.

During this study, the lowest recorded shucking efficiency left 0.85 % of the domoic acid in the product. The shucking efficiency of the processors in producing the roe-on product during this study was not directly measured as no comparable "virtual" whole animal data was produced. Only the single pooled whole animal sample taken by Integrin is available for the whole animal amount and only the gonads from the roe-on product were measured. Presuming that this whole animal concentration holds for the scallops shucked by the processors and that amount of domoic acid in roe-on product is similar to the amount in the gonad, then the worst result for a processor left only 0.30 % of the domoic acid in the roe-on product at 127.6 mg/kg whole animal domoic acid concentration. In the study, the worst shucking efficiencies were associated with the highest whole animal domoic acid concentrations. There was also usually at least some domoic acid associated with the adductor muscles produced by the processors. The shucking efficiency of the processors at 250 mg/kg will, therefore probably be below 99.7 % but probably also above 99 % given the similarity in the amount of domoic acid in the gonads from the same batch shucked by Integrin and by the processors.

During this study, the adductor muscle and gonads formed approximately 55 % of the whole animal tissue mass in those scallops measured. It is, however, likely that at some times the mass ratio between the roe-on product and the whole animal tissue mass drops below 50:50. If the domoic acid content of the roe-on product remains at a maximum of 1 % of the whole animal amount, then the domoic acid content (and hence concentration) of the roe-on product will increase.

Although the assumptions of the model may therefore not hold strictly true in all cases, the theoretical maximum concentration of 5 mg/kg does give a considerable margin of safety. The shucking efficiency would need to fall to 96 % or the mass ratio of adductor muscle and gonad combined to the whole animal would need to fall to 12.5%: 87.5 % before the roe-on product would reach 20 mg/kg at a mean whole animal concentration of 250 mg/kg. Theoretically, scallops could be harvested up to 1000 mg/kg whole animal domoic acid concentration before the roe-on product exceeds 20 mg/kg. However, the shucking efficiency minimum removal of 99 % of whole animal total domoic acid amount may not hold true at such levels. Nevertheless, even with a combination of poor shucking, low roe-on mass

compared with whole animal mass and scallops coming from populations over 250 mg/kg, the roe-on product should be comfortably under the regulatory concentration unless there has been gross breach of the model's assumptions.

Using the model to calculate likely gonad domoic acid concentrations or to back calculate whole animal concentrations from gonad domoic acid concentrations requires more information to be put into the model and additional assumptions to be made. As there is no ramification of the gut present in the adductor muscle there should be no domoic acid associated with the muscle except for contamination from either hepatopancreas juices or from small pieces of tissue other than the muscle that have not been completely shucked off. Theoretically, all the domoic acid in the roe-on product should be associated with the gonad. The actual division of domoic acid into the adductor muscle and gonad compartments was not studied for the processors and the only data we have relates to Integrin's data from the roe-off experiment. While the concentration of domoic acid in the adductor muscles was always low, the large size of the adductor muscle meant that the amount of domoic acid in any one adductor muscle could be quite large and in this data set the adductor muscle contained on average a surprisingly large 28.5 % of the total domoic acid associated with the gonad and muscle combined. We can incorporate this in the model as an assumption that the ratio of domoic acid in the gonad to the muscle will be 70 %:30 %.

We can now apply this model to the actual results of the roe-on experiment. The whole animal concentration of the pooled sample for site 4 was 127.6 mg/kg. The maximum total amount of domoic acid that will be associated with a 250 g portion of scallops at this level will be:

0.5 (mass of scallops) x 127.6 (domoic acid concentration) = 63.8 mg

As only 1 % of domoic acid is left in the shucked roe-on product, the maximum amount left will be:

63.8 mg x 0.01 (shucking efficiency)

= 0.638 mg of domoic acid

In a 250 g serving of roe-on product, the maximum concentration of domoic acid should not exceed:

0.638 mg ÷ 0.25 (mass)

= 2.552 mg/kg

70 % of this will be in the gonad so the gonad amount will be:

0.638 x 0.70

= 0.45 mg

We did not measure the ratio of adductor muscle mass to gonad mass for each processor sample but if we assume that the ratio was approximately 22 % gonad mass: 78 % muscle

mass (taken from the Integrin data for this experiment) then the mass of gonad associated with the 250 g portion will be:

250 (g portion size) x 0.22

= 55 g

The maximum mean concentration of domoic acid predicted by the model will be:

0.45 ÷ 0.055

= 8.18 mg/kg of domoic acid

The highest mean gonad concentration found in the roe-on study (Processor 4) was 5.49 mg/kg. The shucking efficiency of even the lowest performing processor does appear to be better than 99 %. However, the actual ratio of adductor muscle and gonad combined against whole animal tissue mass may have been higher that 50:50 which would have reduced the predicted gonad concentration.

Applying the model to the best data we have, which are the results of the study's initial comparative results as part of the roe-off experiment we find the model predicts that:

With a measured (virtual) whole animal concentration of 382.2 mg/kg and a measured ratio of 55 % adductor muscle and gonad combined to 45 % whole animal tissue mass, the amount of domoic acid associated with a 250 g portion will be:

To achieve 250 g of the roe-on product, the required total whole animal tissue mass will be:

250 (portion size) ÷ 1.22

= 205 (g rest of tissue) + 250 (g portion size)

= 455 g whole animal

the total amount of domoic acid in the scallops will be:

0.455 (mass of scallops) x 382.2 (domoic acid concentration) = 173.9 mg

As only 1 % of domoic acid is left in the shucked roe-on product, the maximum amount left will be:

173.9 mg x 0.01 (shucking efficiency)

= 1.739 mg of domoic acid

In a 250 g serving of roe-on product, the maximum concentration of domoic acid should not exceed:

1.739 mg ÷ 0.25 (mass)

= 6.96 mg/kg of domoic acid

The actual result (adductor muscle and roe combined) was calculated to be approximately 1.3 mg/kg.

70% of this will be in the gonad (calculated from the % of domoic acid in the adductor muscle as found in the study) so the maximum gonad amount will be:

1.739 x 0.70

= 1.22 mg of domoic acid

In this case the gonad was only 10.3% of the combined mass of the adductor muscle and gonad so the predicted maximum concentration in the gonad will be:

1.22 ÷ 0.026 (mass of gonad)

= 46.9 mg/kg domoic acid

The actual mean result was 8.47 mg/kg so analysts in this study were leaving only 0.002 % of the total domoic acid amount associated with whole scallops at this high level. However, the "virtual" whole animal concentration was higher than an actual whole animal concentration measured from 50 individual scallops from the same batch (247.9 mg/kg). If we apply this lower figure rather than the "virtual" figure then the maximum gonad concentration would be predicted to be 30.4 mg/kg and the predicted maximum concentration of domoic acid in the roe-on product would be 4.5 mg/kg.

This model can be expressed more formally thus (see annex 3 for full details):

$$(\frac{1}{r}+1).(cw).(se) = cr$$

Where *r* is the ratio of the mass of the adductor muscle and gonad to the mass of the "rest of tissue", *cw* is the concentration of domoic acid in the whole animal; *se* is the shucking efficiency and *cr* is the domoic acid concentration in the shucked roe-on product.

If *r* is presumed to be 1 (i.e. the adductor muscle and gonad form 50 % of the total tissue mass of the scallops) then the formula becomes:

$2(cw).(se) = cr$

or

$$\frac{cr}{2cw} = se$$

if we wish to calculate the shucking efficiency.

The model can be extended to calculate gonad concentrations, as is shown in the worked examples above. However, the model loses much of its utility as gonad concentration can only be calculated if two additional factors are quantified: the ratio of domoic acid amount in the gonad against that in the adductor muscle (in the current study this was measured at 70:30) and gonad mass The latter is easy to obtain but is very variable while obtaining the amount of domoic acid in the gonad and muscle represents more work than just directly measuring the gonad concentration. As the presence of domoic acid associated with the adductor muscle is essentially an artefact caused by contamination, the ratio between amount of domoic acid in the gonad and the amount in the adductor muscle is likely to be highly variable. In the data set, individual ratios varied from a high of 100:0 to a low of 47:53. The plastic nature of the gonad and the susceptibility to artefact thus makes modelling gonad concentrations an unrewarding exercise.

The roe-on model can be used for both quality control purposes and as a regulatory tool. For the processor, the shucking efficiency (*se*) is the most important factor and the only one under their control. It can be used to compare the performance of different shucking staff and as a quality measure that can be built into quality standards. From a regulatory viewpoint, the upper limits of the domoic acid concentration (*cr*) are determined by the whole animal concentration (*cw*) and the shucking efficiency. From the current study, $se < 0.01$. When the tiered system is in place then $cw \leq 250$. Applying the model, we find that the upper domoic acid concentration limits of the roe-on product is 5 mg/kg. This assumes $r = 1$ but even when this is not true, *r* is a minor factor in determining the concentration of domoic acid in the roe-on product. If the EPT exceeds 5 mg/kg, then either $se > 0.01$ or $cw > 250$. In either case this is concern to the regulator since it suggests that either scallops are being collected from an area where the whole animal concentration is exceeding the maximum limit of 250 mg/kg or the HACCP procedures in a processor are failing to prevent gross contamination of the product.

This model is derived from the study data rather than the study having been designed to test the model. The experiments carried out in this study did not look at the efficiency of processors with the adductor and gonad (roe-on) product directly as the domoic acid analysis was done only on the gonad (roe). Another caveat is that the data relates only to a limited time of the year. Further extensive work would be needed to confirm that processors are consistently able to produce adductor and gonad (roe-on) product with a domoic acid concentration that does not exceed 5 mg/kg when they originate from whole King Scallops with a concentration of less than 250 mg/kg. This data is needed to validate the model before it is applied for regulatory or management purposes. However, the model does show considerable promise as a means to simplify, understand and manage domoic acid in processed scallops.

Considering all this information and working from the basis that protection of public health is the starting point of the exercise, answers to the necessary questions concerning EPT and the tiered system can be suggested.

What should a batch be?

There is spatial and temporal variation of domoic acid within the King Scallop so the batch test must reflect this to an acceptable degree to protect public health. As the EPT result depends on the shucking practice of the processor, the EPT has to be produced from each individual processor. Different processors cannot “share” an EPT.

There is no reason to suspect that different boats produce different results so this can be considered as "fishing effort". The batch test must therefore be based on samples from a prescribed area, from within a prescribed time and to a designated processor.

The study showed that domoic acid amounts in the processed product can change over relatively short time scales, even in the apparent absence of an external domoic acid event. The batch must therefore be based around a reasonably restricted time frame. We suggest that this period is normally a week. At times of an actual phytoplankton bloom, resulting in rapidly rising domoic acid levels, this frequency may have to be increased but weekly and at all times of the year seems the appropriate level.

The spatial variation is more problematic. Clearly scallops from the different sites in J5 had varying levels of domoic acid associated with them. Therefore, an EPT based on scallops from one site may not be representative of scallops from another site within the same box. It would be better that regulation was based around grounds rather than the arbitrary fishing boxes. The grounds should be defined around some measure of homogeneity so that a sample from one part of the ground will be representative of all the ground. In the current study, for instance, the Ross of Mull sample sites could be considered homogeneous. It is likely that on the East Coast of Scotland in particular, areas larger than the current fishing boxes could be considered homogeneous and declared scallop grounds. It would be helpful if the monitoring programme focussed on areas within boxes that were likely to have the highest amounts of domoic acid associated with them so that processors would know what the worst-case scenario for scallops anywhere within a given box was.

In summary, the recommendation would be that a batch is considered as all landings from a single box, landed to a single processor within a week regardless of the gatherer. This may mean that a batch has more than one unique identifier, as it will include all boat codes fishing in that box.

What should the end product test (EPT) consist of?

The EPT should be a pooled homogenate of ten roe-on adductor muscles i.e. the normal product from scallop processors. If any other product is produced (e.g. gonad only or adductor muscle only products) these should be tested separately and usually 100 g of product tested. There appears to be no advantage in using a higher number of individual scallops to form the pool. Although using 20 scallops did produce results that were closer to the population mean, it made insufficient difference to merit the extra labour involved in processing twice the number of scallops. Using larger numbers of scallops may also produce problems of sample homogeneity.

The contamination experiments showed that it was possible to produce considerably elevated levels in the adductor muscle and gonad. While concentration of domoic acid in the adductor muscles was usually lower than in the gonads, the greater bulk of the adductor muscles meant that they could have a higher amount of domoic acid than the gonads. It would be possible to envisage an outcome of contamination where the gonad would have a concentration of less than 20 mg/kg but the adductor muscle would be higher than 20 mg/kg. The end product test should thus focus on the actual end product. Given the 250 mg/kg whole animal upper limit on harvesting, the roe-on product should always be below 5 mg/kg. Anything above this means that the scallops are either grossly contaminated or come from a population with greater than 250 mg/kg whole animal. As noted, this requires confirmation but it could be

used as the basis of the shucking standard so that processors have this level as their internal quality control standard. Additionally, by measuring whole animal domoic acid levels from the same batch, performance can be measured to check on shucking efficiency. The establishment of such a standard would be of benefit to the industry in boosting retailer and consumer confidence, assuring regulators that the industry is taking effective measures to ensure public exposure to domoic acid is being kept to a minimum, and as a possible marketing tool via the establishment of quality assurance schemes.

Passing a roe-on EPT does not necessarily mean that the gonads will have a concentration of below 20 mg/kg if tested separately. Presuming that the end product has been shucked to the best standards, the probability of the gonads separately being above 20 mg/kg will crucially depend on the mass of the gonad and its state of maturation. There is a wider issue over the relevance of gonad concentration, which relates to the question of whether anyone would actually eat 250 g of a product produced only from gonads. A proper risk assessment based on culinary practice and portion sizes may determine that the gonad alone is unimportant in terms of food safety.

How can processors ensure their product has as low domoic acid levels as possible?

The shucking study and the contamination study data provide a great deal of information on how processing of scallops can be optimised in relation to minimising domoic acid load in the shucked product.

Domoic acid is predominantly associated with the hepatopancreas and other gut tissues. Over 99% of the total domoic acid is found in tissues other than the gonad and adductor muscle. Removing these tissues dramatically reduces the domoic acid content and associated risk. The gonad and adductor muscle have very low levels of domoic acid associated with them and the essence of good processing is to ensure that the gonad and adductor muscle are free of contamination from other tissues. This includes ensuring clean physical removal of all offal and preventing fluids from the other tissues contaminating the adductor muscle and gonads.

The HACCP recommendations and procedures (detailed in section 6.3) are designed to achieve best practice in this regard. The shucking study showed that excellent removal standards can be achieved by shuckers working at speeds relevant to the industry's needs. The importance of good training in achieving this cannot be over-stated. It is fortunate in that good shucking is visually easy to check: failure to remove all of the hepatopancreas and other parts of the gut (bad practice) is easy to monitor as the black tissue clearly stands out against the white and orange of the shucked product (Fig. 29). Post-shucking visual inspection is thus a valuable and inexpensive tool in maintaining standards within processors.

Contamination of the shucked meats by fluids from the hepatopancreas is a more insidious problem, as it may not leave a visible mark. It is difficult to assert what the exact degree of risk caused by fluid contamination is. Contamination experiments show that HP fluids can raise the domoic acid levels in the adductor muscle and gonad considerably and that this can be difficult to subsequently remove. However, there are no data on how frequently such contamination actually occurs. Therefore, we are left with having to take a precautionary approach to this by looking to block off potential routes by which contamination can occur. The most important of these is to reject smashed scallop shells where HP fluid leakage may have occurred and to ensure that shucking practices ensure complete separation of the shucked product from HP fluids both during and subsequent to shucking.

Final comments:
This study will hopefully prove useful to both industry and the FSA Scotland. The study has created new knowledge that will aid our understanding of domoic acid in scallops and help to identify the way forward to better regulation and management of the problem.

The study did not set out to examine the scientific rationale behind the EU Commission Decision on the tiered system but much of the new data is relevant to it. The tiered system offers the prospect of a sensible and workable approach to managing elevated levels of domoic acid in scallops. Ensuring that all scallops from affected areas are shucked is the single most important feature of this system and, combined with the 250 mg/kg whole animal upper limit for harvesting, this will guarantee public safety.

However, the main area for concern in the EU decision is the focus on gonad concentrations and in particular the 4.6 mg/kg "trigger level". The whole issue regarding the use of gonads solely in other dishes requires urgent research. The current study has little to contribute to this except to reiterate that the end product from Scottish processors should always be fit for purpose'. The concept of the trigger level was based on the rationale that it could be used as a predictor of the probability of EPT failure. However, the assumptions behind the model used to produce this figure have been shown to be invalid in this study. It has been shown in this study that the EPT result crucially depends on the shucking efficiency within individual processors and this makes it is impossible to predict from gonad monitoring data what the EPT result will be without a very good knowledge of what the shucking efficiency of an individual processor is. It is envisaged that the EU Commission will re-examine the case for the trigger level in the light of the data from this study. It would also be helpful if the use of gonad concentration as a regulatory tool is scrutinised.

Good shucking is paramount to ensuring the quality of scallop end product. Industry should move quickly to establish best practice throughout processors. This should be attainable without excessive costs or severe dislocation from current industry practices.

Main findings, conclusions and recommendations:

- It may be possible to determine from which areas within a fisheries box scallops are most likely to have high levels of domoic acid, making it possible to accurately define scallop grounds where the levels of domoic acid in the scallop will be similar.
- Over 99% of the domoic acid associated with the scallops is consistently removed by shucking.
- Different processors produced significantly different results when shucking scallops from the same batch for both roe off and roe on product. "Shucking" standards are thus very important in determining the outcome of end product testing (EPT), particularly when the scallops gonads are small.
- Thorough washing was important in reducing the domoic acid levels in poorly shucked scallops. It is important to prevent the "shucked scallop meats from coming in contact with the juices from the scallop offal.
- Implementation of full HACCP procedures to deal with domoic acid in scallops should not be difficult or overly expensive for scallop processors
- The analysis that resulted in the 4.6 mg/kg "trigger-level" assumed that the methods used to produced only negligible variation in domoic acid. "Shucking" is in fact the greatest source of variation, making this assumption (and the calculation depending on it) invalid.

- The EPT should be based on the combined roe-on product (i.e on both gonad and muscle simultaneously). If any product is formed from gonads or adductor muscle alone these should be separately tested. A batch should be all the scallops landed from a single box (or similarly defined area) to a single processor within one week regardless of the number or type of scallop gatherers.

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