Risk Assessment for Practical Changes in the Scottish Offshore Scallop Monitoring Programme for Domoic Acid

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SUMMARY

- Data from the FSAS offshore and inshore monitoring programmes were examined to determine seasonal and long term trends in domoic acid levels over different spatial scales to see if there is a case for changing the sampling regimes
- Mean domoic acid concentrations in scallops from Scotland show a year on year increase. It is not clear if this represents a genuine rise in domoic acid production in the environment or is a characteristic of the slow growth and long detoxification times that are peculiar to King Scallops. Areas that were associated with low domoic acid levels at the beginning of monitoring are now showing elevated levels,
- The concept of using Zones of Significant Equivalence to justify using larger box sizes was explored. Arguments can be made for treating some groupings of boxes as equivalent in terms of the regulatory state that scallops will be in at any time either in terms of whole animal concentrations or gonad concentrations but not both at the same time.
- Because of the general rise in domoic acid concentrations, there is a tendency for all scallops in Scottish waters to exceed 20 mg/kg. This means that they can be treated as a unified regulatory state with regard to whole animal concentrations and monitoring effort switched to improved definition of long term trends. Conversely, gonad concentrations are increasingly fluctuating between state 1 (< 20 mg/kg) and state 2 ($\underline{\geq}$ 20 mg/kg) in all areas.
- Mean data from the gross data set and from other species shows that domoic acid production is confined to the months between May and November with a peak in September. Bi-modal production may be occurring in some areas with two high periods of production.
- There is no evidence that domoic acid production is constant in all areas during the domoic acid season. This makes it difficult to predict in which areas domoic acid will rise each month except at very large scales. Detoxification in gonads does, however, occur relatively quickly out of season and is usually below the regulatory level in January for all areas.
- Detoxification rates are approximately 30% per month but there are huge differences between boxes and between different months. This is probably largely due to intrabox variation in domoic acid levels. This makes it impossible to accurately predict what the domoic acid concentration will be in a sample from what it was in the previous sample from the same box.
- It is recommended that the sampling regime be altered so as to collect better data (i.e. more duplicate samples) from fewer areas and to improve the sampling frequency in these “Monitoring Boxes”. Other resources should be switched to supporting industry in developing and improving shucking standards so that consumer protection is achieved through improved HACCP and end product testing.

INTRODUCTION

In 1987, hundreds of Canadians were inflicted by a severe and unprecedented form of shellfish poisoning. 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 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. However, it was shown that domoic acid acts as a powerful glutamate agonist with both gastrotoxic and neurotoxic activity and at high doses capable of producing permanent damage to the victim's central nervous system. 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. The most severe symptoms were seen in elderly victims with renal impairment[1]. A severe impact on 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 diatoms (various *Pseudonitzschia* species). Domoic acid has not been firmly implicated in any further shellfish poisoning incidents since the initial incident in Canada, although it has been suggested that fatalities of wildlife that consume filter-feeding fish (such as anchovies) have been due to domoic acid.

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 lowered.

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 every subsequent year domoic acid has been detected at high levels in scallops over a very wide area of Scotland.

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 processed 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 but 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 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 answers to enable the tiered system to be implemented. 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 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 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 dissected by UK NRL or the processor. The tiered testing system limits were based on laboratory-derived data and the variation encountered in this data. Therefore, more extensive scientific data on the shucking differences by different processors on domoic acid concentration in end products was required.

Integrin were commissioned to undertake these studies and reported in early 2002. Other studies into domoic acid in scallops have shown that the amount of domoic acid can vary considerably even over short distances.

The tiered system has not been introduced. Instead the FSAS is still operating its pragmatic practice of allowing scallops to be taken from areas where the whole animal level exceeds 20 mg/kg providing the scallops are taken for processing. Boxes are only shut when the gonad level exceeds 20 mg/kg. The Commission would like to see all offshore boxes to be sampled weekly. This would be both difficult and very expensive. FSAS have commissioned risk assessments (currently being undertaken by Environ and Integrin) to study the effects of enlarging the boxes used for monitoring in a bid to reduce costs.

As the existing monitoring system is based around sub-division of ICES boxes then there is scope for changing the box size (and or shape) to make monitoring easier. Changing the box size also effects what may reasonably defined as a batch and thus

the cost to industry. FSA have a specific problem in that once a box is closed (either because the gonad level is over 20 mg/kg or the whole animal level exceeds 250 mg/kg – if the latter is implemented) there is little point sampling it again until there is a good probability that the levels will have fallen below the regulatory limits. Resources used to monitor boxes that have little hope of falling below the regulatory limits would be better re-employed elsewhere both for the FSA and industry.

FSA require a critical risk/benefit analysis of changing the box area and guidance on appropriate sampling intervals once a box has been closed. This will be based on a scientific appraisal of the available monitoring data.

Approach

We will model Zones of Significant Equivalence (ZSE's) for the Scottish Scallop harvesting activity. Basically, if there are no differences between two areas with regard to the domoic acid content of the scallops within them then the two areas can be considered to be effectively the same area. This is further defined as an area where there is little likelihood that domoic acid concentration changes within a sub section of the ZSE would lead to one area of the ZSE having a different status from the rest of the area. ZSE's will be defined on a range of parameters including DA levels; geographical location; mean depth and percentage land mass in the ZSE.

The available datasets will also be analyzed to determine both the duration of closures in the past, the robustness of the data underpinning this and the possibility of applying this data to the ZSE approach to define sampling frequency and sampling area after closure.

Integrin will use the Environ database as the principal data source for the analyses.

Workplan:

A1: Modelling ZSE's: The model parameters for ZSE's will be established and the risk/benefits associated with various degrees of adherence to the model will be defined.

A2: Analysis of the monitoring data: The available data will be assessed to determine patterns of domoic acid occurrence within existing boxes both for gonad and whole animal. This will involve looking for seasonality, spawning, amplitude of changes etc and how robust the data is. Where possible a statistical approach will be taken to define similarity between boxes using multivariate techniques. The Environ database will be used where appropriate.

A3: Defining best fit ZSE's: If ZSE's are be shown to be valid then recommendations on redefined box sizes will be made based on the best available data. If the concept has merit but the existing data is inadequate then

recommendations will be made on what data would be necessary to allow implementation of the ZSE approach.

A4: Sampling Frequency after closure: The available data will be analyzed to determine the mean time different boxes and areas were closed in the past and determine how robust the data underpinning these closures were. This information will then be used to determine the probability a box will remain closed and hence the best sampling regime in terms of frequency. If the ZSE approach is upheld by the research in the previous modules then a study will be made to suggest a sampling pattern based on sampling only a single area or reduced sampling within a ZSE.

A5: Reporting: Integrin will deliver a report on its findings to FSA Scotland complete with recommendations on possible box sizes and sampling frequency.

Deliverables:

1: Feasibility assessment of ZSE concept for domoic acid in scallops

2: Analysis of closure timings

3: Recommendations on sampling regimes, particularly focused on after closure

4: Report and presentation of findings to FSA

Results

Data selection and limitations:

In theory, the monitoring programme database should have at least monthly values for gonad and whole scallop samples for each of the 196 quarter ICES boxes that are currently in use. However, many of these boxes have only ever been irregularly sampled (if at all). A decision was made to restrict the analyses to those boxes where there was deemed sufficient data from which to make sensible analyses. The cutoff was set at > 8 samples within at least one year (including duplicate samples within a box on the same date). Boxes with less than 8 samples were not considered further. This restricted the number of boxes for analysis to 65 boxes from all 9 areas (Map 1; table 1). While there is some data available for most of the remaining boxes, this was deemed insufficient to allow meaningful analyses and has been ignored in this study, even for the more general studies.

Another major problem with the dataset is that most of the high values are reported as > 250 mg/kg rather than as their actual values. Inconsistently, some high values are quoted as actual values in the early years. While this can be addressed by treating data given as > 250 mg/kg as a state, it does limit the usefulness of the data in assessing how quickly depuration is occurring as we often do not know what the starting points are.

While there is some duplicate sampling in the database, the number of samples taken within a box at the same time rarely exceeds two. This means that is difficult to statistically assess the intra-box variability. For this we have to rely on the separate studies undertaken to assess domoic acid variability (Campbell et al 2001; McKenzie & Bavington 2002).

A1: Modelling ZSE's (and A3 defining ZSE's):

The Environ database contains within it information on bathymetry for each box (mean depth; maximum depth, minimum depth and standard deviation). To this information we added a % land category (table 1). Rather than laboriously work out the detailed percentage of land mass in each box, each box was assigned a % class (0; 1-20; 21-40; 41-60; 61-80; 81-100). This allows us to rapidly gauge how similar each box is in terms of their bathymetry and influence of islands and mainland.

It is clear that most boxes studied are influenced by the terrestrial environment. This means that they are likely to have complex tidal streams and fractal environments. Only the East coast boxes (E) are relatively free from direct terrestrial influences, with the Moray Firth area (M) being more mixed and West coast and Island areas (J; SM; H; NM; O and S areas) being generally highly influenced by landmasses. As there is only one Clyde box with sufficient data to be included no comparison can be made, although Clyde boxes are obviously also heavily influenced by the terrestrial environment.

With such a great amount of terrestrial influence in most of the studied boxes, it is not surprising to also find great bathymetric variability. The Orkney (O) and Shetland (S) boxes tend to be shallow (both have mean depths of 30m) as are the Hebrides boxes (H: 29.4m). The Jura (J) and Moray (M) boxes tend to be of medium depth (mean depth of all boxes is 53.5m). The South Minch (SM) and North Minch boxes are generally deeper (mean depth is 72m and 63.5m respectively and the east Coast boxes are generally quite deep (E: 66m). The vast majority of boxes, however, extend from shore to over 100m, which is the maximum depth that scallops are likely to occur at (and certainly to be fished from). Only the Orkney boxes and the majority of the Minch boxes tend to show a bathymetric distribution that run from shore to a maximum depth less than or equal to 100m.

Most of the boxes are, therefore, rather similar to each other in that most have some terrestrial influence, most run from the shore and most have a maximum depth in excess of 100m. The most obviously exceptional area is the East Coast as few boxes in this area have any terrestrial influence. The adjacent Moray area boxes tend to be most similar to East Coast boxes.

Comparisons of domoic acid levels between boxes:

When comparing domoic acid levels between boxes in order to define equivalence, the actual values are less important than the regulatory state. For gonad levels there are only two states: state 1 where the gonad concentration is less than 20 mg/kg and state 2 where the concentration exceeds 20 mg/kg. For whole animal there are three states: state 1 where the whole animal concentration is less than 20 mg/kg; state 2 where the concentration is more than 20 mg/kg but less than 250 mg/kg and state 3 where the concentration exceeds 250 mg/kg. The last state is not enforced at present.

The first level of comparison we can make is between all the boxes within the designated areas (e.g. all boxes within Jura area (J), all within East Coast area (E) etc). We can compare both the states for gonad concentration and those for whole animal and see if all the boxes share the same states at the same time. The next level is to look for groups of boxes within an area that share the same states all the time and then compare these groupings with the bathymetric and percentage landmass data.

Gonad states:

Table (2) shows the percentage of boxes within each area that are State 2. As can be seen, there are very few areas where every box is in the same state at the same time (such boxes would always have 100% or 0%). Instead we see a mosaic of values for each state, particularly in the summer months. Shetland was the only area where all the boxes showed the same state throughout the data period, but this was derived from only three boxes and the sample numbers are also low. The East Coast area (E) and Moray Firth (M) had a long run of same states until the summer of 2003 when boxes began to deviate from each other. All the other areas had periods where at least some of the boxes were in a different state from the other boxes within the area.

This means that if all the boxes in any area (other than Shetland) were considered ZOE's and only a single sample taken there would be periods where the sample was likely to be unrepresentative of all the boxes.

If we compare subsets of boxes within each area then a very mixed picture emerges. In the East Coast Area (E) the variability is entirely associated with E1, the remaining boxes for which there was sufficient data all had the same gonad states.

The Moray Firth showed more variability. M2 did not show the same pattern as any other box; M3 and M9 were always the same as each other; M1, M10, M12, M18, M25 and M28 were always the same as each other.

The three Shetland (S) boxes for which there was sufficient data to analyze were always in the same state as each other. In the Orkneys (O), boxes O10, 25, 27 were always the same as each other, but the remaining boxes (O11, O18, O19) differed.

In the North Minch (NM) no boxes showed the same pattern of gonad state as another box. This was also the case for the South Minch (SM) boxes and while Jura (J) 9 and 14 had the same pattern this was on very limited sample numbers. All the other boxes in the Jura area had different patterns. In the Hebrides H6 and H8 were always the same.

Whole animal states:

Looking first at all boxes within each area (Table 6, Fig. 5)), a rather different pattern from that obtained from the gonad data emerges (table). The East Coast (E) area is very variable and all three states (< 20 mg/kg; 20-<250 mg/kg and >250 mg/k) are encountered; the Moray Firth area (M) is very similar. Shetland (S) has a good degree of agreement between the boxes, though there are occasional differences between boxes. Orkney (O) is very variable: initially boxes vary between states 1 and 2 but latterly between 2 and 3. The Hebrides area (H) has a very high percentage of uniformity and the boxes are always in states 2 or 3. The North Minch (NM) is very variable, mostly between states 2 and 3 but in one case there is a state 1 encountered. The South Minch (SM) has a very high percentage of time when all the boxes are in agreement with each other and always fluctuating between states 2 and 3. The boxes in the Jura area tend to show considerable variation and also vary between all three states, though the only box to show state 1 results was J15 which has waters within the Clyde Sea Area and it is possible that these scallops were more representative of those from this area rather than the “Hebridean” Jura area.

Looking at groups of boxes within each areas to see if there is a case for amalgamating sub-areas also provides an interesting picture. No boxes at all in the East Coast area (E) share the same pattern of whole animal concentrations. Only boxes M9 and M12 share total similarity in the Moray Firth area (though the sample numbers are low). For Shetland (S), though the boxes are similar most of the time, they sometimes deviate from each other. In the Orkney area (O), O10, 11 and 27 were always the same and O10 and O11 are well sampled. For the Hebridean area (H) only one box ever shows any differences from the other boxes (H10) and only once. NM 19 and NM 20 in the North Minch area are always equal but no other boxes in this area show equivalence. The South Minch (SM) is the most interesting in that boxes SM 1, 4, 7, 8, 11 and 15 are always equal and these boxes are roughly all contiguous. Boxes SM 2, 5, 9, 10, 12 and 14 each sometimes showed differences from all the other boxes at some time. J1 and 4 were always the same as each other but the other Jura (J) boxes were always different. J15 was conspicuous for having lower values than the other Jura boxes.

Discussion and Conclusions on validity of the ZOE approach:

The principle of defining ZOE’s is sound: if there are no differences between areas then we might as well treat them as a single area. An immediate worry is that because the existing boxes are rather large, every box tends to be similar in its gross bathymetric profile. Most also contain some land mass so will tend to have complex tidal streams. There is thus limited scope in the physical data to define obviously

separate areas. The most promising division is between “Hebridean” (NM, SM, H, J areas) and “North sea” areas (E, M, O and S). Unfortunately we don’t have enough box data to judge if the Clyde Sea area is separate from the general “Hebridean” area but there are good reasons to believe that it is. The North Sea areas tend to have lower levels of domoic acid associated with them – though this does not necessarily mean that they are more homogeneous with regard to domoic acid levels – in some cases the opposite is true. In any case, the situation appears dynamic and there is some evidence that the North Sea areas are becoming more like the Hebridean areas (see below).

However, the tempting prospect of being able to group areas based on their similarities is confounded by the actuality of the domoic acid data (and in some cases the lack of it). By treating all data as states we avoid the problem of some data over 250 mg/kg being recorded as actual figures and most just being recorded as > 250 mg/kg. It is less easy to circumnavigate the problem of weaknesses in the dataset. Only one third of the total possible boxes have enough samples associated with them to sensibly interpret (and even some of those boxes which are included have only limited data associated with them). Another problem is that it is difficult to compare boxes in border zones between adjacent areas as these are rarely sampled in the same months.

The boxes that have insufficient samples associated with them to analyze tend to be offshore boxes which might have been expected to show the best fit with the ZOE approach – though these are also probably the least fished and so the least important. Another feature is that boxes where levels are generally low (such as the Clyde Sea area) are under-represented in the monitoring data. While this is understandable, it does make it impossible to judge if these areas may be amenable to the ZOE approach.

The boxes that have been well sampled do not, however, tend to support the ZOE approach. A major part of the difficulty is that there are two different parameters being observed: gonad concentrations and whole animal concentrations. Areas where the ZOE approach seems feasible for one parameter (e.g. the East Coast area for gonad) show completely the opposite case for the other parameter (e.g. East Coast area for whole animal). Only for Shetland is there a case to be made for amalgamating the boxes based on both gonad data and whole animal data but here the data is quite weak.

The reason why most areas tend to be diametrically opposite each other in terms of whole animal states and gonad states is interesting: when gonad concentrations are generally low, whole animal concentrations are also low but usually fluctuating around the regulatory state (i.e. 20 mg/kg). Most of the areas where the whole animal levels are of the same state it is because they are in state 2 (i.e. > 20 mg/kg < 250 mg/kg). In this case the gonad levels tend to fluctuate around the regulatory limit. So when one parameter shows geographical eveness, the other parameter tends to be uneven. Given the general upward drift in domoic acid concentrations for both whole animal and gonad concentrations (see below) we will probably see increasing

stability in whole animal states (particularly if we ignore the > 250 mg/kg state 3 and only focus on states 1 and 2) but an increasing instability in the gonad states within areas. As closures are currently made on the gonad concentration rather than whole animal concentration this is obviously a concern.

Given the huge amount of seabed that each of the existing areas covers it is perhaps not surprising to find that they are not homogeneous with regard to domoic acid concentrations. It might be hoped that smaller sections of each area might be considered ZOE's. However, here again, the available data makes it difficult to sustain such an argument. To take the East coast boxes as an example again, if we consider gonad data on their own then there is a good case for considering that all the East Coast boxes other than E1 could be considered ZOE's and even E1 is only rarely different from its fellow E boxes. For whole animal data, however, the complete opposite is the case: none of the E boxes showed the same pattern of states as their fellows. The rest of the areas also show similar results: while groupings of boxes can be made on the basis of a single parameter, this is rarely supported by the other parameter.

In summary, if larger boxes were formed from groupings of the existing boxes then there will be an increase in the likelihood that the sample taken will not accurately reflect the state of scallops in the other areas within the new box. What we don't know is whether a single sample area could be made more representative by taking multiple samples within it. It is tempting to think that this may be the case but the current data does not allow us to judge this.

A pertinent point is that the samples from within a box may not represent the state of all scallops within that box, never mind be representative of scallops from adjacent boxes. Indeed the available data suggests that intra-box variability is likely to be high, though we have no hard data on just how representative each sample is. This leads us to an interesting situation: if the existing sampling regime is already producing data with a good margin of error in it, can we justify moving to larger boxes on the basis that these will contain the same degree of error and so are no worse than taking single samples from smaller areas? The data does not allow us to make this argument on the grounds of ZOE's but we can make it in terms of sampling efficiency. This will be explored in the general discussion below.

A2: Analysis of the monitoring data: The available data will be assessed to determine patterns of domoic acid occurrence within existing boxes both for gonad and whole animal. This will involve looking for seasonality, spawning, amplitude of changes etc and how robust the data is.

The dataset was analyzed to look for trends in the datasets to see what predictive capability the data can give us that may be useful in determining monitoring strategies. The 65 boxes that were judged to have sufficient data for reasonable analysis were again used here but this time actual data values (rather than states) were used. This means that the effect of data values being reported as > 250 mg/kg

rather than as actual values has to be taken into account. Such records are treated as being 250 mg/kg.

We were interested in several basic questions:

1: when is domoic acid produced in the environment?

2: how quickly does it get in the scallops and how quickly is it lost?

3: can the effects of spawning on gonad and whole animal domoic acid concentration be discerned?

4: are there long-term trends in the data?

5: how robust are the datasets for detailed evaluation?

For the first point we considered not only scallop data but also the monitoring data for other species (particularly mussels). King scallops (*Pecten maximus*) are perhaps unusual in that they retain domoic acid in their tissues for much longer intervals than other species. While there has been some speculation as to why this should be, there is no known reason for this. The corollary of this is that other species only contain domoic acid when there is recent domoic acid in their diets. They thus make good indicators of domoic acid production on the basis of mere presence while in scallops we have to rely on interpreting changes in domoic acid level – which may be obscured by concentration changes effected by spawning and gonad maturation rather than actual changes in the amount of domoic acid present in the animals.

Results

Large scale trends: The temporal and spatial distribution of records of domoic acid in shellfish other than *Pecten maximus* is shown in Fig. 2. Very few of the values exceed the regulatory limit but domoic acid is frequently encountered in other species. Most of the records are from the West Coast and Shetland but this merely reflects the distribution of inshore monitoring sites which are predominantly on the West Coast and there are records of domoic acid seen as far south as the Forth Estuary.

In 2001, domoic acid starts being detected in June and records of its occurrence occur every month until October. The database shows records in March of this year but this seems to be an artifact of way results were reported at that time.

In 2002 the “season” started earlier, with the earliest record in May, and the last records were in November. The numbers of records (25) was higher than for 2001 (11).

In 2003 the earliest records were again in June and ended in November. Total number of samples where domoic acid was recorded was again 25.

2004 data has not been fully analyzed (and the "season" has not yet finished at the time of writing) but over 20 samples with domoic acid levels over the limit of detection have been encountered and the earliest records were from June.

There are sufficient samples to be reasonably satisfied that domoic acid is being produced between the months of May/June and November in sufficient quantities for it to show up in the flesh of species other than *Pecten maximus*. For *P.maximus* itself we have two forms of data: gonad concentration and whole animal concentration. Because the gonad undergoes rapid and radical changes in size during gametogenesis and spawning, the concentration of domoic acid can vary independently of the amount of domoic acid so this has to be borne in mind when looking at the data.

Whole animal concentrations for all 65 sites studied between April 2001 and October 2004 is shown in Fig. 3 A clear periodicity is seen, with domoic acid concentrations rising in spring (usually June) and falling in the early winter (November or December). The values tend to show a degree of noise in their trends from month to month and it has to be borne in mind that not all areas are sampled each month so that the data is not uniform. Despite this, the overall trends are clear enough. An interesting observation is that domoic acid levels appear to be rising year on year. This trend is even more real than evident as the monitoring data is only recorded as being > 250 mg/kg. If the actual values were available it is very probable that the 2004 data would be considerably higher than is shown. Another interesting feature is that after initial falls, the domoic acid concentration tends to reach a shallow "valley" before rising again. This in itself is not remarkable but the "altitude" of the "valley" is increasing each year. This suggests that either domoic acid production starts even earlier than we think (in February or March) and there is a balancing of new production versus depuration or that the domoic acid is not following a simple diffusion model of depuration. An alternative hypothesis is that this "valley" is produced by spawning at this time that tends to increase concentration but balanced by continued loss of domoic acid from the animals.

The gonad concentration data (Figure 4) shows very similar trends to the whole animal data, suggesting that any influence of spawning is masked by the overall trends. That said, the values for October 2004 are extraordinarily high and may represent the effect of spawning in these samples. McKenzie and Bavington 2002 showed that small gonads are more difficult to shuck effectively and tend to have small pieces of hepatopancreas still attached. Given the high whole animal values, even tiny bits of hepatopancreas can make a huge difference to the overall domoic acid concentration in the gonads. As with the whole animal concentrations, there is a year on year increase in mean gonad concentrations. Levels start to elevate in the Spring (usually June) and fall in the late Autumn/early Winter (October to December).

Fine scale results: There are no individual boxes that have a complete monthly run of samples (never mind any with a complete weekly run). There are a few boxes that have a good run (such as O19) but on the whole the dataset is very fragmented with lots of missing data. This makes it difficult to judge trends within a single box.

By combining the data into areas the position is improved (though there are still occasions where there is no data from a particular area for a particular month). Fig.5 and Fig.6 show the whole animal mean concentrations and gonad mean concentrations for each area. One of the difficulties of high results being reported just as > 250 mg/kg is evident. In many of the graphs it looks as if 2002 saw higher levels than in 2003 but this is solely because values in 2002 were often reported as actual values. In 2003 all high values were reported as being > 250 mg/kg and this has resulted in the whole animal data "saturating" in 2003 for areas such as Jura (J); South Minch (SM) and Hebrides so that there is no useful trend information available.

As might be expected, the area data shows the same trends as the combined dataset with rises in the Spring and falls in late Autumn/ early Winter. There are, however, anomalous periods where the monthly value either rises or falls outside of the normal pattern (e.g. Orkney (O) March 2003; Moray (M) September 2003. This almost certainly reflects intra-area (and intra-box) variability being picked up by the monitoring sampling. (Where individual boxes have been sampled sufficiently frequently to look at such trends on a box level, such anomalies are very common). An interesting feature is the large rise in overall whole animal levels in the East Coast (E) area in 2003 compared to the earlier years. It may be that the East Coast is becoming more similar to the other areas.

Gonad concentrations follow the same trends as for the whole animal concentrations but with more fluctuations between months. There is, however, no obvious effect of spawning on the results. This may because the spawning periods are coinciding with periods of domoic acid production and so is being masked.

The speed at which domoic acid is taken up and expelled is discussed in the next section.

Discussion and conclusions:

The data obtained from species other than scallops shows very similar trends to the scallop data. Shellfish start to show elevated levels of domoic acid in their tissues in either May or June and these levels start to fall in November or December. This does not necessarily mean that domoic acid is only produced by *Pseudonitzschia* in this period as this period also coincides with peak feeding activities of the shellfish as the waters warm up in the Spring then start to cool down in the Winter. The available phytoplankton monitoring data does support peak *Pseudonitzschia* production during this period but the relationship between *Pseudonitzschia* abundance and domoic acid production is still obscure. What is certain is that all shellfish are likely to show elevated levels of domoic acid from May or June through to December.

There is no obvious evidence from the data of a frontal system of domoic acid production i.e. elevated levels of domoic acid first start in one area then spread to other areas. In fact, it is logical that such a system does exist (most likely linked to seasonal effects on the phytoplankton) but the monitoring data does not give us sufficient resolution to discern it. We cannot, therefore, select to adjust the monitoring strategy to target the front as we do not know where it is. In any case any frontal effect is likely to be evident only over a few weeks.

A worrying trend in the data is the year on year increase in domoic acid concentrations. The fact that high values are just expressed as > 250 mg/kg tends to mask some of this dramatic rise and FSA should ensure that future monitoring data determines the true value so that this trend can be properly followed in the future.

Extrapolating back in time from the data would immediately lead to the conclusion that domoic acid production in Scottish waters is a recent phenomenon, possibly starting in the mid 1990's. Given that scallops are relatively long-lived and that they are not usually fished until they are at least five years old, it may be that what we are seeing is annual additions to the domoic acid burden in each scallop rather than evidence that the actual level of domoic acid production in any one year is increasing. The data from other species is weak but does not suggest that levels are obviously increasing in the environment as a whole. This annual addition model can be described as:

$$Y1 (x-y) + Y2 (x1-y1) + Y3 (x2-y2) + Y4 (x3-y3) + Y5 (x4-y4)$$

Where Y is year x is domoic acid input and y is domoic acid loss. It seems unlikely that domoic acid did actually commence just recently but the observed increases must be the result of some change. It is possible that in the past x was balanced by y in any particular year and that this relationship has become disturbed comparatively recently. It is unlikely that y will have changed so x is more likely. It may be that there has been a relatively modest increase in x reflecting an environmental rise but this is amplified by the 5 year growth cycle to allowed catch size. If so, then a plateau may be reached as older scallops are lost from the population and replaced with younger scallops. However, it may also be that there is genuinely more domoic acid being produced each year and that the area affected is increasing. The large rise in domoic acid concentrations in scallops from the East Coast area in 2003 would suggest the latter.

As the situation is clearly dynamic, the results of previous studies may not hold under the current conditions.

A4: Sampling Frequency after closure:

The intention here was to use the available data to determine the mean time different boxes and areas were closed in the past and determine how robust the data underpinning these closures were. This information would then be used to determine the probability a box will remain closed and hence the best sampling regime in terms of frequency. As the ZSE approach is not supported as a valid approach, alternative approaches to simplifying the monitoring regime need to be explored.

Closures are only made on gonad concentrations at present so the whole animal data can be ignored. The designation of shucking boxes is determined by whole animal concentrations but the number of boxes that are below 20 mg/kg is rapidly falling so we will not consider whole animal data further here.

An immediate problem is that few boxes have anywhere near complete sets of sampling series. This means that the timing of some closures were affected not just by the domoic acid concentrations but also by sample frequency. This is especially problematic where there is a gap between the initial closure result and the next sample result if the latter is below 20 mg/kg as it is impossible to know when the value dropped. As only one third of the boxes have sufficient data to consider their analysis, we have to be careful in extrapolating any generalized conclusions onto these boxes where we have insufficient data.

To identify periods of closure we have taken these as being between months when the gonad state moved from state 1 (< 20 mg/kg) to state 2 (≥ 20 mg/kg). We have not checked that closures and re-opening actually took place at these times (table 3).

It is clear from the data that whether boxes were open or closed was rather arbitrary depending on if the box had been sampled or not at the appropriate times. To take the Jura (J) area as an example (table 8): In 2002, boxes J1, J2, J3 and J5 were closed because they were in state 2 mostly between September to December. Boxes J4, J6, J7, J8, J9, J11, J14 and J15 were theoretically open but only because they had not been sampled (most of these boxes were sampled only to June of this year). In 2003 a very similar story was seen. However, in 2001 a more complete sampling programmes was made. J1, J3, J5, J6, and J8 all showed a closure pattern in the autumn/ early winter (September-December). J5 showed a longer closure period (July-January). J2, J9 and J11 all showed earlier closure periods (July-August) but did not show closure levels in the Autumn despite being adequately sampled. From this we can see that boxes that are not sampled in the late Autumn my be incorrectly left open but it cannot be exactly predicted that they should be closed from the results from adjacent boxes.

One interesting feature in 2002 was J6 where there was a change in state from 1 to 2 seen in February-March. As there had been state 2 samples taken from this box in November (but not in December in January) it may be that the samples taken in February were more representative of those taken in November than those in December and January. However, in general boxes that are state 2 in November or December have reduced to state 1 by January. This is true of all areas.

One obvious way to determine post-closure sampling levels is to look at the percentage fall in domoic acid concentration once domoic acid uptake has ceased. It should in theory be easy to calculate how quickly a box has a good chance of falling below regulatory levels by applying the percentage fall to the starting point and extrapolating forward.

There are three levels of information that can be used to inform this: the total dataset (Figs. 3,4); data for specific areas (table 6) and the data for individual boxes (table 1). Unfortunately as we move into higher resolutions (total, area, box) the predictability decreases. While there is a general decline month on month from the late Autumn (usually by October) individual boxes show considerable variation in the percentage decrease and it is not unusual to see boxes increasing rather than decreasing in gonad concentration (the same result is seen with whole animal data). While we can be confident that gonad concentrations generally will fall we cannot predict that a specific box or even a specific area will fall in line with the general trend. This is most probably because of the intra-box variability in concentrations.

Another problem is that the gonad data is not consistent between the different years (Fig. 4). While the whole animal concentrations have shown very consistent patterns every year, the gonad data is quite different for 2003 than it is for 2001 and 2002 where the mean concentrations were much lower. The earlier data appears to have more noise associated with it but by 2003 the overall data produces a much clearer trend. It is too earlier to see how 2004 compares though the first part of the year resembles 2003. The gonad data does not show the “shallow valley” effect seen in the whole animal data, instead it continues to fall sharply towards zero until the new production in May reverses this trend. There is approximately a 30% fall per month in mean gonad concentration in 2003-2004.

In summary, while we can predict where the mean domoic concentration will be and hence the proportion of boxes that will become open, we cannot accurately predict which boxes will actually fall below regulatory limits because of the limitations of the sampling regime.

General Discussion and Conclusions

The primary interest of the FSA in this study is to have a scientific justification for a rationale approach to determining when boxes should be re-sampled after closure. A secondary interest is in seeing if there is an argument for changing the size of boxes used as the basis of the monitoring programme.

The Monitoring Programme gives us sufficient information and resolution to be certain of a number of points:

1: Scallops in Scotland generally show a consistent pattern of approximately six months of domoic acid uptake (May to October) followed by approximately six months of domoic acid release (November to April). This pattern is observed throughout Scotland and is also observed in other species.

2: Gonad and whole animal data are in good agreement with regard to gross trends

3: There has been a year on year increase in domoic acid concentrations in both whole animal and gonad concentrations in scallops.

The Monitoring Programme dataset does, however, have major drawbacks when we attempt to derive more detailed information from it. Many boxes have been sampled rarely so that good time series from any boxes or areas are difficult to obtain. The recording of data that is higher than 250 mg/kg merely as > 250 mg/kg rather than as the actual figure imposes a further hurdle in understanding what the true position is.

The greatest problem, however, is the extreme individual variability that scallops can exhibit with regard to both whole animal and gonad concentration. This introduces considerable difficulties that are only really overcome by treating all the data as one set to produce a general picture. Attempts to produce a finer resolution analysis founder on this problem.

The concept of Zones of Significant Equivalence (ZSE's) would be extremely useful in defining an improved monitoring programme. Unfortunately the scientific case for treating large areas as significantly equivalent cannot be upheld by the data except for a few small areas.

The first difficulty is that most boxes in Scotland all share very similar characteristics: strong terrestrial influences, depth ranges from 0 to > 100m. There are differences between the North Sea areas and those in the West Coast areas but these may be diminishing in relation to domoic acid concentrations. Isothermal data might show interesting relationships to domoic acid patterns and trends. However, any attempt to tie physical oceanography to domoioc acid concentrations is likely to be hindered by the limitations of the available data (single or double measurements made at often irregular time periods) and the underlying variability of individual scallops.

Domoic acid concentration in scallops from one box or area cannot be accurately predicted from a knowledge of domoic acid in scallops from another box or area by the current monitoring methods. Nor can the trend in domoic acid concentration within a box or even an area be accurately predicted from a knowledge of previous concentrations. Indeed, a sample from a box cannot be used to accurately predict what the values of other samples from within that box will be even if the samples are taken at the same time. Gross generalizations can be made but the data does not allow us to put any confidence limits on these predictions because of the limitations

of the data. Of course, samples from within an area or box are representative at some scale but the current monitoring data does not allow us to determine how representative these samples are. We cannot say how often boxes are erroneously allocated to a state either because the sample is unrepresentative or merely not taken but the suspicion is that boxes are frequently open when they should be shut.

These problems with the data are why that we cannot simply apply a notional "detoxification" rate to monitoring data to predict when a box should re-open. In theory samples within the domoic acid production season will either increase, stay the same or fall (depending if there is active production at the time) but samples outwith the production season should only fall. The data is, however, so variable that it is not possible to define what the detoxification rate should be. The data does, however, suggest that in almost all cases, and at current levels, gonad concentrations will fall below regulatory limits by January and not genuinely rise again before May. However, the gonad concentrations can also fall below regulatory limits before January, so this cannot be used as an argument for not taking action before then.

The next problem in using the ZOE approach is that two regulatory variables are simultaneously in play: gonad concentration and whole animal concentration. While gonad concentration does show reasonable correlation with whole animal concentration, at least at gross levels, this is not true of regulatory states. When gonad levels are very low within an area the whole animal levels tend to be around the whole animal regulatory level. When the whole animal concentrations are consistently above the regulatory level it is likely that gonad concentrations will exceed regulatory limits for at least some of the time. If the two states were decoupled then it would be possible to designate ZOE's for single variables and particularly for whole animal concentrations.

In summary, there is no scientific case for increasing the box sizes that can be made for both variables simultaneously. Nor does the data support the application of a "detoxification" rate to individual boxes or areas except as a gross generalization.

To resolve these problems of sample rate and size so that the Monitoring Programme would accurately define the appropriate regulatory states would require much more frequent sampling of each box plus multiple samples to be taken within each box so that confidence limits could be placed on the data. This is obviously not practical given the resource issues.

Recommendations and suggestions:

1: Establish representative monitoring boxes for each area

At present the Monitoring programme is being driven by the need to comply with the appropriate EU legislation as enshrined in UK law. There is, however, an additional need to provide robust time series data that can be use to genuinely monitor long term change in domoic acid concentrations in bivalves. For this purpose it is better that a few areas are frequently sampled rather than more areas are infrequently sampled.

It is recommended that one box in each of the areas is designated the long term monitoring box and that this box is sampled at least every month and preferably each week during the domoic acid season. Multiple samples should be taken at each sampling occasion so that confidence limits can be estimated for the concentrations. All values should be recorded as actual values rather than as states (i.e. not as > 250 mg/kg). This box should be chosen on the basis of how easily samples can be consistently obtained from it and on geographic relevance. It may be necessary to have more than one Monitoring Box for some of the larger existing areas.

2: Focus sampling intensity on a seasonal basis and use the Monitoring Box as a Reference Box for re-opening. .

Outside of the domoic acid season, levels should be falling and sampling the Monitoring Box should be adequate to pick up any unusual production of domoic acid. Additional sampling should be only directed at achieving samples to allow revocation of FEPA orders.

In April effort should be aimed at sampling as many boxes as possible to detect the onset of the domoic acid season. Effort should be focused on areas where the whole animal concentration is below 20 mg/kg and areas where the gonad concentration is likely to rise above 20 mg/kg.

Most of the areas in Scotland are now over 20 mg/kg for whole animal concentration and much of the West Coast is unlikely to return below 20 mg/kg in the foreseeable future. (The > 250 mg/kg state is not currently used for any official purpose though if this were to change then this would have to be taken into account). In these areas the monitoring box will suffice to look at long term changes in whole animal concentration and it is not necessary to measure whole animal levels in each box.

Resources can be redeployed into better measurement of the few areas where some boxes are below 20 mg/kg for whole animal and areas where gonad concentrations are likely to increase beyond 20 mg/kg. Once either gonad or whole animal concentrations in these boxes exceeds 20 mg/kg, these boxes are assigned to the Monitoring box and not resampled until the Monitoring Box indicates over at least two sampling dates that domoic acid concentrations are dropping in that area (the actual status of the monitoring box is irrelevant, it is the trend in the data that matters). Boxes that are re-opened within the domoic acid season must be re-sampled at least fornightly to maintain their open status.

Some boxes as currently defined could be combined to reduce overall box numbers – particularly in the North Sea areas and in West Coast areas where some boxes consist of very small sea areas which could be legitimately combined with adjacent open sea areas..

If implemented, these measures would improve the predictive value of the Monitoring Programme and focus sampling resources on the areas of greatest need. They would not preclude boxes being ascribed to the wrong status but it should allow a degree of prioritization that would help prevent boxes being wrongly ascribed just because they have not been sampled. These recommendations would probably not result in much saving sin terms of sampling costs but they would see the resources being used more effectively.

Another approach would be to abandon detailed measurement of offshore areas altogether; focus long-term monitoring around a few Monitoring Boxes chosen for their ease of sampling and general representativeness and concentrate the remaining resources on policing (and perhaps supporting the funding of) end product testing.

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