Project S14036

Risk assessment of the FSA Scotland inshore shellfish monitoring programme based on historical toxin data from 2004-2006

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EXECUTIVE SUMMARY

The aim of this study was to assess the monitoring programme conducted by the Food Standards Agency Scotland (FSAS) for determining the prevalence of toxins responsible for diarrhetic shellfish poisoning (DSP), paralytic shellfish poisoning (PSP) and amnesic shellfish poisoning (ASP), in shellfish harvested from classified inshore production areas in Scotland. The toxicity patterns observed at designated sites throughout the year were established using data collected over a three-year period from April 2004 to November 2006. The current study is a follow-up on a previous risk assessment (report S01026), which was concerned with assessment of the inshore monitoring programme during April 2001 – March 2004.

The current (as implemented in April 2006) FSAS monitoring programme was assessed for the risk of a toxic event at a particular site being undetected. Alternative schemes that offered a more targeted allocation of resources or an improved level of public health protection were also considered.

Mussels are currently used as indicator species, and as it was the only species with data from all across Scotland, findings from this report tend to focus on mussels.

Analysis of monitoring data

The data analysis was concerned with toxin concentrations of ASP, DSP and PSP detected in mussels, pacific oysters, king scallops, queen scallops, and cockles (1788, 518, 31, 95, 26, and 30 samples, respectively). The numbers of samples collected during April 2004 – November 2006 were similar to those during the earlier period of April 2001 – March 2004, except for king scallops and cockles, which were only 20 to 30% of the numbers collected during April 2001 – March 2004.

Initial analysis of the monitoring data from 2004/6 revealed the following.

- Prevalence of toxins during April 2004 – November 2006 was markedly different from the prevalence observed during April 2001 – March 2004. Not only was the average prevalence lower during 2004-2006, the pattern of prevalence of toxin during the 12 months of the year was different for these two data sets also.

To get a better understanding of variation in toxin patterns from year to year, it was decided to analyse the entire data set (from April 2001 to November 2006) in a combined, coherent, manner. All findings mentioned hereafter relate to the 2001/6 period, and are based on the model assumptions outlined in the report.

All samples were assigned to Pods (these are designated sample locations, introduced by FSAS in November 2006). In some instances the sample site was not covered by an existing pod, in which case a new pod was introduced. This resulted in 84 pods. To allow for modelling of toxin patterns, pods with limited data that had similar toxin patterns and were in close proximity of each other, were grouped. This resulted in 25 sites.

Analysis of the monitoring data from 2001/2006 revealed the following.

- Toxin levels of DSP, PSP and ASP varied significantly over time (between months and between years), across sites (with some sites showing a tendency for higher toxin levels) and across shellfish species.
- DSP
    - DSP was most often detected in queen scallops (10%) and mussels (5%). For the remaining species, fewer than 4% tested positive.
    - Prevalence of DSP was high in 2001, 10% in mussels (20% in queen scallops), but gradually declined to 4% in mussels in 2006 (3% in queen scallops). This decline with time was seen in all shellfish species.
    - DSP in mussels was present throughout the year but peaked at 13%, for an average site, during June – August. For certain sites prevalence went up to 31%.
    - Nearly all sites tested positive for DSP in mussels at some time during 2001/6.
- PSP
    - PSP levels exceeding the regulatory limit of 80 µg/100g only occurred in mussels, except for 1 cockle and 2 clam samples in 2006 that exceeded 80 µg/100g.
    - From 2001 to 2005 there was a decline in the proportion of mussel samples exceeding the regulation limit; 2.6% in 2001 down to 0.5% in 2005. In 2006 levels went up again (1.7%).
    - A peak occurred in early summer (May and June, up to 4.6%) and a lesser peak in September (1.2%), although for some sites in Shetland this went up to 20% in June. Prevalence was low during the winter months.
    - There were several sites for which PSP in mussels tested negative during the entire period of investigation.
    - PSP was nearly always absent in pacific oysters, with only 2 samples (out of 852) testing between 0 and 40µg/100g (test results exceeding 40 µg/100g result in more frequent sampling).
- ASP
    - King scallops is the only shellfish species for which ASP levels exceeded regulatory limits (> 20µg/g), although low levels were detected in all shellfish species.
    - There were only four sites at which king scallops were sampled and the ASP prevalence was similar for these sites.
    - Prevalence of ASP exceeding regulatory limits in king scallop gonads was high during 2001/3 (up to 50%, for an average site and average month) then dropped down to 3% in 2005 and increased to 17% in 2006.
    - In mussels, although approximately 40% of samples tested positive for ASP, only 3 out of 3791 samples exceeded field closure limits.
    - Prevalence of positive ASP levels in mussels was high in 2001/4 at around 50%, but dropped to 20% in 2005 and 8% in 2006.
    - ASP was detected in mussels at all 25 sites and prevalence across sites was similar.
    - ASP in king scallop gonads and mussels tended to be present throughout the year although prevalence tended to be lower in late winter and early spring.

- For a given toxin, toxicity patterns over time (e.g. decline in DSP over the 2001/6 period) were similar for all shellfish species, although the actual level varied from species to species.
- Toxin prevalence varies between sites, even for sites that are close geographically.

Risk assessment of present and alternative monitoring schemes

The monitoring programme in place in 2006 consists of

- PSP: weekly all year round for all species
- DSP: weekly from April to November, fortnightly in December, monthly from January to March for all species
- ASP: weekly from July to November, fortnightly from April to June, monthly from December to March for all species. The only exception is king scallops, which are tested weekly all year round.

The monitoring data from April 2001-November 2006 provided sufficient information on levels of DSP in mussels, PSP in mussels and ASP in mussels and king scallops for each site during each month to enable a risk assessment to be carried out. The risk assessment was concerned with the monitoring programme failing to detect a toxic event, i.e. that a site is not tested while toxin levels exceed field closure (for example, a monthly sampling scheme would fail to detect that a site might become toxic only one week after a negative test result). This is referred to as the risk of non-detection. It was assumed that the test result is valid for one week.

- As PSP was tested on a weekly basis, the risk of non-detection in mussels was zero. As several sites always tested negative, alternative schemes were developed that considered reduced sampling effort at those sites.
- The maximum risk of non-detection was 3.7% for DSP in mussels during December (currently fortnightly sampling). Monthly testing during January-March seems acceptable as the maximum risk of non-detection was only 1.4%. Overall, the current scheme appears to be largely appropriate.
- ASP in king scallop gonads was tested weekly throughout the year and hence the risk of non-detection was zero. As high levels of ASP were prevalent throughout the year the current scheme appears to be appropriate.

<u>The risk assessment enabled the following recommendations to be made.</u>

- For the monitoring of PSP in mussels, sampling effort could be made more efficient by either reducing the sampling frequency or using simple screening methods for sites that have always tested negative for PSP.
- For a given toxin, toxicity patterns were similar for all shellfish species, supporting the use of mussels as indicator species.
- It is important to note that these findings are based on only six years' of data and therefore there is a considerable amount of uncertainty in the estimates. There is no guarantee that sites, species, or months that were clear during this six-year period will remain clear in the future as toxin patterns may change. Therefore,
    - Some level of shellfish monitoring should be continued at all sites in order to reduce the risk of toxic events being overlooked.
    - Sampling schemes should be flexible so that adjustments in sampling frequency can be easily and quickly made when necessary.

CONTENTS

LIST OF TABLES AND FIGURES

TABLES

FIGURES

FIGURES APPENDIX A (raw data)

TABLES AND FIGURES APPENDIX B (ASP > 5µg/g in mussels)

GLOSSARY

AHA	:	Associated Harvesting Area. These are harvest areas represented by a representative monitoring point.
ASP	:	Amnesic Shellfish Poison, measured in units of µg/g shellfish. Field closure when ASP levels exceed 20 µg/g.
ASP>0	:	Also referred to as 'positive for ASP' and refers to all samples that had a non-zero test result, including samples for which ASP was at the limit of detection.
Clams	:	Combination of clams (including surf, venerupis, carpet, pullet and venus clams), ensis, native oysters, razors and spisula.
DSP	:	Diarrhetic Shellfish Poison, measured as absence or presence. Field closure when DSP is present.
Extreme year	:	Based on year-to-year variation, an extreme year is defined as a year during which prevalence of toxin is extremely high. It is assumed that such a year happens only once every 20 years.
FSAS	:	Food Standards Agency Scotland.
G8	:	Group of pods. The number indicates the pod in the group that had most samples, and the 'G' indicates that is a group of pods. See also P5.
GLM	:	Generalised Linear Model.
HGLM	:	Hierarchical Generalised Linear Model.
HPLC	:	High Performance Liquid Chromatography. Used for ASP testing.
LOD	:	Limit of Detection
Log-odds	:	Natural logarithm of the odds (= ln(p/(1-p)).
LOQ	:	Limit of Quantification
MBA	:	Mouse Bioassay, used for testing of DSP and PSP.
Odds	:	$p / (1 - p)$
p	:	Probability that toxin levels exceed field closure limit.
P5	:	Pod identification number (pod 5). The 'P' indicates that this data is obtained from single pod. See also G8.
p_{high}, p_{low}	:	Cut-off levels that are used to construct alternative sampling schemes. Weekly sampling is applied when p exceeds p_{high}, while monthly sampling is sufficient when p is less than p_{low}.
Pod	:	A group of shellfish harvesting sites, where sites within a pod are thought to be similar hydrographically and environmentally. Pods are defined by FSAS. For the risk assessment it is assumed that the risk of a toxic event is similar for sites within a pod.
PSP	:	Paralytic Shellfish Poison, measured in units of µg/100g shellfish. Field closure when PSP levels exceed 80µg/100g.
PSP>0	:	Also referred to as 'positive for PSP' and refers to all samples that had a non-zero test result, including samples for which PSP was at the limit of detection.

Risk of non-detection	:	Used in assessment of existing and alternative sampling schemes, and is defined as the probability that a field is unknowingly toxic.
R_{max}	:	Maximum acceptable risk of non-detection.
RMP	:	Representative Monitoring Point. This is an official control monitoring point representative of a classified shellfish production area. This point will be monitored to a time table for all toxin groups throughout the year.
2001/4 data	:	Data from 1 April 2001 to 31 March 2004, used in previous risk assessment.
2004/6 data	:	Data from 1 April 2004 to 30 November 2006.
2001/6 data	:	Data from 1 April 2001 to 30 November 2006.
Site	:	Either a pod or a group of pods. Based on the mussel data, if a pod did not have sufficient samples for statistical modelling, it was combined with neighbouring pods. Such a group of pods is referred to as a site. If a pod had sufficient data it was not combined with others, but is still referred to as a site. See also the G5 and P8 definitions.

1 AIMS AND OBJECTIVES OF THE INVESTIGATION

1.1 Introduction

Shellfish harvested from inshore classified production areas in Scotland are monitored by the Food Standards Agency Scotland (FSAS, as the competent authority in Scotland under Regulation (EC) No. 854/2004 for the toxins responsible for amnesic shellfish poisoning (ASP), diarrhetic shellfish poisoning (DSP) and paralytic shellfish poisoning (PSP).

In the UK, ASP toxins are monitored in bivalve molluscs using High Performance Liquid Chromatography (HPLC) as specified in Commission Regulation (EC) No. 2074/2005. If ASP levels in sampled shellfish are detected at a concentration of 20 µg/g or above, harvesting areas are closed. Directive 91/492/EEC requires that both DSP and PSP toxins be monitored in shellfish using a mouse bioassay (MBA) method. For DSP, a positive MBA result (indicating the presence of toxin) leads to the closure of a harvesting area. In the case of PSP, harvesting areas are closed when toxin levels are found to be greater than or equal to 80 µg/100 g tissue by MBA. To reduce the use of the numbers of animals used in the MBA, a simple screening test (Jellet Rapid Test) was introduced in April 2005, and was replaced HPLC in November 2006. These screening tests allow for detection of PSP at low levels. When the screening test result is positive, the sample is retested using the MBA.

In 2004, FSAS commissioned a research project to perform a risk assessment of the Scottish inshore shellfish sampling scheme (Holtrop & Horgan, 2004) using monitoring results from April 2001 to March 2004. This study revealed that the monitoring frequencies as implemented at that time were inadequate and alternative monitoring frequencies were proposed. Based on the recommendations from this report, FSAS implemented a revised monitoring scheme in April 2006. This revised scheme is based on weekly sampling all year round for PSP. For ASP, weekly sampling takes place during July – November (April – November for DSP), fortnightly sampling takes place during April – June (for DSP fortnightly sampling occurs during December only), with monthly sampling otherwise.

This project is a follow up of project S01026 (Holtrop & Horgan, 2004) and assessed the current monitoring programme (as implemented in 2006). It also considered alternative monitoring regimes that may offer increased levels of confidence in toxin detection, or may be more economical or practical for FSAS to implement. Using historical data from April 2004 to November 2006, both existing and any revised schemes were assessed by looking at the following:

- The distribution of toxin levels in shellfish from different sites.
- The occurrence of shellfish toxins over the 12 months of the year, and whether these patterns have changed between 2001/4 and 2004/6.
- The risk that shellfish with toxin levels exceeding the accepted threshold are not detected through the monitoring programme.
- Whether there are regions or sites that are more likely to experience toxic events that are not detected by the current monitoring programme.

This information will aid the FSA in the design and refinement of the inshore shellfish toxin monitoring programme in the future.

1.2 State of the art

Levels of PSP, DSP and ASP toxins have been monitored in Scottish shellfish for several years. However, although the existing monitoring programme appears to provide a sufficient level of public health protection there have been isolated cases of toxic samples reaching the consumer market. In June 2006, a DSP outbreak occurred in London, affecting individuals who had eaten Scottish harvested mussels in several restaurants around the city. Onset of illness generally occurred 2-12 hours following eating. Notably, while the restaurants identified 171 cases, only six individuals reported their illness to the relevant local authority, demonstrating the under-reporting of food poisoning incidents. The Official Control sample from this area was found to be positive that same week and the area was subsequently subject to a closure which lasted 4 months. Three mussel samples from the suspected batch were obtained from the restaurants' supplier for analysis two samples tested positive for the presence of DSP toxins by mouse bioassay. Cases of shellfish poisoning are rare in the UK and this is only the third incident of DSP to be reported in the UK. One in Scotland in 2002 which led to a recall of mussels originating from Highland area and incidents reported in 1994 and 1997.

Based on recommendations made in Holtrop & Horgan (2004), the shellfish monitoring scheme was revised in 2006. To our knowledge, the shellfish toxin test results obtained since April 2004 (i.e. following on from the data analysed in the previous risk assessment) have not been summarised, statistically analysed and assessed for risk of not detecting toxic events.

1.3 Scientific work undertaken

1. Historical monitoring data from April 2004- November 2006, provided by FSAS, were used to develop statistical models that describe the deterministic and random components of variation in shellfish toxin levels. These included variation between sites and regions, seasonal variation, and variation between years. The statistical techniques used include hierarchical generalised linear models that include fixed and random effects. These approaches allowed the development of models that are capable of describing toxicity patterns over time and location for each of the ASP, DSP and PSP toxins.
2. These models were then used to compare toxin patterns observed during April 2001 – March 2004 to those observed during April 2004 – November 2006.
3. These models were also used to assess the effectiveness of the existing monitoring programme in detecting toxic events.
4. Alternative monitoring schemes (which may provide an increased level of public health protection, or which are more practical or more economical) were assessed for their effectiveness in detecting toxic events.
5. Similarity of sites that are in close proximity of each other was assessed in detail for mussel test results from Shetland.

1.4 Outcomes of the study

The following information has been provided by this study.

1. Models describing the toxicity patterns of ASP, DSP and PSP over time (based on data from April 2001 to November 2006).
2. Assessment of the effectiveness of the existing monitoring regime (in terms of the risk of a contaminated field being undetected).
3. Assessment of alternative monitoring schemes which may provide an improved level of public health protection, or which are more economical or more practical.

Based on this information, a list of recommendations was constructed that will aid the FSA in developing more effective monitoring schemes.

2 MATERIALS AND METHODS

2.1 Data Handling

2.1.1 Toxin Data

The data consisted of toxin levels recorded for Paralytic Shellfish Poison (PSP), Diarrhetic Shellfish Poison (DSP) and Amnesic Shellfish Poison (ASP), over a three-year period from 1 April 2004 to 30 November 2006 (referred to as 2004/6).

- For DSP the data were recorded simply as absent (ascribed zero) or present (ascribed one).
- For PSP the toxin level is expressed as µg/100g shellfish. The limit of detection is approximately 30 µg/100g and the field closure limit is set at 80 µg/100g.
- For ASP the toxin level is given as µg/g. The limit of detection is approximately 1.0 µg/g and field closure occurs at toxicity levels exceeding 20 µg/g.
- If DSP is present or PSP or ASP levels exceed field closure limits, the field is closed until 2 consecutive results below the closure level are obtained which are at least 7 days apart.

Each test result has an identification number that uniquely identifies the location the sample came from.

2.1.2 Categorisation of toxin levels

For presentation purposes as well as development of statistical models, PSP and ASP levels have been categorised as follows.

For PSP:

- 0
- > 0 and < 40 µg/100g (40 µg/100g is an arbitrary alert level)
- ≥ 40 and < 80 µg/100g
- ≥ 80 µg/100g (field closure).

For ASP:

- 0
- > 0 and < 10 µg/g (10 µg/g is an arbitrary alert level)
- > 10 and < 20 µg/g
- ≥ 20 µg/g (field closure).

Note that any reference to ‘PSP>0’, ‘ASP>0’, ‘positive for PSP’, and ‘positive for ASP’ include those samples for which the test result was at the limit of detection.

2.1.3 Definition of Sites

The classification of shellfish harvesting areas was revised by FSA Scotland in November 2006. Shellfish harvesting sites were assigned to ‘pods’, where locations

within a pod are thought to be similar hydrographically and environmentally, so that the risk of a toxic event is assumed similar within a pod. For each pod, one of the locations was assigned as a Representative Monitoring Point (RMP) status, with the remaining locations being assigned Associated Harvesting Area (AHA) status. For each RMP, a representative shellfish species (usually mussels) is sampled according to a set time table, and the test result is assumed to represent the entire pod (i.e. the RMP itself as well as the associated AHA locations). If the test result requires field closure, then the shellfish beds in the entire pod are closed. It is possible for an individual AHA, however, to have samples tested to contest closure of their AHA.

As one of the project objectives was to compare the 2004/6 toxin results against those obtained previously (April 2001 to March 2004, referred to as 2001/4 data), samples from 2001/4 were assigned retrospectively to pods. This was done by FSAS. When samples could not be assigned to existing pods, new pods were introduced and were given a number exceeding 100. As a result, all samples from 2001/6 were uniquely assigned to a pod.

Prior to analysis, duplicate data entries were removed. For 2004, there were some inconsistencies and these were checked by FSAS. This resulted in 1878, 2093 and 2397 samples for PSP, DSP and ASP, respectively for April 2004 – November 2006 (compared with 2701, 2746 and 2990 samples for April 2001- March 2004 respectively).

The FSAS definitions of pods were taken as a starting point. To allow for statistical investigation of differences between sites, years and months, the aim was to have, on average, at least 1 – 2 mussel samples per (group of) pod(s) per month per year. Where necessary, pods were grouped based on

- Proximity
- Similarity of hydrographical and environmental conditions
- Similarity in toxin profiles

Pods with limited data were only combined with other pods if their toxicity patterns were similar, as assessed by examination of plots of the toxin profiles. Possible groupings of pods were suggested by FSAS and the final groupings were agreed on in close collaboration with the Agency. This resulted in 25 (groups of) pods, with full details given in Table 1 and Figure 1[1]. In this report, each group of pods will be referred to as a site. Note that although the groupings are similar to those used for the previous risk assessment (Holtrop & Horgan 2004), they are not identical.

2.1.4 Definition of species

The data sets included toxin values for the following species, with the maximum number of samples given in parentheses: carpet clams, clams, pullet carpet clams, surf clams, venerupis clams, venus clams (9 for 2001/4, 11 for 2004/6), cockles (79 for 2001/4, 22 for 2004/6), mussels (2161 for 2001/4, 1705 for 2004/6), native oysters (15 for 2001/4, 9 for 2004/6), pacific oysters (405 for 2001/4, 489 for 2004/6), queen scallops (83 for 2001/4, 91 for 2004/6), razors (12 for 2001/4, 20 for 2004/6), king scallops (149 for 2001/4, 26 for 2004/6), spisula (15 for 2001/4, 4 for 2004/6). Due to

[1]Tables and Figures are numbered and located according to when they first appear in the Results and Discussion Sections.

limited numbers of samples, clams (including surf, venerupis, carpet, pullet and venus clams), ensis, native oysters, razors and spisula were combined into one species denoted by the term 'clams'.

2.1.5 King scallops: gonads versus whole scallop

For DSP and PSP, testing of king scallops takes place on the whole animal. For ASP, testing is conducted on both the whole animal and the gonad. Previous scientific studies have indicated that most of the domoic acid (the toxin responsible for ASP) in contaminated king scallops is associated with the offal (hepatopancreas, mantle and gills). The contamination of edible tissues (adductor muscle and gonad) can be minimised, if the offal is completely removed by shucking (McKenzie and Bavington, 2002). Effective shucking allows scallops harvested from a particular site which are found to contain over 20 µg/g ASP in the whole animal, but below 20 µg/g in the gonad, to be placed on the market. However, they must be taken to an approved processor for shucking before they may be considered safe for consumption.

In the present study, 89% (156/175) of the king scallop samples tested for ASP gave a whole animal test result exceeding the field closure limit of 20 µg/g, whereas 34% of the gonad samples were found to contain toxin levels which were above the field closure limit. Because the majority of whole scallop samples would have resulted in field closure, the whole scallop results for ASP were excluded, and only the gonad results were included in the analysis. Therefore, for the purposes of this study, the risk of high ASP levels occurring in whole scallops was estimated as 100%.

2.2 Estimation of the probability that toxicity exceeds the field closure limit

As mussels are regarded as indicator species, and had the highest numbers of samples, model development was based on mussel test results.

2.2.1 Model

For each species and each toxin, the following model was constructed. Let p be the probability that a sample is positive (i.e. the toxin level exceeds the field closure limit). This probability is likely to depend on the time of year (e.g. high values are more likely to occur in summer than in winter). Likewise, p may depend on the location it was taken from (e.g. a sample is less likely to be positive when taken from the East Coast). To investigate such relationships, a binomial model with logistic link, which is a special case of a Generalised Linear Model (GLM, see McCullagh & Nelder, 1989 for details) was constructed. Let y_{ms} and n_{ms} be the number of positive samples and the total number of samples respectively, for month m at site s. Then y is assumed to follow a binomial distribution, where the probability of a sample being positive is modelled as a function of month and site:

$$y_{ms} \sim \text{Binomial}(n_{ms}, p)$$

Let the odds be defined as p/(1-p). A linear model was formulated for the log-odds as follows:

$$\ln [\ p/(1-p)\ ] = \text{constant} + \text{Month}_m + \text{Site}_s \qquad (1)$$

with ln denoting the natural logarithm. This model formed the basis for determination of the significance of Month and Site in the previous assessment, where Month was regarded as a fixed effect and Site as a random effect, i.e. on the log-odds scale, Site effects were assumed to have a normal distribution with a mean of zero and unknown between-site variance σ_s^2. A model containing both fixed and random effects is referred to as a Hierarchical Generalised Linear Model (HGLM, see Lee & Nelder (1996, 2001)).

All statistical analyses were conducted using the HGLM routine in Genstat 10th edition, release 10.1 (VSN International Ltd, Hemel Hempstead, Herts., UK).

2.2.2 Model fitted to 2004/6 mussel data

In the first instance, a HGLM model with Month as a fixed effect and Site as a random effect was fitted to the 2004/6 mussel data. For PSP, models were developed for

- Probability that PSP is positive (y = number of samples for which PSP > 0)
- Probability that PSP exceeds 40µg/100g (y = number of samples for which PSP > 40µg/100g)
- Probability that PSP exceeds field closure (y = number of samples for which PSP > 80 µg/100g)

For each of these models, n was given as the total number of mussel samples tested for PSP. For ASP in mussels, models were developed for

- Probability that ASP is positive (y = number of samples for which ASP > 0)
- Probability that ASP exceeds 10µg/g (y = number of samples for which PSP > 10µg/g)

For each of these models, n was given as the total number of mussel samples tested for ASP.

To allow for comparison of results against the 2001/4 mussel data, the latter data set was re-analysed using the new groupings as outlined in Table 1. It was found that the fitted values for this earlier data set were similar for both the old and the new groupings.

When the model fit for the 2004/6 data was compared against that of the re-analysed 2001/4 data, it was found that there were major differences in the estimated toxin patterns. For example, DSP in mussels showed high prevalence in November and December for the 2001/4 data, while for the new data the prevalence was estimated to be nearly zero. For PSP in mussels, the earlier data set showed high prevalence during early summer, while the new data set indicated an additional peak in August and September. For ASP in mussels, the 2001/4 data showed a high prevalence during the second half of the year, while for the 2004/6 data the prevalence was estimated to be much lower and was nearly constant throughout the year.

2.2.3 Models for 2001/6 mussel data

The findings above suggest that the toxin pattern observed during a year varies from year to year. To increase our understanding of such annual variations, it was decided to analyse the 2001/4 and 2004/6 data sets in a combined manner. This allows for investigation of statistical significance of variations between Sites, Years, Months and interactions between these terms.

Based on Lee & Nelder (1996, 2001), HGLM models with Month as fixed effect and random effects (assumed to be Normally distributed) for Year (Y), Site (S), Year by Site (Y.S), Year by Month (Y.M) and Site by Month (S.M) interactions were fitted to the 2001/6 mussel toxin data. The significance of the random effects was investigated by comparing the h-likelihoods using Akaike's Information Criterion. This resulted in the following random models for the mussel data:

DSP > 0:	Y + S + Y.S + Y.M
PSP > 0:	Y + S + Y.M
PSP > 40:	Y + S
PSP > 80:	Y + S
ASP > 0:	Y + S + Y.M
ASP > 10:	Y + S

For king scallop gonads:

ASP > 10:	Y+S
ASP > 20:	Y+S

For pacific oysters:

ASP > 0:	Y+S

Finally, there were some species/toxin combinations that allowed for limited modelling of a Year effect (random) but no inclusion of a fixed term for Month, namely; DSP > 0 for pacific oysters and queen scallops; ASP > 0 for clams, cockles and queen scallops.

Interpretation of the random effects is as follows:

1. Random variation between years (Y). Toxin levels are thought to vary randomly between years, with some years showing high toxin levels while other years show low levels. It is not known in advance whether toxin levels are going to be high or low in any specific year.
2. Random variation between sites (S). Toxin levels are thought to vary randomly between sites, with some sites having a tendency for high toxin levels while other sites have a tendency for low toxin levels. Some of these differences may be explained by hydrographical and local environmental conditions, but even so, we might expect there to be some unexplained variation between sites, even if local conditions are similar.

3. Interaction between Year and Month (Y.M): Toxin levels are thought to vary between years, as described under 1, but the effects depend on the time of year. For example, in May there may be a large difference between two years while in Jan there is only a small difference between these two years.
4. Interaction between Year and Site (Y.S): Toxin levels are thought to vary between years (as described under 1) as well as between sites (as described under 2), but in addition the effect of Year varies from site to site. For example, year 1 gives high toxin levels overall while year 2 gives low toxin levels overall (variation between years), but the difference between years 1 and 2 may be much smaller for some sites and much larger for other sites (it may even be the case that for certain sites year 2 gives higher toxin levels than year 1).

The first practical consequence of including random effects such as Site is that although the estimated probability of a sample being toxic for a particular site is based on the data obtained from that site, the estimate is slightly shrunk towards the overall mean value. The amount of shrinkage depends on the number of samples and the magnitude of the random variation between sites. As a consequence, site effects were never estimated to be exactly zero, even if all the samples from a site were clear. This seems sensible as data from only six years were available, and the absence of toxin at a site during a particular period does not indicate that the site will always be clear.

The second consequence of including random effects, such as Year, is that, in addition to looking what would happen during an average year, we can also look at what might happen during an 'extreme' year during which prevalence of toxin is extremely high (see Section 2.2.5 for details).

To better understand the effects of Month, Site and Year let us look at model (1) again:

$$\ln [\, p/(1-p) \,] = \text{constant} + \text{Month} + \text{Site} + \text{Year} \qquad (2)$$

This model is linear in terms of the log-odds, ln[p /(1-p)]. In terms of the odds, defined as p/(1-p), we have

$$p/(1-p) = \exp(\text{constant}) \exp(\text{Month}) \exp(\text{Site}) \exp(\text{Year}) \qquad (3)$$

showing that the effects of Month, Site and Year have a multiplicative effect on the odds. So a bad site (in terms of toxin prevalence) will have a high multiplication factor exp(Site).

2.2.4 Averaging model predictions over months or years

Based on the parameter estimates, it is possible to estimate what the toxicity would have been during Jan-Mar 2001. When calculating the mean for 2001, these estimates have been included (to avoid a bias towards higher toxin levels for 2001). Likewise, when calculating the mean for 2006, the estimated toxicity for December 2006 has been included. When calculating the mean for January (over all 6 years), the

estimated values for Jan 2001 have been included (the same holds for Feb 2001, Mar 2001 and Dec 2006).

2.2.5 Random variation between years, introducing an 'extreme' year

Based on the estimated random variation between years (and interactions with Month and Site), it is possible to estimate the toxin levels for an extreme year. Let an extreme year be defined as one which happens only once every 20 years, on average. Let σ^2 denote the variation over time (sum of variation between years and interaction terms involving year). Inserting a Year effect of 1.64 σ in equation (2) then gives an estimate of the log-odds for such an extreme year. This represents an upper 95% limit on the log-odds, due to variation between years. Note that this does not account for model uncertainty.

2.3 Risk assessment of current and alternative sampling schemes

2.3.1 Present monitoring scheme

Under the present (2006) monitoring scheme, the sampling frequencies are as follows:

- PSP: weekly all year round
- DSP: weekly from April to November, fortnightly in December, monthly from January to March
- ASP: weekly from July to November, fortnightly from April to June, monthly from December to March.

These frequencies are applicable to all sites and all shellfish species.

2.3.2 Risk assessment

The aim of the sampling strategy employed in the monitoring programme is to maximise confidence that a harvesting site is clear (i.e. toxin levels are below field closure). This is equivalent to minimising the risk that a site is unknowingly toxic. For the purposes of this study, this will be referred to as the 'risk of non-detection', and can be applied to any of the three toxins.

> Risk of non-detection is defined as the chance that a site is unknowingly toxic

In other words, it looks at the probability that the site is not sampled while toxin levels exceed field closure limits.

If a site is tested and is discovered to contain toxin levels that exceed the field closure limit, then it will remain closed until two consecutive samples, taken one week apart, are clear. Consequently, actively harvested sites at which the levels of ASP, DSP, or PSP are found to exceed the field closure limit are usually tested on a weekly basis until two negative results are obtained. Therefore, the risk that a site is unknowingly toxic is more likely to be associated with sites that are considered negative, and so

may be sampled less frequently. Examination of the data shows that it is possible for a clear sample to be followed by a toxic sample only one week later. For example, in September 2006, PSP levels in mussels harvested from pod 70 in the North-West of Shetland, were found to increase from non-detectable levels to 138 μg/100g within 8 days (Table 23[2]). Had the second sample not been taken from this site, it would have been unknowingly toxic.

The risk of non-detection was calculated as follows. Let the chance that the field is toxic be denoted by p. For each toxin/species combination, the HGLM model (see section 2.2) provides an estimate of p for each site for each of the twelve months of the year. For simplicity, it was assumed that a negative test result (i.e. toxin level below field closure limit) was valid for one week. This implies that if samples were to be taken every week, the risk of the field being unknowingly toxic was zero. Likewise, if samples were taken every fortnight, the risk was 0.5p (for every four weeks there were two weeks that the risk of non-detection was zero and two weeks that the risk of non-detection was p, so is (0+p+0+p)/4 = 0.5 p on average). If samples were taken every four weeks, the risk of non-detection was 0.75 p (for every four weeks there was one week with zero risk of non-detection and three weeks with risk of p, which gives (0 + p + p + p)/4 = 0.75 p on average). To summarise:

- Weekly sampling: risk of non-detection is zero
- Fortnightly sampling: risk of non-detection is 0.5p
- Monthly sampling: risk of non-detection is 0.75p.

The risk of non-detection depends on two factors, namely

a) the chance that the field is toxic (i.e. probability that toxin levels exceed the closure limit), and
b) the sampling frequency.

An increase in the chance that the field is toxic, and/or a decrease in the sampling frequency lead to an increased risk of non-detection.

Note that the risk assessment is concerned with the chance of non-detection for a given monitoring scheme, i.e. the chance that a toxic field is not detected because no sampling takes place. The situation where repeated sampling takes place following a positive test result is not considered here. Such repeated sampling is regarded as the responsibility of the shellfish farmer and falls outwith the aims of the monitoring scheme (namely monitoring of toxin levels over time).

2.3.3 Risk assessment of the present monitoring scheme

For each toxin/species combination, the HGLM model (see Section 2.2) provides an estimate of p (the chance that toxin levels exceed the field closure limit) for each site for each of the twelve months of the year. Based on the sampling frequencies employed in the current monitoring scheme, the risk of non-detection was calculated for each site and each month.

[2]Tables and Figures are numbered and located according to when they first appear in the Results and Discussion sections.

2.3.4 Risk assessment of alternative sampling schemes

Alternative sampling schemes were developed considering three possible frequencies, namely once per month when toxin levels are unlikely to exceed the field closure limit, once per week when toxin levels are likely to exceed the field closure limit, and fortnightly otherwise. To put this in a mathematical context, let p_{low} and p_{high} be fixed values that are set in advance, so that for small p (which is the chance that toxin levels exceed the field closure limit), less than p_{low} say, monthly monitoring will suffice, while for high p, exceeding p_{high} say, weekly monitoring would be required:

- when low toxin levels occur (p less than p_{low}) monthly monitoring may be carried out,
- when high toxin levels occur (p exceeding p_{high}) monitoring should be carried out once per week,
- when intermediate toxin levels occur (p between p_{low} and p_{high}), monitoring may be carried out once per fortnight.

For a given p_{low} and p_{high}, and in combination with the estimated values of p for each site and each month (from the HGLM model), monitoring schemes can be developed that are site and time specific. This results in each site having its own monitoring scheme where sampling frequency may vary during the year.

Instead of choosing cut-off levels p_{high} and p_{low} and then deriving the corresponding risk of non-detection, it is more convenient to select an acceptable risk level first, and then derive the corresponding cut-off levels p_{high} and p_{low}. This is done as follows. For a given maximum acceptable risk of non-detection, denoted by R_{max}, the most efficient (i.e. requiring the least samples) monitoring scheme is given by: monthly sampling for $p \leq 4/3\ R_{max}$, fortnightly sampling for $4/3\ R_{max} < p < 2\ R_{max}$, and weekly sampling for $p \geq 2\ R_{max}$, so that the corresponding cut-off points are given by $p_{high} = 2\ R_{max}$ and $p_{low} = 2/3\ p_{high}$.

To summarise, alternative sampling schemes were developed as follows.

- Let R_{max} denote the maximum acceptable risk of the field being unknowingly toxic (to be decided by FSAS).
- Calculate $p_{high} = 2\ R_{max}$.
- Calculate $p_{low} = 2/3\ p_{high}$.
- Based on estimates of p (which is the chance that toxicity levels exceed the field closure limit), develop a new monitoring scheme that is site and month specific, as follows.
    - When $p \leq p_{low}$, monthly monitoring is carried out.
    - When $p_{low} < p < p_{high}$, fortnightly monitoring is carried out.
    - When $p \geq p_{high}$, weekly monitoring is necessary.

Appropriate values for the maximum acceptable risk of non-detection (R_{max}) should be set by FSAS, but to illustrate the approach outlined above, two alternative sampling schemes were used in this report, based on $R_{max} = 5\%$ and $R_{max} = 1\%$.

- Maximum acceptable risk of non-detection is 5%, so that $p_{high} = 10\%$ and $p_{low} = 6.67\%$. Sampling frequency should be once a week when $p \geq 10\%$, once a fortnight when $6.67\% < p < 10\%$ and once a month when $p \leq 6.67\%$.
- Maximum acceptable risk of non-detection is 1%, so that $p_{high} = 2\%$ and $p_{low} = 1.33\%$. Sampling frequency should be once a week when $p \geq 2\%$, once a fortnight when $1.33\% < p < 2\%$ and once a month when $p \leq 1.33\%$.

3 RESULTS

As initial model explorations indicated large differences between toxin level patterns over months for the 2001/4 and 2004/6 data, it was decided to present the combined data over 6 years (i.e. from April 2001 until the end of November 2006).

As for many of the pods only a limited amount of data was available, pods were combined into 25 sites (see Table 1 and Figure 1). Please note that any reference to 'site' is with respect to the sites defined in Table 1. Furthermore, site names mentioned in the text correspond to the shortened names provided in Table 1 (for example, the site based on the pod in the Vaila Sound in Shetland will be referred to in the text as 'Shetland-SW-Vaila').

3.1 Summary of monitoring data

3.1.1 Numbers of samples per species

Mussels were the most frequently tested species over the examined six years (as shown in Table 2), followed by pacific oysters, queen scallops, king scallops, cockles, and clams. Mussels and pacific oysters were most widespread with samples taken from 25 and 14 sites, respectively. The remaining species were less widespread, with samples obtained from 5-7 sites.

The number of mussel samples tested for PSP was low in 2004 (109 samples for 2004, compared to an average of 613 samples per year for the remaining 5 years). The numbers of king scallops tested dropped from 47 in 2003 to 13 in 2004 and 11 in 2005. In 2006 (until 30 Nov) no king scallops were tested for DSP or PSP, and only 2 batches were tested for ASP (Table 2).

Table 1: Definition of site, pods covered by each site and a brief description of locations covered by each site. ID starting with G indicates a group of pods, followed by the pod number that had most data for that grouping. ID starting with P indicates an individual pod, and is followed by the corresponding pod number. Areas highlighted in bold relate to the dominant pod of the grouping. Pod numbers less than 100 refer to the FSAS definitions of pods as introduced in November 2006, while pod numbers exceeding 100 were introduced to refer to areas that had data for 2001/4 only. Sites are arranged starting with the East Coast of Scotland, and then follow the coastline in approximately clockwise manner.

Site[1]	ID	Pods	Description
Eastcoast	G80	20, 80, 107,111	**Forth Estuary**, Eyemouth, Montrose, Tay Estuary
Dumfries	G26	26, 27	**Loch Ryan**, Solway Firth
Ayr-LochStriven	G8	8, 52, 53, 108	**Loch Striven**, Arran, Clyde
WC-LochFyne	G16	13, 14, 15, 16, 17, 109	**Loch Fyne**, Colonsay, West Loch Tarbert
WC-LochEtive	G10	4, 6, 10	**Loch Etive**, Seil Sound
WC-LochCreran	G9	9, 11	**Loch Creran**, Loch Linnhe
Mull-LochSpelve	P5	5	**Loch Spelve**
Mull-LochScridain	P7	7	**Loch Scridain**
Mull-LochnaKeal	G1	1, 2, 12, 32	**Loch na Kael**, Tobermory, Aros, Loch a Chumhainn, Loch Teacuis, Loch Sunart
WC-LochLeven	G31	29, 31, 34	**Loch Leven**, Loch Eil
WC-Lochaber	G28	28, 30, 33	**Ardtoe, Fascadale Bay, Glenuig Bay, Loch Ailort, Loch Moidart**, Arisaig, Loch Nevis, Loch Hourn
Skye	G41	40, 41, 42, 43, 45	**Loch Eishort**, Loch Bracadale, Loch Dunvegan, Loch Snizort, Loch Ainort, Kyle, Loch Sligachan, Scalpay
NWC-LochTorridon	G35	35, 37	**Loch Torridon**, Loch Toscaig, Loch Kishorn
NWC-Ullapool	G39	36, 39	**Loch Ewe**, Ullapool, Little Loch Broom
NWC-other	G48	38,47, 48, 49, 50, 51, 78, 110	**Loch Laxford**, Kylesku, Kyle of Tongue, Kinlochbervie,Lochinver, Enard Bay, Tain
Lewis-LochRoag	G23	23, 24, 102	**Loch Roag,** Loch Tamnabaigh
LewisHarrisUist	G21	21, 22, 25, 26, 77, 101	**Loch Leurbost**, Broad Bay, Killegray, Loch Ceann Dibig, Loch Seaforth, Loch Stockinish, Liernish, Loch Carnan, Loch Eport, Loch Eynort, Benbecula, Sound of Eriskay
Orkney	G54	54, 103, 104, 105, 106	**Orkney;** Bay of Firth, Burray, Hatston, Inganess, Mill Sands, Otterswick, Scapa Flow, Stromness
Shetland-SE	G57	57, 59, 60, 62, 63, 67	**Sandsound Voe**, Stromness Voe, Wadbister Voe, Catfirth, Clift Sound
Shetland-SW-Gruting	P61	61	**Braewick Voe, Browland Voe, Gruting/Seli Voe**
Shetland-SW-Vaila	P68	68	**Vaila Sound**
Shetland-W	G58	58, 72	**Clousta Voe, Vementry Voe**, Papa Little
Shetland-NW	G64	64, 70, 71, 79	**Busta Voe**, Olna Firth, Ronas Voe, Ura Firth
Shetland-NE	G56	56, 65, 66, 81, 82	**Dales Voe, Scarva Ayre**, Basta Voe, Whalefirth Voe, North + South Uyea, Mid Yell Voe
Shetland-N-Balta	P69	69	**Baltasound Voe**

[1]WC, West Coast; NWC, North West Coast; S, South; W, West; N, North; E, East.

Figure 1: Scotland with individual pods (indicated by their pod number) and grouping of pods (Figure 1b), where closed circles in red indicate individual pods (2 pods on Mull and 3 pods in Shetland), open circles, in blue, indicate pods that are grouped.

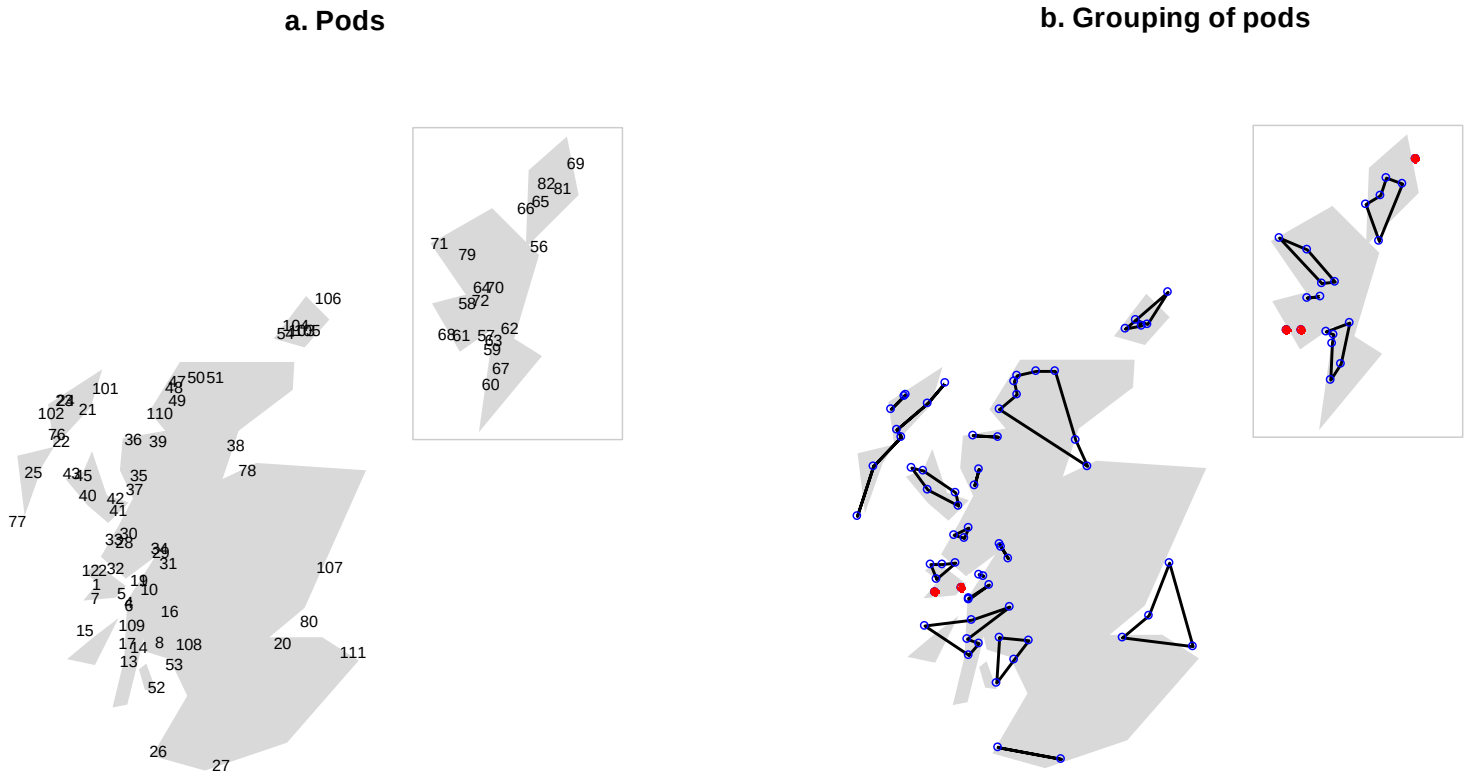


Table 2: Numbers of samples tested for each species, from Apr 2001 to Nov 2006.

		Mussels	Clams[1]	Cockles	King.scallops[2]	Pacific oysters	Queen scallops
# sites[3]		25	7	7	7	14	5
DSP	2001	665	4	15	20	83	41
	2002	612	6	8	9	99	15
	2003	760	29	21	19	156	29
	2004	624	15	8	8	64	38
	2005	427	8	8	5	60	18
	2006	778	8	2	0	155	21
	total	3866	70	62	61	617	162
PSP	2001	623	7	20	36	82	38
	2002	569	6	15	33	112	12
	2003	706	32	36	22	155	27
	2004	109	20	13	7	176	30
	2005	434	15	10	10	139	27
	2006	736	13	7	0	188	27
	total	3177	93	101	108	852	161
ASP	2001	627	9	23	44	77	39
	2002	582	7	14	47	117	14
	2003	728	30	29	47	164	25
	2004	574	22	11	13	157	20
	2005	541	19	11	11	162	38
	2006	739	11	2	2	217	39
	total	3791	98	90	164	894	175

[1]Clams: includes clams, razors, spisula and native oysters.
[2]DSP and PSP tested on whole king scallops, ASP tested on king scallop gonads.
[3]As defined in Table 1.

3.1.2 Prevalence of toxins in each species

DSP

During 2001 through to 2006, the largest percentages of positive DSP samples were observed in queen scallops and mussels (11% and 9%, respectively, Table 3). For pacific oysters less than 1.5% were positive (9 out of 617 samples), while for cockles all 62 samples tested negative. For clams and king scallops 2/70 and 1/61 samples were positive, respectively.

From the data it appears that the prevalence of DSP has decreased in recent years, with no clam, cockle, king scallop, pacific oyster or queen scallop samples testing positive for DSP during 2005 and 2006 (Table 3 and Figure 2a). In mussels, prevalence dropped from 20, 11 and 7% in 2001, 2002, 2003 down to 6 and 2% in 2004 and 2005, respectively, but increased again in 2006 to 9% (Figure 2a).

DSP in mussels tended to occur at most sites (Table 4), but prevalence varied from 1 to 21%, with the North West Coast (except for Loch Torridon), Ayr-LochStriven, WC-LochFyne, WC-Lochaber, Skye and Orkney all exceeding 15%.

PSP

None of the PSP test results for pacific oysters, queen scallops and king scallops led to field closure over the examined time period. For cockles, 1 sample in 2001 and 1 in 2006 resulted in field closure while for clams there were 2 samples in 2006 that exceeded field closure limits. In mussels, 1.5% of samples taken between 2001 and 2006 (47/3177) exceeded the closure limit, although no samples exceeded 80 μg/100g during 2004 and 2005. In 2006, levels of PSP in mussels started to increase again (Figure 2b) with 11/736 exceeding 80 μg/100g (Table 3). It was observed that PSP tended to be more prevalent during the summer months (Figure 2b).

PSP in mussels tended to vary over sites, with 7/25 sites having no positive PSP test results (Dumfries, WC-LochFyne, WC-LochEtive, WC-LochCreran, WC-LochLeven, Mull-LochSpelve and NWC-Ullapool), and with another 4 sites having test results below the field closure limit. All Shetland sites had positive test results at some time during 2001-2006 (Table 4).

ASP

ASP was a major problem in scallops (tested for gonads) during 2001-2003, with one-third of all samples tested exceeding the field closure limit of 20μg/g. For 2004, 2005 and 2006, however, only 10, 11 and 2 samples were tested respectively, with none of the 2005 and 2006 samples exceeding 20 μg/g. For the other shellfish species, ASP levels were also below field closure limit during these two years (Figure 2c). The only other two species that had test results over 20 μg/g were mussels (3/3791) and queen scallops (3/175), with all of these instances occurring during 2001-2003 (Table 3).

ASP was found in mussels at all 25 sites (pod groupings) applied during this study. Levels exceeding 10 μg/g were observed at 10 sites (Mull, WC-Lochaber, Outer Hebrides and 4 sites in Shetland). In king scallops all 7 sites from which this species were sampled tested positive, with 4 sites having results exceeding 20 μg/g (Table 4).

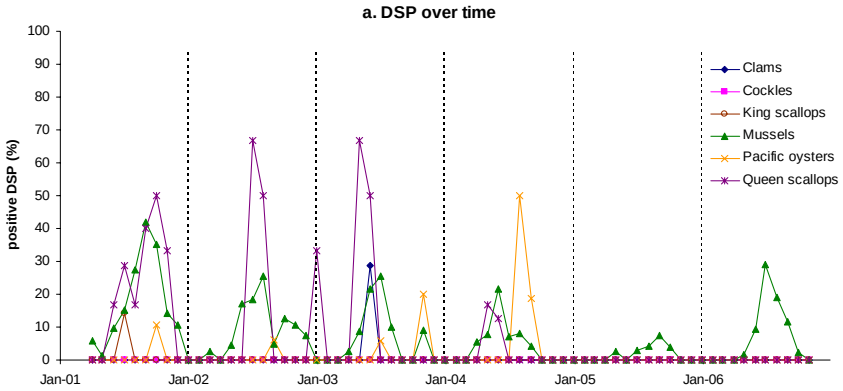
Of the three toxins, ASP tended to be least seasonal with toxic events occurring throughout the year (Figure 2c).

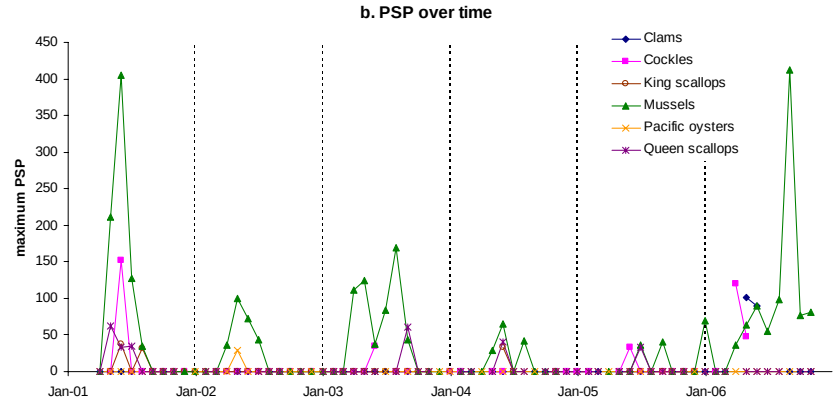
Table 3: Numbers of samples per toxin level for each species for each year. Values resulting in field closure are shown in bold.

		DSP		PSP category[1]				ASP category[2]			
		0	**1**	0	0-40	40-80	**80+**	0	0-10	10-20	**20+**
Clams[3]	2001	4		7				3	6		
	2002	6		6				2	5		
	2003	27	**2**	32				11	19		
	2004	15		20				9	13		
	2005	8		15				12	7		
	2006	8		8		3	**2**	11			
Cockles	2001	15		18		1	**1**	14	9		
	2002	8		15				6	8		
	2003	21		35	1			13	15	1	
	2004	8		13				6	5		
	2005	8		9	1			7	4		
	2006	2		4		2	**1**	2			
King scallops[4]	2001	19	**1**	32	4			2	23	8	**11**
	2002	9		33					25	12	**10**
	2003	19		22					9	7	**31**
	2004	8		6	1				7	3	**3**
	2005	5		10					8	3	
	2006								2		
Mussels	2001	533	**132**	552	20	23	**28**	323	303		**1**
	2002	547	**65**	551	5	11	**2**	211	369	1	**1**
	2003	708	**52**	681	7	12	**6**	314	407	6	**1**
	2004	587	**37**	102	3	4		229	341	4	
	2005	417	**10**	430	3	1		438	102	1	
	2006	708	**70**	668	18	39	**11**	661	78		
Pacific oysters	2001	81	**2**	82				42	35		
	2002	98	**1**	110	2			44	72	1	
	2003	154	**2**	155				77	87		
	2004	60	**4**	176				75	82		
	2005	60		139				139	23		
	2006	155		188				201	16		
Queen scallops	2001	32	**9**	35	2	1		8	30	1	
	2002	12	**3**	12				3	10	1	
	2003	25	**4**	26		1		1	21		**3**
	2004	36	**2**	29		1		6	14		
	2005	18		26	1			5	33		
	2006	21		27				29	10		

[1]Categories for PSP are 0 µg/100g; >0 and < 40 (denoted by 0-40); ≥ 40 and < 80 (denoted by 40-80); ≥ 80 (denoted by 80+, is also field closure limit).
[2]Categories for ASP are 0 µg/g; >0 and < 10 (denoted by 0-10); ≥ 10 and < 20 (denoted by 10-20); ≥ 20 (denoted by 20+, is also field closure limit).
[3]Clams: includes clams, razors, spisula and native oysters.
[4]DSP and PSP tested on whole king scallops, ASP tested on king scallop gonads.

Figure 2: Toxin patterns over time for each species. For DSP, the percentage of positive samples for each month is plotted (Fig 2a), for PSP and ASP the maximum observed toxicity is plotted for each month (Figs 2b and 2c). DSP and PSP in king scallops refers to the whole scallop, while for DSP the gonad test results have been used. Vertical lines indicate January of each year.





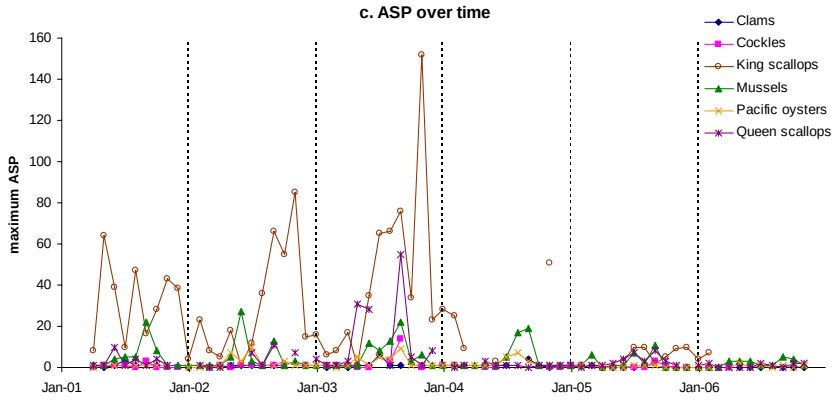


Table 4: Maximum toxin level for each shellfish toxin species and each site.

		Clams[1]			Cockles			King scallops[2]			Mussels			Pacific oysters			Queen scallops		
ID	GroupName	DSP	PSP	ASP	DSP	PSP	ASP	DSP	PSP	ASP	DSP	PSP	ASP	DSP	PSP	ASP	DSP	PSP	ASP
G80	Eastcoast	**1**	101	**1**	0	0	**1**				**1**	142	3						
G26	Dumfries	0	0	0	0	0	**1**				**1**	0	**1**						
G8	Ayr-LochStriven	0	0	**1**							**1**	124	**1**	**1**	0	**1**			
G16	WC-LochFyne	0	0	6				0	0	152	**1**	0	5	**1**	28	**1**	**1**	0	5
G10	WC-LochEtive										0	0	8	**1**	0	7		0	
G9	WC-LochCreran										**1**	0	5	**1**	0	5			
P5	Mull-LochSpelve										**1**	0	22		29	0			
P7	Mull-LochScridain										**1**	89	27						
G1	Mull-LochnaKeal								0	8	**1**	56	10	0	0	4			
G31	WC-LochLeven										**1**	0	**1**						
G28	WC-Lochaber	0	0	4	0	0	0	0	0	8	**1**	214	14	0	0	10			
G41	Skye	0	0	**1**	0	0	3	**1**	38	64	**1**	405	6	**1**	0	3	**1**	40	11
G35	NWC-LochTorridon										**1**	220	3		0	**1**			
G39	NWC-Ullapool							0	0	85	**1**	0	3						
G48	NWC-other				0	120	3				**1**	277	5	0	0	**1**			
G23	Lewis-LochRoag										**1**	127	22						
G21	LewisHarrisUist	0	0	4	0	35	14				**1**	38	17	0	0	3			
G54	Orkney				0	152	**1**				**1**	299	**1**	0	0	0			
G57	Shetland-SE							0		51	**1**	70	10						
P61	Shetland-SW-Gruting										**1**	33	17						
P68	Shetland-SW-Vaila										**1**	84	19						
G58	Shetland-W										**1**	321	6						
G64	Shetland-NW							0	34	10	**1**	413	6				**1**	62	55
G56	Shetland-NE										**1**	130	13	0	0	**1**	0	0	0
P69	Shetland-N-Balta										**1**	169	**1**	0	0	**1**			

[1]Clams: includes clams, razors, spisula and native oysters.
[2]DSP and PSP tested on whole king scallops, ASP tested on king scallop gonads.

3.2 Comparing models for 2001/4 versus 2004/6 mussel data

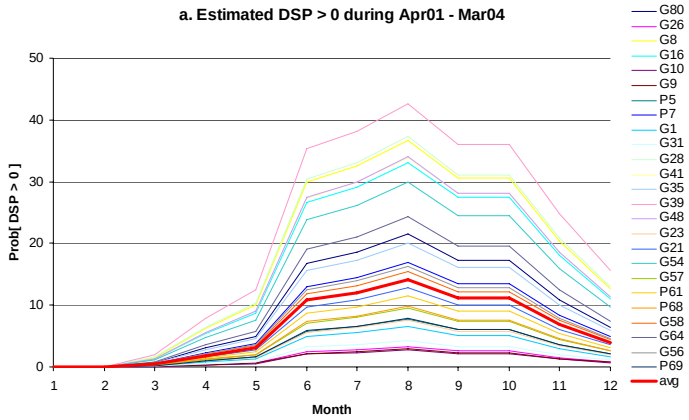
With the previous risk assessment, the April 2001 – March 2004 data (referred to as 2001/4 data) were analysed using Hierarchical Generalised Linear Models (HGLM, see Materials & Methods for details), where Site was regarded as a random effect and Month as a fixed effect. This resulted in an estimated probability of a sample being toxic (i.e. toxin levels exceed closure limit) for each site during each month of the year. As the grouping of sites has changed somewhat (only 25 as opposed to 33 previously), the 2001/4 mussel data were reanalysed using the new groupings. It was found that results were similar for both groupings. These models were then fitted to the data from April 2004 – November 2006 (referred to as 2004/6 data), and compared against the model results from the 2001/4 data. Results are described below.

3.2.1 Comparison of 2001/4 versus 2004/6 results

DSP

The raw data for DSP in mussels are presented as an Appendix (Figure A1). Figure 3 shows the estimated probability of mussel samples testing positive for DSP, for each site for each of 12 months of the year. Based on the 2001/4 data, high levels are predicted for June through to December, whereas for the 2004/6 data, the peak season appears to be much shorter, from June to September. The biggest difference is that for the second data set the predicted likelihood of DSP being positive is nearly zero for November and December, whereas for the 2001/4 data the corresponding likelihood ranges from zero to 20%, depending on the site. Furthermore, the prevalence over sites is also different, with five Shetland sites in the top ten toxic sites for 2004/6 whereas this was the case for only one Shetland site in 2001/4.

Figure 3: Estimated probability (%) of DSP in mussels testing positive for each site and each month using data from April 2001 to March 2004 (Fig 3a) and using data from April 2004 to November 2006 (Fig 3b).



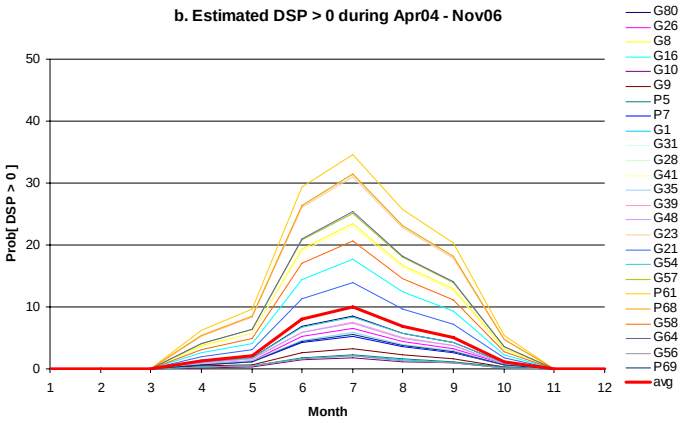
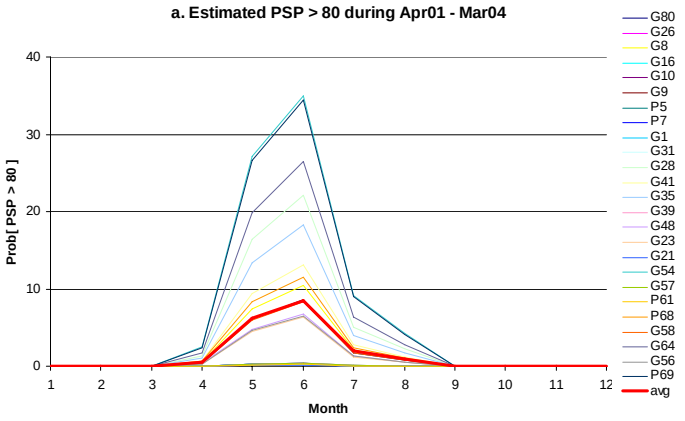
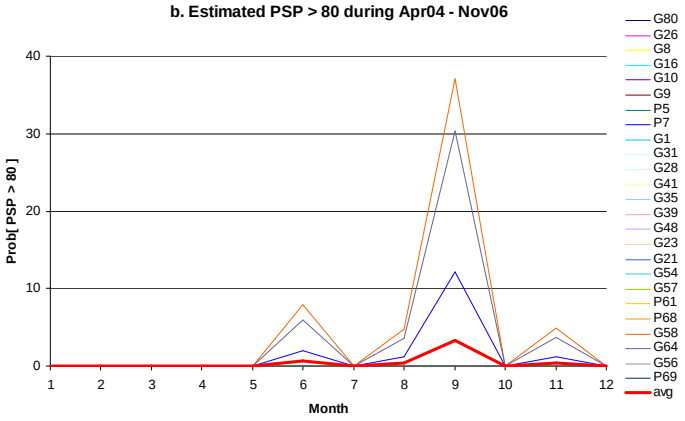


Figure 4: Estimated probability (%) of PSP levels in mussels exceeding field closure (80 µg/100g) for each site and each month using data from April 2001 to March 2004 (Fig 4a) and using data from April 2004 to November 2006 (Fig 4b).





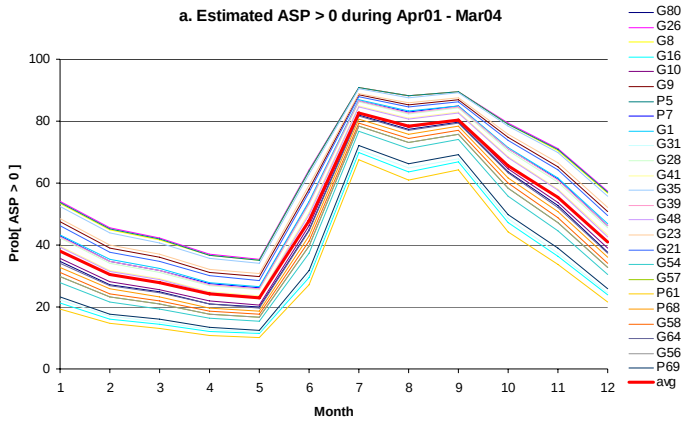
PSP

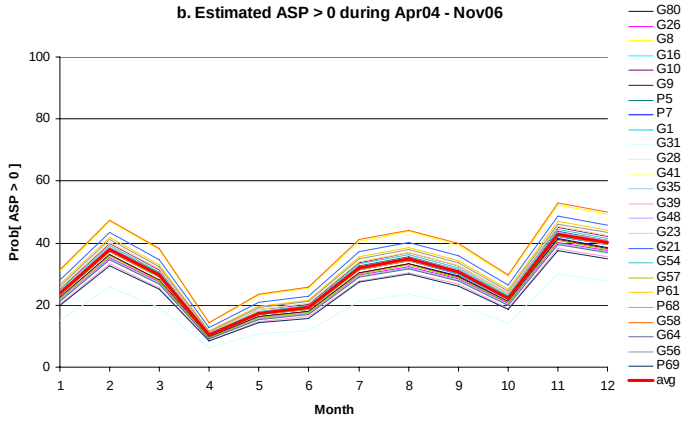
The raw data for PSP > 80 µg/100g in mussel flesh are shown in Appendix A (Figure A4). The predicted probability of PSP in mussels exceeding field closure limit (shown in Figure 4) is also different for the two data sets. For the earlier data, PSP was predicted to peak in early summer, whereas in the later data the main peak occurs in September. Furthermore, only 3 sites (Shetland-W, Shetland-NW and Mull-LochScridain) were predicted to have a probability exceeding 5% of field closure during the height of the season in 2004/6, whereas for the earlier data, 14 sites gave predicted toxin levels exceeding field closure.

ASP

The raw data for ASP > 0 µg/g in mussels are shown in Appendix A (Figure A5). The probability of testing positive (>0) for the 2001/4 data ranged from 40 to 90% during July – November, whereas for the 2004/6 data this probability was much more evenly distributed with time and did not exceed 50% (Figure 5). For the latter data, three of the Shetland sites (W, SW-Gruting and NE) were among the top 5 whereas in the 2001/4 data these sites only appeared in the bottom half of the ranking.

Figure 5: Estimated probability of ASP levels in mussels testing positive (>0 µg/g) for each site and each month using data from April 2001 to March 2004 (Fig 5a) and using data from April 2004 to November 2006 (Fig 5b).





3.2.2 Conclusions

From these initial statistical analyses it became clear that there are considerable differences between the two time periods.

- There appear to be differences between years, with some years having high levels of toxin detected and other years with low toxin levels (e.g. Figure 5, ASP in mussels).
- There also appear to be differences in toxin prevalence over time among sites, with some sites showing high prevalence in certain years and other sites showing high prevalence in other years (e.g. Figure 3, DSP in mussels).
- Toxin patterns during the year can vary, with sometimes a peak occurring in early summer and sometimes a peak occurring in late summer (e.g. Figure 4, PSP in mussels).

These findings, together with raw data plots (Figures 2, A1, A4 and A5) suggested that it was appropriate to analyse the 2001/6 data in a combined manner, allowing for random fluctuations between years.

3.3 Models for the 2001/6 data for PSP, DSP and ASP in mussels

3.3.1 PSP > 80 µg/100g in mussels

Following the model selection criteria described in Section 2.2.3 the following model described the PSP data best. It allowed for

- Random variation between sites, i.e. some sites are more likely for PSP levels to exceed field closure than others.
- Random variation between years, i.e. some years tend to have high prevalence of PSP while other years tend to have low prevalence.

Furthermore, the model contains a term to allow for modelling of toxicity for each month of the year. This is relevant for assessing and developing monitoring schemes, where the frequency of sampling may vary during the year.

Figure 6a shows the estimated probability of PSP in mussels exceeding field closure over time for each site. Two aspects are worth pointing out. First, the predicted pattern over the 12 months was the same for each year, but the actual levels differed, reflecting the random variation between years. For example, during 2001 there was a high chance of field closure, whereas for 2002 this was much lower, but the pattern of toxin occurrence (peak in May-June and secondary peak in September) was the same for both years. For 2004 and 2005 the estimated chance of exceeding 80µg/100g was practically zero, so the pattern was no longer prominent (enlarging this part of the graph would still show the same pattern as before, but at very low values). The second aspect is that some sites consistently showed a relatively low chance of exceeding field closure, while other sites consistently showed a relatively high probability of exceeding field closure. This is a consequence of allowing for variation between sites in the model and implies that the some sites consistently had an above average chance of PSP > 80µg/100g.

Figure 7a shows the estimated toxin pattern over the 12 months of the year, averaged over sites, in more detail. The predicted field closure (for an average site), shows a large peak in June and a smaller peak in September. Furthermore, the pattern of a peak in May-June and a second peak in September is the same as shown in Figure 6. The interpretation of Figure 7a is that, for a future year and an average site, the best prediction we can make for any given month is the one given by the bold red curve, showing 3-4.4% chance of exceeding field closure during May and June and 1.2% in September.

Looking at the individual sites in more detail, Table 5 summarises the estimated chance of field closure for each site and each month, averaged over 6 years. It shows that Orkney and the Shetland sites W, NW and N-Balta had the highest probability of PSP in mussels exceeding field closure, ranging from 10 to 20% during the peak month of June. If a prediction during the course of a future year for a given site were to be made, then Table 5 gives the best available estimate of the chance of PSP levels exceeding field closure levels.

Figure 6: Estimated probability (%) of PSP > 80 ug/100g (Fig 6a), PSP > 40 µg/100g (Fig 6b) and PSP > 0µg/100g (Fig 6c) for each site.

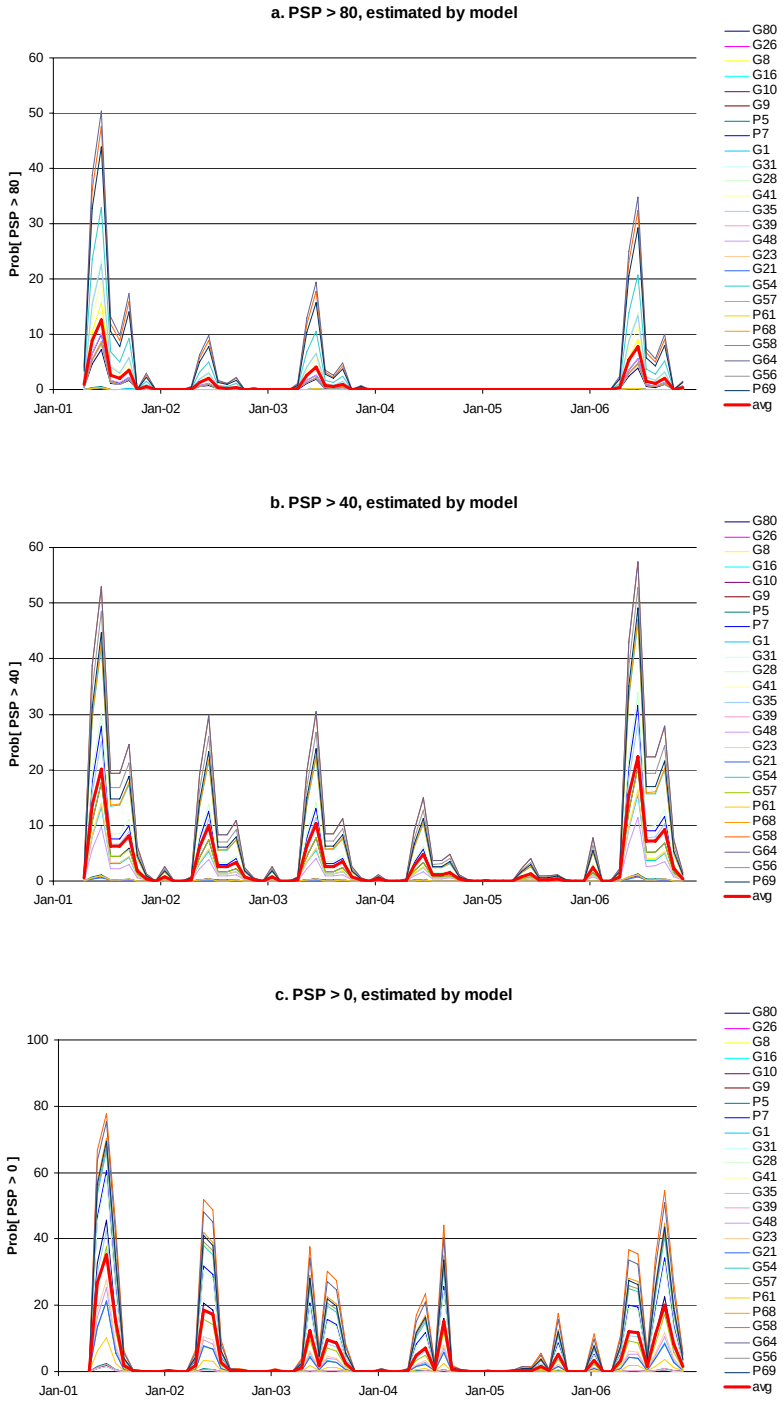


Figure 7: Estimated probability (%) of PSP in mussels exceeding field closure limit, 40µg/100g and exceeding 0 µg/100g, for each of 6 years.

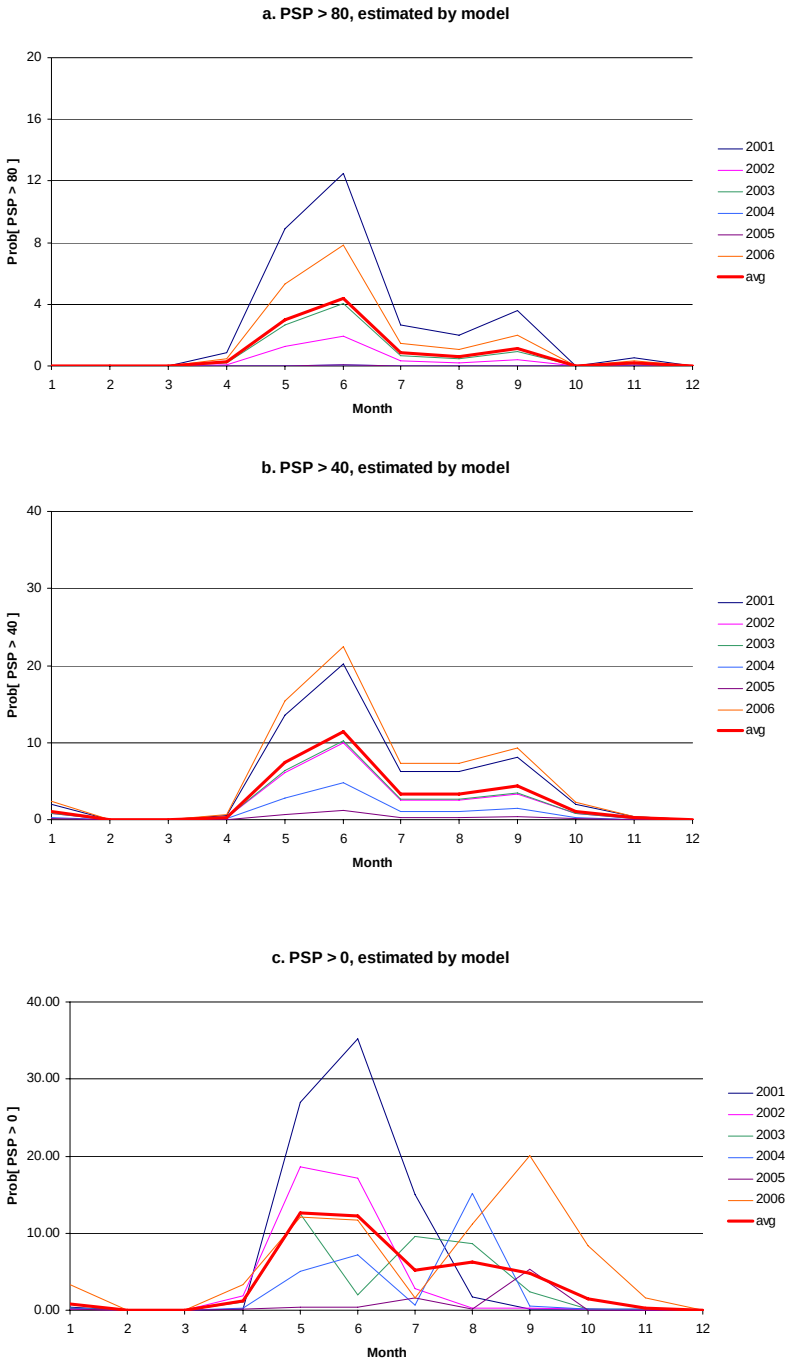


Table 5: Estimated[1] probability (%) that PSP levels in mussels exceed 80µg/100g, for each site per month, averaged over 6 years. The value 0% represents a small positive number having a value of less than 0.5%. Probabilities of 1% and higher are shown in bold.

			Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec
ID	Site	Avg[2]	0	0	0	0.3	**3.0**	**4.4**	0.9	0.6	**1.2**	0	0.2	0
G80	Eastcoast	0.4	0	0	0	0.1	**1.5**	**2.3**	0.4	0.3	0.5	0	0.1	0
G26	Dumfries	0.0	0	0	0	0	0.1	0.1	0	0	0	0	0	0
G8	Ayr-LochStriven	1.0	0	0	0	0.3	**3.4**	**5.2**	0.9	0.6	**1.2**	0	0.2	0
G16	WC-LochFyne	0	0	0	0	0	0.1	0.1	0	0	0	0	0	0
G10	WC-LochEtive	0	0	0	0	0	0.1	0.1	0	0	0	0	0	0
G9	WC-LochCreran	0	0	0	0	0	0.1	0.1	0	0	0	0	0	0
P5	Mull-LochSpelve	0	0	0	0	0	0.1	0.2	0	0	0	0	0	0
P7	Mull-LochScridain	0.6	0	0	0	0.2	**2.1**	**3.2**	0.5	0.4	0.7	0	0.1	0
G1	Mull-LochnaKeal	0	0	0	0	0	0.1	0.1	0	0	0	0	0	0
G31	WC-LochLeven	0	0	0	0	0	0.1	0.1	0	0	0	0	0	0
G28	WC-Lochaber	**1.5**	0	0	0	0.4	**5.2**	**7.9**	**1.4**	**1.0**	**1.9**	0	0.3	0
G41	Skye	**1.2**	0	0	0	0.3	**4.3**	**6.6**	**1.1**	0.8	**1.5**	0	0.2	0
G35	NWC-LochTorridon	**1.4**	0	0	0	0.4	**5.0**	**7.6**	**1.3**	1.0	**1.8**	0	0.3	0
G39	NWC-Ullapool	0	0	0	0	0	0.1	0.1	0	0	0	0	0	0
G48	NWC-other	0.6	0	0	0	0.2	**2.1**	**3.3**	0.5	0.4	0.7	0	0.1	0
G23	Lewis-LochRoag	0.5	0	0	0	0.1	**1.7**	**2.6**	0.4	0.3	0.6	0	0.1	0
G21	LewisHarrisUist	0	0	0	0	0	0.0	0.1	0	0	0	0	0	0
G54	Orkney	**2.3**	0	0	0	0.7	**7.9**	**11.6**	**2.2**	**1.6**	**3.0**	0	0.5	0
G57	Shetland-SE	0	0	0	0	0	0.1	0.1	0	0	0	0	0	0
P61	Shetland-SW-Gruting	0	0	0	0	0	0.1	0.1	0	0	0	0	0	0
P68	Shetland-SW-Vaila	0.5	0	0	0	0.1	**1.8**	**2.8**	0.4	0.3	0.6	0	0.1	0
G58	Shetland-W	**3.7**	0	0	0	**1.2**	**12.8**	**17.8**	**3.9**	**2.9**	**5.2**	0	0.8	0
G64	Shetland-NW	**4.0**	0	0	0	**1.4**	**13.9**	**19.1**	**4.3**	**3.2**	**5.7**	0	0.9	0
G56	Shetland-NE	0.5	0	0	0	0.1	**1.7**	**2.7**	0.4	0.3	0.6	0	0.1	0
P69	Shetland-N-Balta	**3.3**	0	0	0	**1.1**	**11.5**	**16.2**	**3.4**	**2.5**	**4.6**	0	0.7	0

[1]From HGLM with Site and Year as random effects and Month as fixed effect.
[2]For each site the average probability over 12 months over 6 years was calculated, and for each month the probability over all sites over 6 years was calculated.

3.3.2 PSP > 40 µg/100g in mussels

Based on the raw data collated in Appendix A (Figure A3), the model that gave the best fit is the same as for PSP 80µg/100g, i.e. allowing for random variation between years and sites. Figure 6b shows the estimated probability of mussel samples exceeding 40µg/100g over time for each site. The pattern is similar to that observed for the probability of PSP exceeding field closure, except that the probability tended to be somewhat higher.

Figure 7b shows the estimated probability of mussel samples exceeding 40µg/100g over 12 months in more detail. When compared to the probability of exceeding field closure limit (Figure 7a) the patterns were similar for 2001-3 and 2006, except that the proportion is (obviously) higher for PSP > 40 µg/100g. When predicting the toxin pattern for a future year (for an average site), the best prediction that can be given is given by the bold red line, i.e. 8- 11% chance of a positive sample in May and June, with 4-5% chance for July-September.

When looking at individual sites (Table 6) it can be seen that five out of the seven Shetland sites had a relatively high estimated chance of exceeding 40µg/100g. During the peak months of May and June this probability reached 16 to 32%, and stayed at 7% or above during July – September. Skye and Orkney tended to have high probabilities as well, with up to 16% of the samples estimated to be positive during May.

Table 6: Estimated[1] probability (%) that PSP levels in mussels exceed 40µg/100g, for each site per month, averaged over 6 years. The value 0% represents a small positive number having a value of less than 0.5%. Probabilities of 1% and higher are shown in bold.

ID	Site	Avg[2]	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec
			1	0	0	0	**8**	**11**	**3**	**3**	**4**	**1**	0	0
G80	Eastcoast	**2**	1	0	0	0	**6**	**10**	**2**	**2**	**3**	1	0	0
G26	Dumfries	0	0	0	0	0	0	0	0	0	0	0	0	0
G8	Ayr-LochStriven	**2**	1	0	0	0	**4**	**8**	**2**	**2**	**2**	1	0	0
G16	WC-LochFyne	0	0	0	0	0	0	0	0	0	0	0	0	0
G10	WC-LochEtive	0	0	0	0	0	0	0	0	0	0	0	0	0
G9	WC-LochCreran	0	0	0	0	0	0	0	0	0	0	0	0	0
P5	Mull-LochSpelve	0	0	0	0	0	0	1	0	0	0	0	0	0
P7	Mull-LochScridain	**3**	**1**	0	0	0	**10**	**15**	**4**	**4**	**5**	**1**	0	0
G1	Mull-LochnaKeal	**2**	0	0	0	0	**4**	**7**	**2**	**2**	**2**	0	0	0
G31	WC-LochLeven	0	0	0	0	0	0	0	0	0	0	0	0	0
G28	WC-Lochaber	**4**	**1**	0	0	0	**11**	**17**	**4**	**4**	**6**	**1**	0	0
G41	Skye	**6**	**2**	0	0	1	**16**	**24**	**7**	**7**	**10**	**2**	0	0
G35	NWC-LochTorridon	**3**	**1**	0	0	0	**8**	**14**	**4**	**4**	**5**	**1**	0	0
G39	NWC-Ullapool	0	0	0	0	0	0	0	0	0	0	0	0	0
G48	NWC-other	**1**	0	0	0	0	**3**	**5**	**1**	**1**	**2**	0	0	0
G23	Lewis-LochRoag	**2**	1	0	0	0	**4**	**7**	**2**	**2**	**2**	0	0	0
G21	LewisHarrisUist	0	0	0	0	0	0	0	0	0	0	0	0	0
G54	Orkney	**6**	**2**	0	0	1	**16**	**24**	**7**	**7**	**9**	**2**	0	0
G57	Shetland-SE	**2**	1	0	0	0	**6**	**10**	**2**	**2**	**3**	1	0	0
P61	Shetland-SW-Gruting	0	0	0	0	0	0	1	0	0	0	0	0	0
P68	Shetland-SW-Vaila	**6**	**2**	0	0	1	**16**	**25**	**7**	**7**	**10**	**2**	0	0
G58	Shetland-W	**8**	**3**	0	0	1	**22**	**32**	**11**	**11**	**13**	**3**	1	0
G64	Shetland-NW	**8**	**3**	0	0	1	**22**	**32**	**11**	**11**	**13**	**3**	1	0
G56	Shetland-NE	**7**	**3**	0	0	1	**19**	**28**	**9**	**9**	**12**	**3**	1	0
P69	Shetland-N-Balta	**6**	**3**	0	0	1	**17**	**26**	**8**	**8**	**10**	**2**	0	0

[1]From HGLM with Site and Year as random effects and Month as fixed effect.

[2]For each site the average probability over 12 months over 6 years was calculated, and for each month the probability over all sites over 6 years was calculated.

3.3.3 Positive PSP test result in mussels

Only 1 to 4 % of the mussel samples exceeded toxin levels of 40µg/100g, offering limited scope to investigate the various sources of random variation. For PSP testing positive (i.e. > 0µg/100g), there was more scope to investigate such sources as over 6% of the samples tested positive (Table 3, Figure A2). The following model described the probability of a positive PSP test result best. In addition to a month effect allowing for modelling of toxicity for each month of the year, it also allowed for

- Random variation between sites, i.e. some sites tend to have a high chance of a positive PSP test result, while other sites tend to have a low chance.
- Random variation between years, i.e. some years tend to have high prevalence of PSP while other years tend to have low prevalence.
- Year by month interaction. This allows for the month effect to vary over years, i.e. it allows the toxin pattern over the 12 months to be different for each year, so that for some years a peak may occur in early summer, say, whereas for other years a peak may occur in late summer.

Figure 6c shows the estimated probability of positive PSP samples over time. It shows that there is random variation between sites, with some sites consistently being more likely to have positive PSP samples than others. It was also noted that there was variation between years, with 2001 showing high probability of positive samples while in 2005 this probability was low. Finally, it was also observed that the pattern of probability within a year could differ across years, e.g. 2001 and 2002 had one peak only whereas during 2003 – 2006 there were two peaks. This is a reflection of the year by month interaction included in the model.

Figure 7c shows the estimated prevalence of mussel samples that tested positive for PSP over 12 months in more detail. From this graph it can be seen that the estimated probability of mussels testing positive for PSP was similar to that for PSP > 80 (Figure 7a) and PSP > 40µg/100g (Figure 7b), except that the predicted chance of PSP > 0 µg/100g is greater. The model allowed for the toxin pattern to be different for each year. When predicting the toxin pattern for a future year (for an average site), the best prediction that can be made is given by the bold red line, i.e. up to 13% chance of a positive sample for May and June, with 5-6% chance for July-September.

For individual sites (Table 7) the pattern was similar to that observed for PSP > 40µg/100g (Table 6).

Table 7: Estimated[1] probability (%) that PSP levels in mussels tested positive (>0µg/100g), for each site per month, averaged over 6 years. The value 0% represents a small positive number having a value of less than 0.5%. Probabilities of 10% and higher are shown in bold.

			Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec
ID	Site	Avg[2]	1	0	0	1	**13**	**12**	5	6	5	1	0	0
G80	Eastcoast	4	1	0	0	1	**14**	**14**	5	6	5	1	0	0
G26	Dumfries	0	0	0	0	0	0	1	0	0	0	0	0	0
G8	Ayr-LochStriven	1	0	0	0	0	5	6	2	2	2	0	0	0
G16	WC-LochFyne	0	0	0	0	0	0	1	0	0	0	0	0	0
G10	WC-LochEtive	0	0	0	0	0	0	0	0	0	0	0	0	0
G9	WC-LochCreran	0	0	0	0	0	0	0	0	0	0	0	0	0
P5	Mull-LochSpelve	0	0	0	0	0	1	1	0	0	0	0	0	0
P7	Mull-LochScridain	6	1	0	0	2	**21**	**21**	9	**10**	8	2	1	0
G1	Mull-LochnaKeal	2	0	0	0	0	5	6	2	2	2	0	0	0
G31	WC-LochLeven	0	0	0	0	0	0	0	0	0	0	0	0	0
G28	WC-Lochaber	6	1	0	0	2	**20**	**20**	8	10	8	2	0	0
G41	Skye	8	2	0	0	2	**26**	**25**	**11**	**13**	10	3	1	0
G35	NWC-LochTorridon	4	1	0	0	1	**12**	**13**	5	6	5	1	0	0
G39	NWC-Ullapool	0	0	0	0	0	0	1	0	0	0	0	0	0
G48	NWC-other	2	0	0	0	0	6	7	2	3	2	1	0	0
G23	Lewis-LochRoag	2	0	0	0	0	7	8	3	3	3	1	0	0
G21	LewisHarrisUist	2	0	0	0	0	5	6	2	2	2	0	0	0
G54	Orkney	8	2	0	0	2	**26**	**24**	**11**	**13**	10	3	1	0
G57	Shetland-SE	3	0	0	0	1	**11**	**11**	4	5	4	1	0	0
P61	Shetland-SW-Gruting	1	0	0	0	0	2	3	1	1	1	0	0	0
P68	Shetland-SW-Vaila	8	2	0	0	3	**28**	**27**	**12**	**14**	**11**	4	1	0
G58	Shetland-W	**11**	3	0	0	4	**35**	**32**	**16**	**19**	**14**	5	1	0
G64	Shetland-NW	10	2	0	0	3	**33**	**30**	**15**	**17**	**13**	4	1	0
G56	Shetland-NE	8	2	0	0	2	**26**	**25**	**11**	**13**	**10**	3	1	0
P69	Shetland-N-Balta	8	2	0	0	3	**28**	**26**	**12**	**14**	**11**	3	1	0

[1]From HGLM with Site, Year and Year by Month interaction as random effects and Month as fixed effect.
[2]For each site the average probability over 12 months over 6 years was calculated, and for each month the probability over all sites over 6 years was calculated.

3.3.4 Comparison of the three PSP toxin level ranges (>0, >40, > 80 µg/100g)

Figure 8 compares the estimated chance of PSP levels exceeding 0, 40 and 80µg/100g throughout a year, for an average year and average site, based on the six years worth of data (2001-2006). The predicted pattern was similar for each of the toxin levels, except that the chance of PSP testing positive was greater than that of PSP exceeding 40µg/100g, which in turn was greater than the chance of PSP levels being more than 80µg/100g. Figure 9 summarises the average prevalence of PSP across sites, showing hat it is most prevalent in Shetland. From these comparisons we can conclude that the three statistical analyses are coherent and are in agreement with what we would expect from biology.

Figure 8: Comparison of the estimated probability (%) of PSP exceeding various toxin levels.

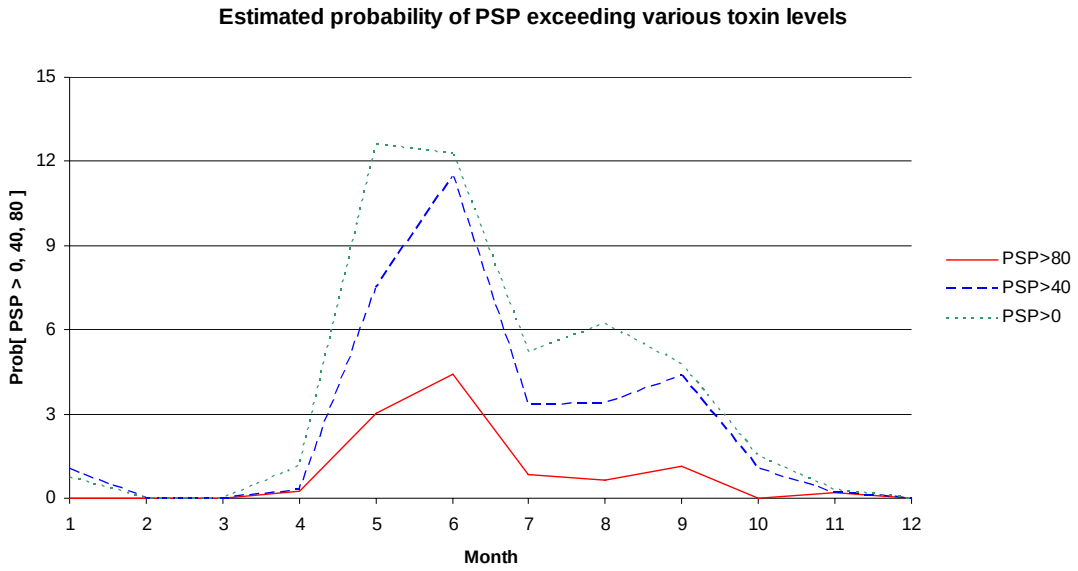
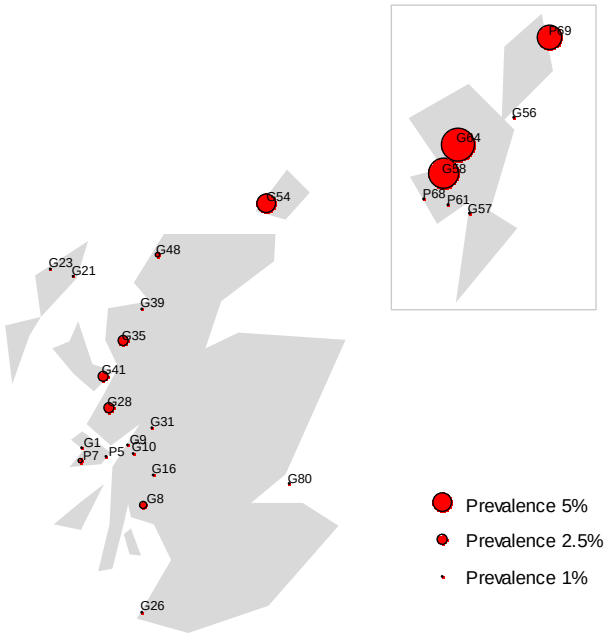
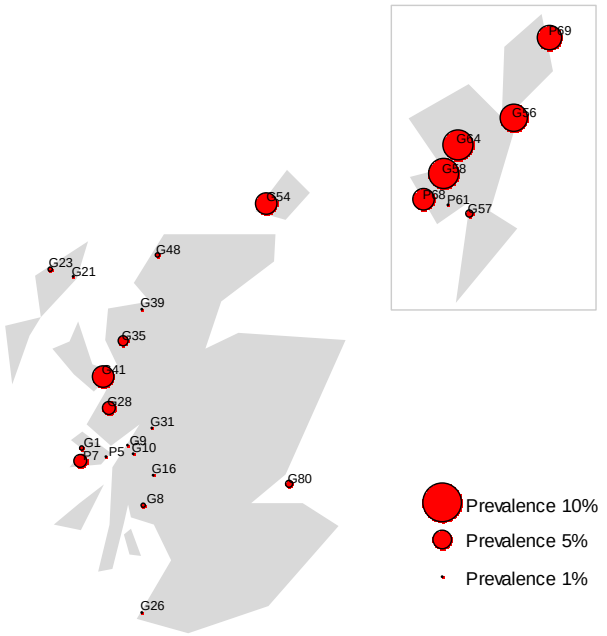


Figure 9: Estimated prevalence (%) of PSP in mussels (PSP > 80, PSP > 40, PSP > 0 in Figures 9a, 9b and 9c, respectively), for an average year and an average month. Although the outline of Shetland is shown enlarged, to keep findings compatible the sizes of the symbols are the same as for mainland Scotland.

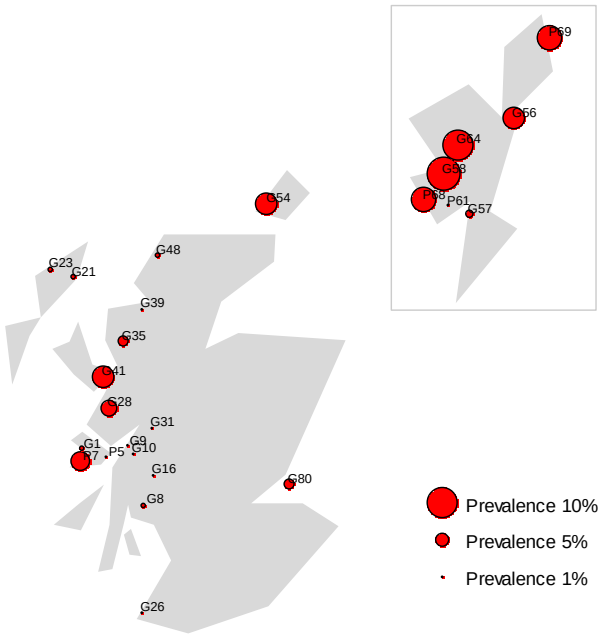
a. Average prevalence PSP > 80 in mussels



b. Average prevalence PSP > 40 in mussels



c. Average prevalence PSP > 0 in mussels



3.3.5 Positive DSP test result in mussels

The DSP test results from mussels allowed for detailed modelling of effects of Month, Year, Site and various interactions as both positive and negative samples were found across many sites, months and years. As a consequence, the following model described the probability of a positive DSP test result best. In addition to a month effect allowing for modelling of toxicity for each month of the year, it also allowed for

- Random variation between sites, i.e. some sites tend to have a high chance of a positive DSP test result, while other sites tend to have a low chance.
- Random variation between years, i.e. some years tend to have high prevalence of DSP while other years tend to have low prevalence.
- Year by month interaction. This allows for the month effect to vary over years, i.e. it allows the toxin pattern over the 12 months to be different for each year, so that for some years a peak may occur in early summer, say, whereas for other years a peak may occur in late summer.

These three terms are the same as in the model for PSP > 0 in mussels. In addition, however, the following term was also included for the DSP results:

- Year by site interaction. This allows for the year effect to vary over sites.

Figure 10 shows the estimated probability of positive DSP over time. There was variation between sites, e.g. in 2004 there were sites low prevalence (5% or less) but there were also sites with high prevalence, up to 60% in late summer. This is also demonstrated in Figure 11. There was also variation between years, with DSP less likely to occur in 2005. The pattern within a year changed from year to year (year by month interaction), with e.g. a peak in early summer of 2004 whereas in 2005 the

main peak occurred later in the year. Finally, the variation between sites varied over time. An example of this is WC-LochFyne (G16) which in 2003 had the highest prevalence of all sites, whereas in e.g. 2006 it was below average. This is a reflection of the year by site interaction that was allowed for in the model.

Figure 10: Estimated probability (%) of positive DSP in mussels, for each site.

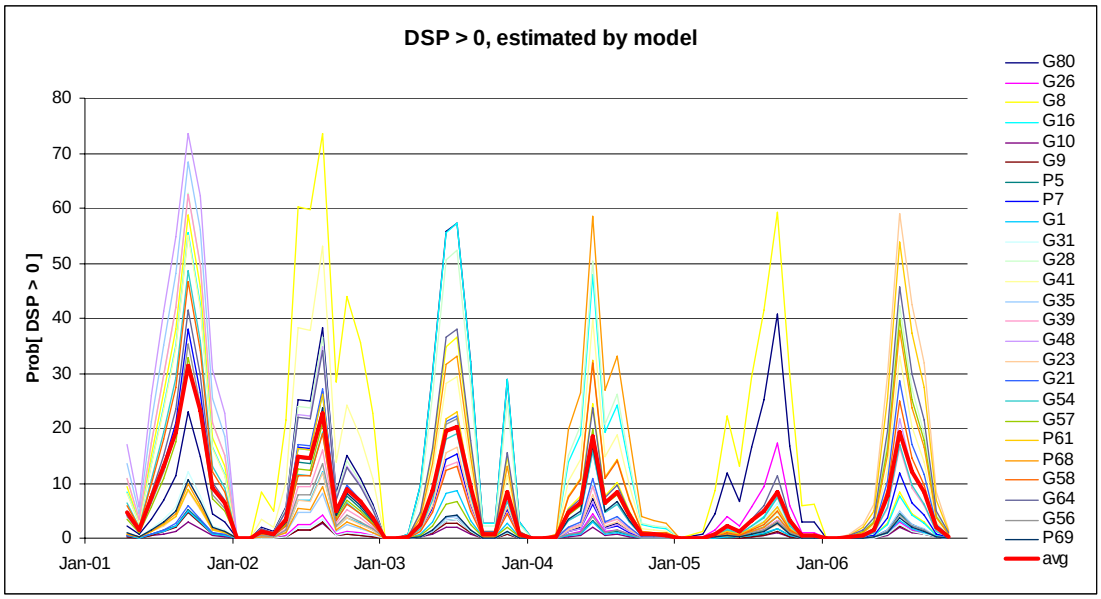


Figure 11: Estimated prevalence (%) of DSP in mussels, for an average year and an average month. Although the outline of Shetland is shown enlarged, to keep findings compatible the sizes of the symbols are the same as for mainland Scotland.

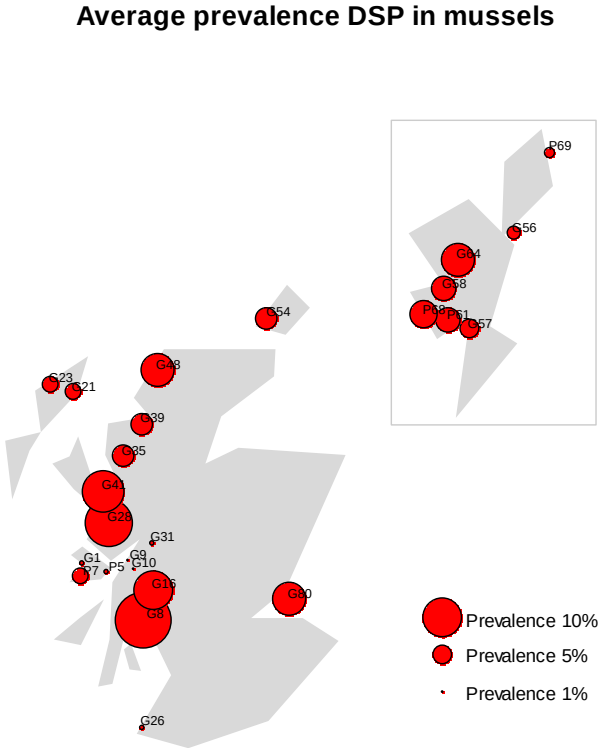


Figure 12 shows the estimated probability of positive mussel samples for each month for each of 6 years. For 2001 there was a peak in late summer, which was also the case for 2005, albeit to a lesser extent. For the remaining years DSP tended to peak earlier in the summer. Prevalence was predicted to be highest during June-September, exceeding 10% for an average year and average site. Table 8 shows a further breakdown by site. DSP tended to occur at the majority of sites, with only Dumfries, WC-LochEtive, WC-LochCreran, Mull-LochSpelve, Mull-Lochnakael and WC-Lochleven staying below 5% all year round. Furthermore, Table 9 gives the average prevalence per site for each year. It shows how e.g. Lewis-LochRoag had relatively low prevalence in 2001-2 and relatively high prevalence in 2006, reflecting a year by site interaction. If we want to make a prediction of DSP prevalence for a given site during the course of a future year then the information provided in Table 8 can be used.

Figure 12: Estimated probability (%) of DSP in mussels testing positive for each of 6 years.

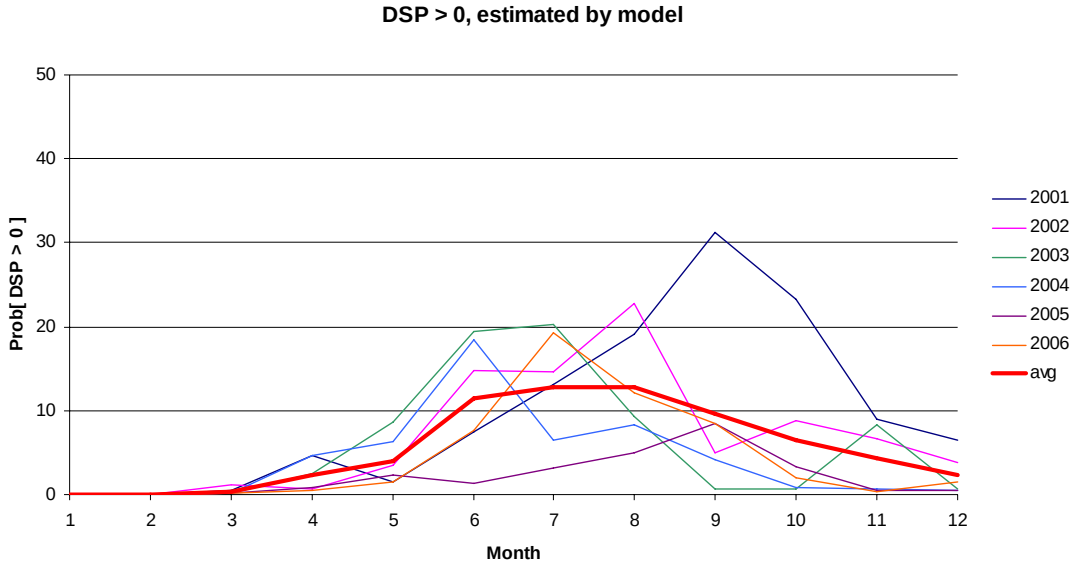


Table 8: Estimated[1] probability (%) that DSP levels in mussels tested positive, for each site per month, averaged over 6 years. The value 0% represents a small positive number having a value of less than 0.5%. Probabilities of 10% and higher are shown in bold.

ID	Site	avg[2]	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec
			0	0	0	2	4	**12**	**13**	**13**	10	6	4	2
G80	Eastcoast	8	0	0	1	3	8	**17**	**19**	**19**	**13**	8	8	3
G26	Dumfries	1	0	0	0	0	1	2	3	3	4	2	1	0
G8	Ayr-LochStriven	**14**	0	0	2	5	**12**	**25**	**28**	**31**	**26**	**20**	**13**	7
G16	WC-LochFyne	10	0	0	1	6	9	**23**	**21**	**21**	**14**	10	9	3
G10	WC-LochEtive	0	0	0	0	0	0	1	1	1	1	1	0	0
G9	WC-LochCreran	1	0	0	0	0	1	3	2	2	1	1	0	0
P5	Mull-LochSpelve	2	0	0	0	0	1	4	4	5	2	2	1	1
P7	Mull-LochScridain	4	0	0	0	1	2	8	10	**11**	9	6	4	2
G1	Mull-LochnaKeal	1	0	0	0	0	1	3	4	4	2	1	1	0
G31	WC-LochLeven	1	0	0	0	1	1	3	3	3	4	2	1	0
G28	WC-Lochaber	**12**	0	0	1	6	10	**27**	**28**	**27**	**18**	**12**	10	5
G41	Skye	**10**	0	0	1	4	7	**22**	**23**	**24**	**16**	**12**	8	5
G35	NWC-LochTorridon	5	0	0	0	3	2	8	9	**11**	**13**	**10**	5	3
G39	NWC-Ullapool	6	0	0	0	2	2	9	**12**	**13**	**13**	10	5	3
G48	NWC-other	8	0	0	1	4	4	**14**	**18**	**19**	**16**	**13**	8	5
G23	Lewis-LochRoag	5	0	0	0	1	3	**10**	**15**	**11**	8	3	2	2
G21	LewisHarrisUist	4	0	0	0	1	3	**10**	**12**	**10**	5	3	3	1
G54	Orkney	6	0	0	0	2	3	**10**	**12**	**13**	**12**	8	4	2
G57	Shetland-SE	5	0	0	0	2	3	**10**	**13**	**13**	**10**	6	3	2
P61	Shetland-SW-Gruting	6	0	0	0	2	5	**16**	**18**	**16**	9	4	3	2
P68	Shetland-SW-Vaila	7	0	0	0	4	7	**19**	**18**	**15**	9	3	3	2
G58	Shetland-W	6	0	0	0	3	4	**12**	**13**	**14**	**12**	8	4	2
G64	Shetland-NW	9	0	0	1	3	6	**19**	**22**	**20**	**15**	9	6	3
G56	Shetland-NE	4	0	0	0	1	2	7	8	8	7	5	3	1
P69	Shetland-N-Balta	2	0	0	0	1	2	6	5	7	4	3	2	1

[1]From HGLM with Site, Year, Year by Month and Year by Site as random effects and Month as fixed effect.

[2]For each site the average probability over 12 months over 6 years was calculated, and for each month the probability over all sites over 6 years was calculated.

Table 9: Estimated[1] probability (%) that DSP levels in mussels tested positive, for each site and each year, averaged over 12 months. The value 0% represents a small positive number having a value of less than 0.5%. Probabilities of 10% and higher are shown in bold.

ID	Site	avg[2]	2001 10	2002 7	2003 6	2004 4	2005 2	2006 4
G80	Eastcoast	8	6	**11**	**19**	1	**11**	1
G26	Dumfries	1	1	1	1	1	4	1
G8	Ayr-LochStriven	**14**	**19**	**30**	**11**	5	**18**	2
G16	WC-LochFyne	10	**18**	7	**19**	**12**	2	2
G10	WC-LochEtive	0	1	1	1	0	0	0
G9	WC-LochCreran	1	1	1	1	2	0	0
P5	Mull-LochSpelve	2	1	6	1	1	0	1
P7	Mull-LochScridain	4	**11**	7	4	1	1	2
G1	Mull-LochnaKeal	1	1	3	2	1	0	1
G31	WC-LochLeven	1	3	1	1	2	1	0
G28	WC-Lochaber	**12**	**21**	**11**	**16**	**13**	1	10
G41	Skye	**10**	**17**	**18**	8	9	1	7
G35	NWC-LochTorridon	5	**24**	2	1	3	1	1
G39	NWC-Ullapool	6	**21**	4	4	2	1	3
G48	NWC-other	9	**28**	**10**	6	1	1	5
G23	Lewis-LochRoag	5	2	3	4	2	1	**16**
G21	LewisHarrisUist	4	1	8	6	2	1	6
G54	Orkney	6	**15**	5	5	3	2	4
G57	Shetland-SE	5	9	6	2	4	1	9
P61	Shetland-SW-Gruting	6	2	7	6	7	1	**14**
P68	Shetland-SW-Vaila	7	2	2	9	**16**	1	9
G58	Shetland-W	6	**14**	5	3	7	1	5
G64	Shetland-NW	9	**12**	10	**11**	5	3	**11**
G56	Shetland-NE	4	10	4	6	1	1	1
P69	Shetland-N-Balta	3	3	7	1	3	1	1

[1]From HGLM with Site, Year, Year by Month and Year by Site as random effects and Month as fixed effect.
[2]For each site the average probability over 12 months over 6 years was calculated, and for each year the probability over all sites over 12 months was calculated.

3.3.6 Positive ASP test result in mussels

As there were only 3 mussel samples exceeding 20µg/g, and 17 samples exceeding 10 µg/g, these two toxin levels were not modelled. Forty-three percent of the mussel samples tested positive for ASP (i.e. ASP>0 µg/g). It was found that the following model described the probability of a positive ASP test result best. In addition to a month-term to allow for modelling of toxicity for each month of the year, it also allowed for

- Random variation between sites, i.e. some sites tend to have a high chance of a positive ASP test result, while other sites tend to have a low chance.
- Random variation between years, i.e. some years tend to have high prevalence of ASP while other years tend to have low prevalence.
- Year by month interaction. This allows for the year effect to vary over months, i.e. it allows the toxin pattern over the 12 months to be different for each year, so that for some years a peak may occur in early summer, say, whereas for other years a peak may occur in late summer.

Note that this model is the same as for PSP > 0.

Figure 13 shows the estimated probability of testing positive for ASP over time. The estimated prevalence was much lower for 2005 and 2006. Furthermore, prevalence across sites was estimated to be similar (see also Figure 14).

Figure 15 shows the estimated percentage of mussel samples testing positive for ASP, over each of 6 years. For 2001-4 the pattern was rather similar, whereas for 2005 the percentage of samples testing positive was much lower, and in 2006 this percentage came down to almost zero. Towards the end of 2006, however, levels started to increase again.

When looking at each site and each month (Table 10), it can be seen that all sites had a high chance of positive ASP test results. Furthermore, on average, the likelihood of ASP was lowest during the first half of the year, with probabilities below 34%.

Figure 13: Estimated probability (%) of positive ASP in mussels, for each site.

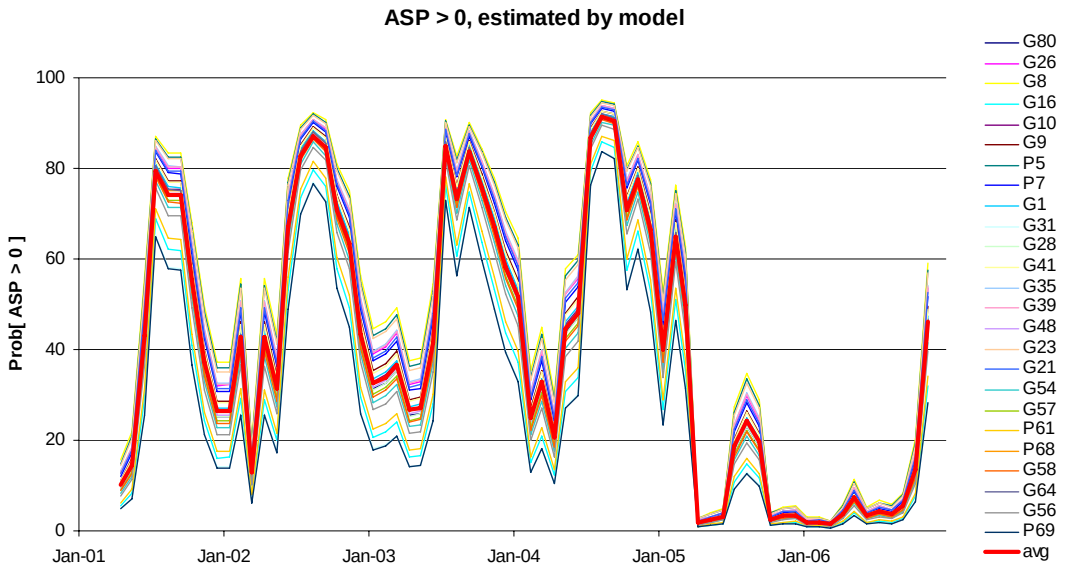


Figure 14: Estimated prevalence (%) of positive ASP in mussels, for an average year and an average month. Although the outline of Shetland is shown enlarged, to keep findings compatible the sizes of the symbols are the same as for mainland Scotland.

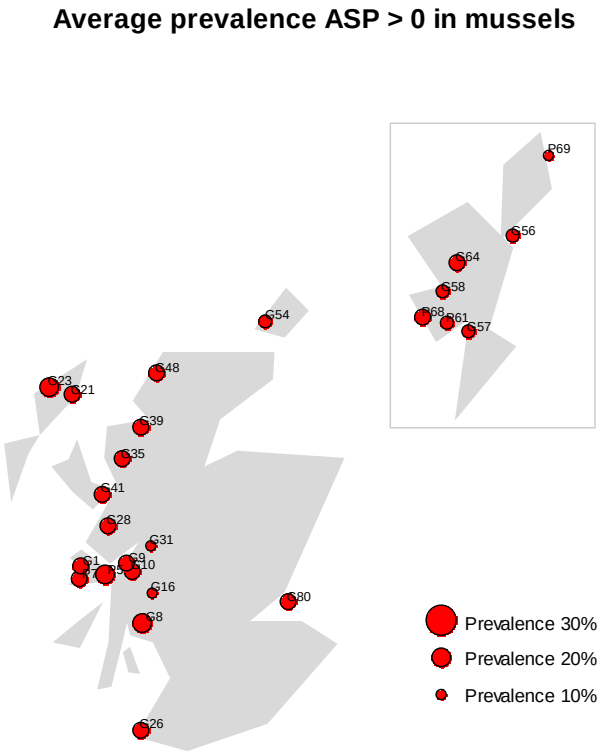


Figure 15: Estimated probability (%) of ASP in mussels testing positive (> 0µg/g), for each of 6 years.

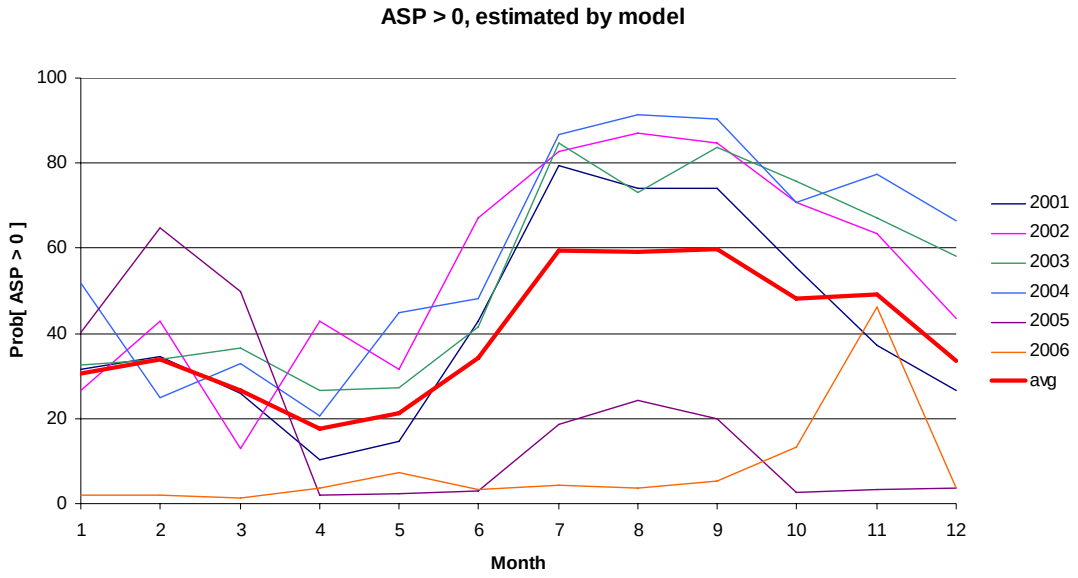


Table 10: Estimated[1] probability (%) that ASP levels in mussels tested positive, for each site per month, averaged over 6 years. Probabilities of 50% and higher are shown in bold.

ID	Site	avg	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec
			31	34	27	18	21	34	**59**	**59**	**60**	48	49	34
G80	Eastcoast	39	30	33	26	17	20	34	**59**	**59**	**59**	48	48	33
G26	Dumfries	44	36	39	32	21	26	39	**63**	**63**	**64**	**53**	**55**	39
G8	Ayr-LochStriven	48	41	44	36	25	29	43	**66**	**66**	**66**	**56**	**59**	42
G16	WC-LochFyne	30	20	23	17	11	13	25	**52**	**51**	**52**	38	38	24
G10	WC-LochEtive	40	31	34	27	18	22	35	**60**	**60**	**60**	49	50	34
G9	WC-LochCreran	42	33	36	29	19	23	37	**61**	**61**	**62**	**51**	**52**	36
P5	Mull-LochSpelve	47	40	43	35	24	28	42	**65**	**65**	**65**	**56**	**58**	41
P7	Mull-LochScridain	43	35	38	30	20	25	38	**62**	**62**	**63**	**52**	**54**	37
G1	Mull-LochnaKeal	40	32	35	27	18	22	35	**60**	**60**	**61**	49	**50**	35
G31	WC-LochLeven	28	18	21	15	10	12	22	49	48	50	35	35	22
G28	WC-Lochaber	39	30	33	26	17	20	34	**59**	**59**	**59**	48	48	33
G41	Skye	41	33	36	28	19	23	36	**61**	**61**	**61**	**50**	**51**	36
G35	NWC-LochTorridon	45	37	40	32	22	26	40	**63**	**63**	**64**	**53**	**55**	39
G39	NWC-Ullapool	44	37	40	32	21	26	40	**63**	**63**	**64**	**53**	**55**	39
G48	NWC-other	39	30	33	26	17	21	34	**60**	**59**	**60**	48	49	33
G23	Lewis-LochRoag	46	39	42	34	23	28	42	**65**	**65**	**65**	**55**	**57**	41
G21	LewisHarrisUist	44	36	39	31	21	25	39	**63**	**63**	**63**	**53**	**54**	38
G54	Orkney	37	27	30	23	15	19	31	**58**	**57**	**58**	45	46	31
G57	Shetland-SE	38	29	32	25	16	20	33	**59**	**58**	**59**	47	48	32
P61	Shetland-SW-Gruting	32	22	25	19	12	15	26	**53**	**52**	**54**	40	40	26
P68	Shetland-SW-Vaila	40	31	34	27	18	21	35	**60**	**59**	**60**	49	50	34
G58	Shetland-W	38	28	31	24	16	19	32	**58**	**58**	**59**	46	47	32
G64	Shetland-NW	40	31	34	27	18	21	35	**60**	**60**	**60**	49	50	34
G56	Shetland-NE	35	26	29	22	14	17	30	**57**	**56**	**57**	44	44	30
P69	Shetland-N-Balta	28	18	21	15	10	12	22	49	48	49	35	34	22

[1]From HGLM with Site, Year, Year by Month interaction as random effects and Month as fixed effect.
[2]For each site the average probability over 12 months over 6 years was calculated, and for each month the probability over all sites over 6 years was calculated.

3.4 Models for the 2001/6 data for ASP in king scallop gonads

As there were only two samples which tested negative for ASP in king scallop gonads, no models were developed for ASP > 0. The raw data for ASP > 20 and ASP > 10 μg/g are shown in Appendix A (Figures A7 and A8, respectively). There were only seven sites with king scallops, three of which had one sample only. This leaves four sites to consider for modelling, namely WC-LochEtive, NWC-Ullapool, Skye and Shetland-NW. Furthermore, numbers of samples tested were low for 2004-6 so comparing model fits for 2001/4 versus the 2004/6 data were not conducted. Nevertheless, it was possible, using data from all six years and the four aforementioned sites, to develop a model allowing for random variation between sites and between years, while the model also contained a term to allow for modelling of toxicity for each month of the year.

Figure 16a shows the estimated probability of ASP levels in king scallop gonads exceeding field closure (>20μg/g) over time for each site, while Figure 16b shows the same for ASP > 10 μg/g. Although the four sites are geographically far apart, the estimated toxin profile was similar for the four sites (i.e. the curves are close together). The predicted probability of field closure was highest in 2003 and 2004. When looking at the pattern over the 12 months of the year, the estimated probability was low for March and April, and high for May-Dec, often exceeding 50%. This is also illustrated in Figures 17a and 17b, as well as Tables 11 and 12.

Figure 18 compares the estimated toxin profiles for ASP > 20 and ASP > 10μg/g in king scallop gonads. The pattern was similar, with the probability of ASP exceeding 10μg/g consistently higher than for of 20μg/g.

To make a prediction of ASP exceeding field closure in king scallop gonads for a given site during the course of a future year then the information provided in Table 11 can be used.

Table 11: Estimated[1] probability (%) that ASP levels in king scallop gonads exceeded 20 µg/g, for each site per month, averaged over 6 years. Probabilities of 50% and higher are shown in bold.

			Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec
ID	Site	Avg[2]	8	12	0	0	22	11	17	**56**	41	**52**	38	23
G16	WC-LochFyne	20	6	9	0	0	18	8	14	**50**	35	46	32	19
G41	Skye	24	8	13	0	0	23	12	18	**58**	43	**54**	39	24
G39	NWC-Ullapool	29	12	17	0	0	30	16	24	**66**	**51**	**61**	47	30
G64	Shetland-NW	20	6	9	0	0	18	8	14	**50**	35	45	32	18

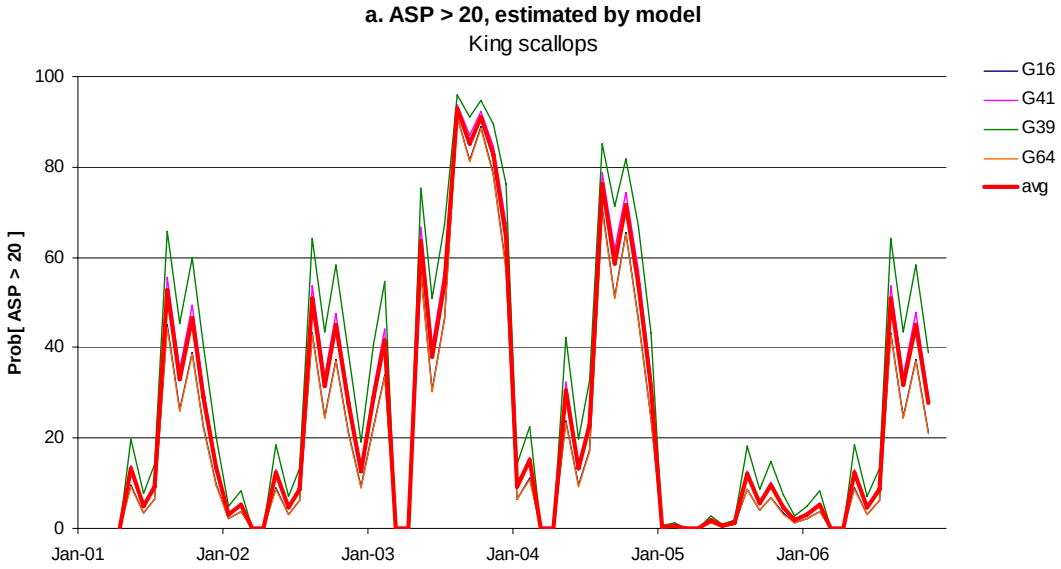
[1]From HGLM with Site and Year as random effects and Month as fixed effect.
[2]For each site the average probability over 12 months over 6 years was calculated, and for each month the probability over all sites over 6 years was calculated.

Table 12: Estimated[1] probability (%) that ASP levels in king scallop gonads exceeded 10 µg/g, for each site per month, averaged over 6 years. Probabilities of 50% and higher are shown in bold.

			Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec
ID	Site	Avg[2]	44	22	0	7	50	19	**51**	**71**	**85**	**68**	42	**65**
G16	WC-LochFyne	47	48	24	0	8	**54**	20	**55**	**77**	**89**	**73**	46	**70**
G41	Skye	40	38	17	0	5	44	14	45	**68**	**84**	**64**	36	**60**
G39	NWC-Ullapool	**58**	**63**	36	0	14	**69**	32	**70**	**87**	**94**	**84**	**61**	**82**
G64	Shetland-NW	30	26	11	0	3	32	9	32	**55**	**74**	50	25	47

[1]From HGLM with Site and Year as random effects and Month as fixed effect.
[2]For each site the average probability over 12 months over 6 years was calculated, and for each month the probability over all sites over 6 years was calculated.

Figure 16: Estimated probability (%) of ASP in king scallop gonads > 20µg/g and > 10µg/g for each site.



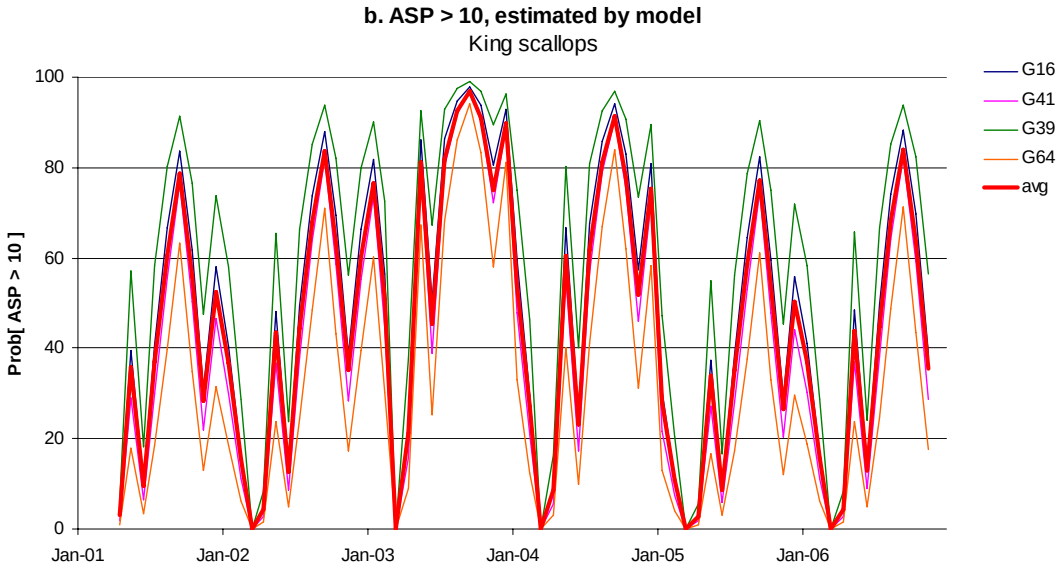


Figure 17: Estimated probability (%) of ASP in king scallop gonads exceeding 20 or 10µg/g, for each of 6 years.

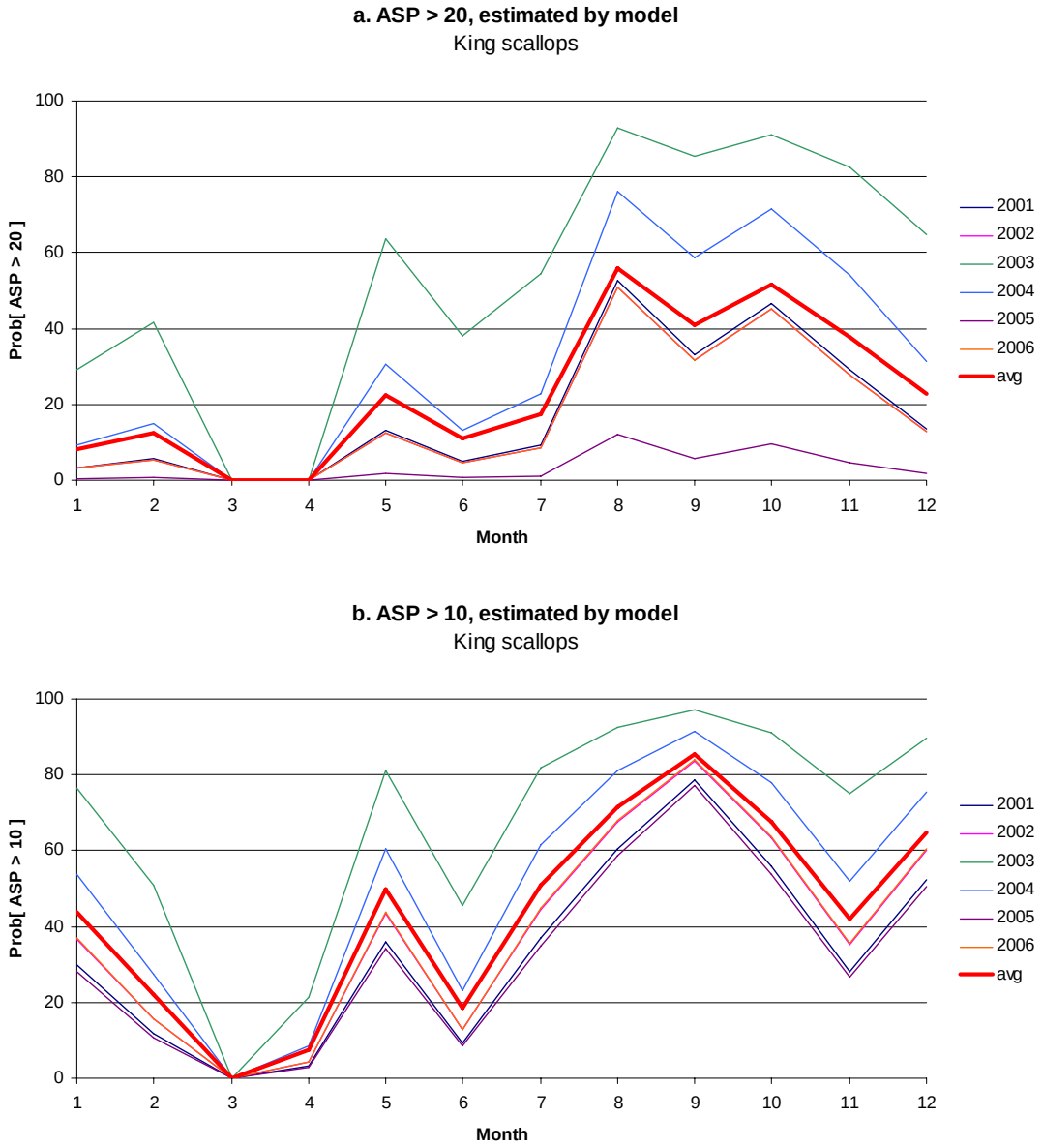
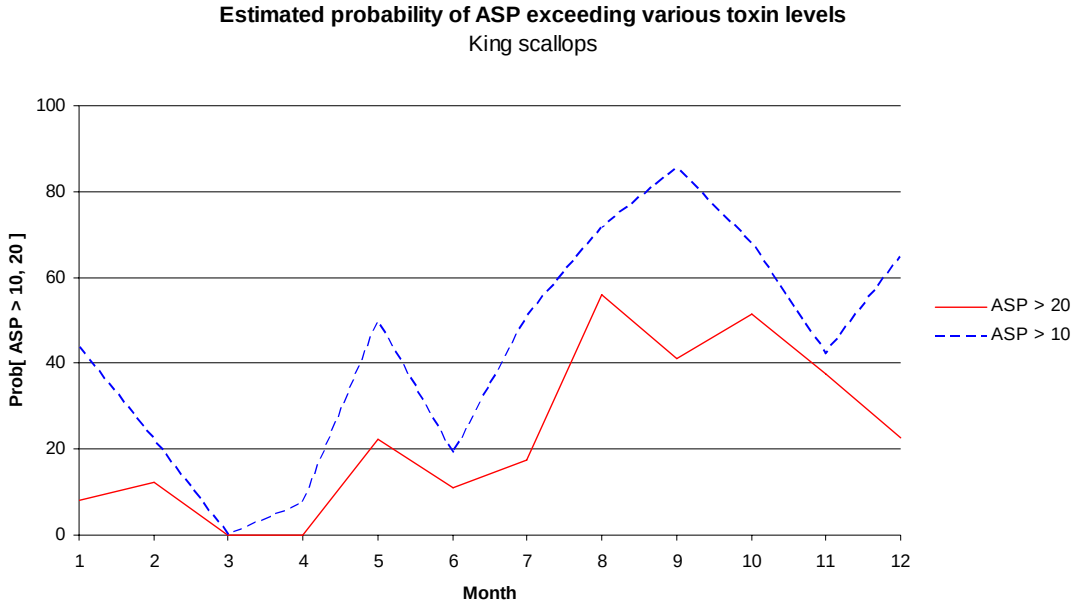


Figure 18: Comparison of the estimated probability (%) of ASP in king scallop gonads exceeding 10 or 20 µg/g, for an average year.



3.5 Models for the 2001/6 data for other species/toxin combinations

3.5.1 Model for positive ASP test results in pacific oysters

No models were developed for ASP > 10 (one sample only) and ASP > 20 (no samples) in pacific oysters (Table 3). For ASP > 0, 35% of the samples (316/894) tested positive. Pacific oysters were tested from 14 sites (as described in Table 4) but for six of these, there were 5 samples or less (see also Figure A9 in Appendix A). As a result, data from the remaining eight sites, having 38 or more samples per site, were included in the models, namely: Ayr-LochStriven, WC-LochFyne, WC-LochEtive, WC-LochCreran, Mull-LochnaKeal, WC-Lochaber, Skye and NWC. A model allowing for random variation between sites and between years, which also contained a term to allow for modelling of toxicity for each month of the year, gave the best fit.

Figures 19 and 20 show that prevalence was high in 2001/4 and much lower in 2005 and 2006. There was little difference between the eight sites. ASP tended to be present all year round with a prevalence of 20%, on average, which went up to 60%, on average, during July-September (Table 13).

Figure 19: Estimated probability (%) of ASP in pacific oysters > 0µg/g for each site.

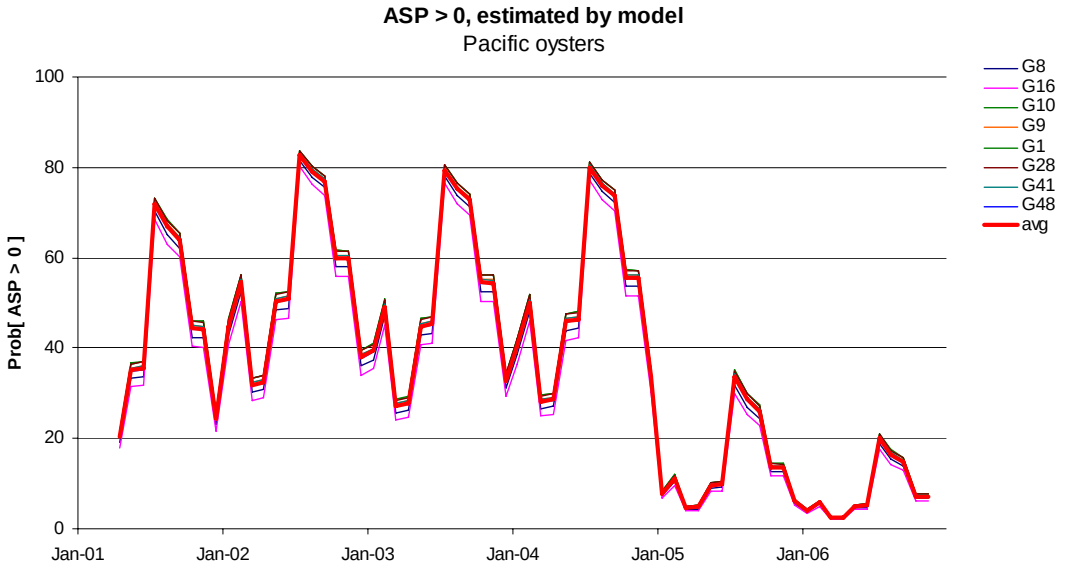


Figure 20: Estimated probability (%) of ASP in pacific oysters > 0 µg/g, for each of 6 years.

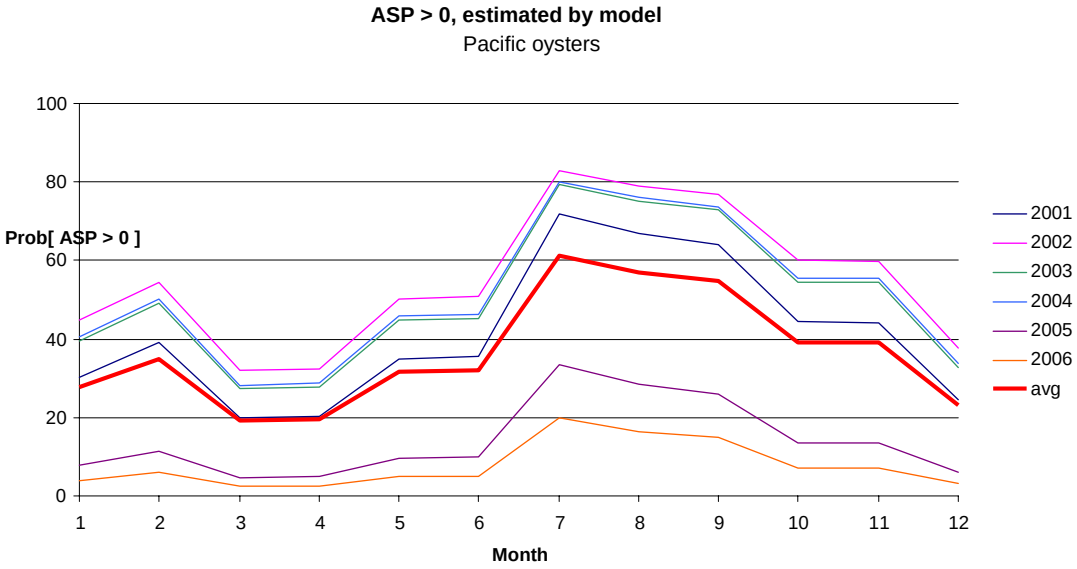


Table 13: Estimated[1] probability (%) that ASP levels in pacific oysters exceeded 0 μg/g, for each site per month, averaged over 6 years. Probabilities of 50% and higher are shown in bold.

ID	Site	avg	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec
			28	35	19	19	32	32	**61**	**57**	**55**	39	39	23
G8	Ayr-LochStriven	35	26	33	18	18	30	31	**60**	**56**	**53**	38	38	22
G16	WC-LochFyne	34	25	32	17	17	29	29	**58**	**54**	**52**	36	36	20
G10	WC-LochEtive	38	29	36	20	20	33	33	**62**	**58**	**56**	41	40	24
G9	WC-LochCreran	37	28	35	19	20	32	33	**62**	**58**	**55**	40	40	23
G1	Mull-LochnaKeal	38	29	36	20	20	33	33	**62**	**58**	**56**	41	41	24
G28	WC-Lochaber	38	29	36	20	20	33	33	**62**	**58**	**56**	40	40	24
G41	Skye	37	28	35	19	20	32	33	**62**	**58**	**55**	40	40	23
G48	NWC	37	28	35	19	19	32	32	**61**	**57**	**55**	39	39	23

[1]From HGLM with Site and Year as random effects and Month as fixed effect.
[2]For each site the average probability over 12 months over 6 years was calculated, and for each month the probability over all sites over 6 years was calculated.

3.5.2 Simple models for positive ASP test results in clams, cockles and queen scallops

For the remaining species and toxin combinations no detailed models could be fitted. This was due to lack of data (clams, cockles, queen scallops) or due to nearly all samples testing negative (pacific oysters). For clams, cockles and queen scallops (raw data shown in Figures A10, A12, A13), however, the positive ASP test result data contained sufficient information to allow for estimation of a Year effect (although it was not possible to include an effect of Month or Site). Results are shown as part of Figure 22 but will be discussed in Section 3.6.

3.5.3 Simple models for DSP test results in pacific oysters and queen scallops

DSP data for pacific oysters and queen scallops (raw data shown in Figures A13, A14) allowed for modelling of a Year effect (but no effects of Month or Site). These results are shown as part of Figure 21 and will be discussed in Section 3.6.

3.5.4 Remaining species/toxin combinations

For the remaining combinations, an estimate was provided of the prevalence of the toxin based on the proportion of samples that tested positive (ignoring Month, Site and Year effects), that is, toxin levels were at or above the limit of detection (LOD) or limit of quantification (LOQ) fort the ASP HPLC test. These findings form part of Table 14 and will be discussed in Section 3.6.

Table 14: Average prevalence (p, %), based on model estimates. Also shown are the number of positive samples (y) and the total number of samples.

						Terms in models		
toxin level	species	y	total	p	95% upper limit[1]	month	site	year
DSP>0	clams	2	70	2.9	8.8			
	cockles	0	62	0.0	4.8			
	king scallops	1	61	1.6	7.6			
	mussels	366	3866	5.5	28.0	y	y	y[2]
	pacific oysters	9	617	1.8	25.9			y
	queen scallops	18	162	10.3	38.0			y
PSP>0	clams	5	93	5.4	11.0			
	cockles	7	101	6.9	12.7			
	king scallops	5	108	4.6	9.5			
	mussels	193	3177	3.7	20.7	y	y	y[3]
	pacific oysters	2	852	0.2	0.8			
	queen scallops	6	161	3.7	7.3			
PSP>40	clams	5	93	5.4	11.0			
	cockles	5	101	5.0	10.2			
	king scallops	0	108	0.0	2.8			
	mussels	137	3177	2.7	10.1	y	y	y
	pacific oysters	0	852	0.0	0.4			
	queen scallops	3	161	1.9	4.8			
PSP>80	clams	2	93	2.2	6.7			
	cockles	2	101	2.0	6.2			
	king scallops	0	108	0.0	2.8			
	mussels	47	3177	0.9	13.4	y	y	y
	pacific oysters	0	852	0.0	0.4			
	queen scallops	0	161	0.0	1.9			
ASP>0	clams	50	98	49.4	82.7			y
	cockles	42	90	38.9	60.4			y
	king scallops	162	164	98.8	100.0			
	mussels	1615	3791	39.5	89.1	y	y	y[3]
	pacific oysters	316	894	36.6	76.8	y	y	y
	queen scallops	123	175	72.5	96.2			y
ASP>10	clams	0	98	0.0	3.1			
	cockles	1	90	1.1	5.2			
	king scallops	88	164	44.6	71.1	y	y	y
	mussels	15	3791	0.4	0.7			
	pacific oysters	1	894	0.1	0.6			
	queen scallops	5	175	2.9	6.0			
ASP>20	clams	0	98	0.0	3.1			
	cockles	0	90	0.0	3.3			
	king scallops	55	164	23.3	63.9	y	y	y
	mussels	3	3791	0.1	0.2			
	pacific oysters	0	894	0.0	0.4			
	queen scallops	3	175	1.7	4.4			

[1]This is the prevalence for an extreme year (1 in 20 years) for an average site and an average month. For entries that were not modelled it is based on the binomial variation (and does not include variation between years).
[2]Includes Year by Month and Year by Site interactions.
[3]Includes Year by Month interaction.

3.6 Comparison of predicted toxin levels across species

3.6.1 Comparison of average prevalence

Table 14 shows a summary of the average estimated prevalence for all species/toxin combinations. This is the average over all sites, years and months, and as a consequence the estimated prevalence may differ from the proportion of positive samples in the data. This is the case for e.g. DSP in mussels (data 366/3866 = 9.5% versus 5.5% from the model) and is caused by sampling being more frequent during toxic periods of the year. Inclusion of Year, Month and Site effects in the models allowed for such biases to be taken into account appropriately.

DSP
DSP prevalence was highest in queen scallops, 10%, followed by 5.5% in mussels.

PSP
PSP was nearly always absent in pacific oysters, with only 2/852 samples with PSP concentrations between 0 and 40 μg/100g. Even when taking into account uncertainty in the data, the likelihood of PSP in pacific oysters exceeding field closure levels was still less than 0.5%. For the other species, the average prevalence of positive PSP samples was low, ranging from 3.7 to 6.9%, although, when taking random variation into account, in a bad year the average probability of PSP exceeding field closure could be as high as 13%.

ASP
Prevalence of positive ASP samples was high in all species. ASP exceeding 10 μg/g was estimated to happen in less than 3% of the samples, except for king scallops, where prevalence remained high, on average.

3.6.2 Comparison of prevalence over time

DSP over 2001/6. When comparing the estimated DSP prevalence over 2001/6 (Figure 21), for those species that had a Year effect included in the models, it can be seen that there was a decline in DSP with time, except for pacific oysters in 2004, where a statistically significant increase was observed with respect to 2003 (4/64 vs 2/154, P-value=0.042).

ASP over 2001/6. For all species, ASP patterns were similar in that prevalence tended to be high in 2001/4 and much lower in 2006 (Figure 22). Queen scallops was the only species that continued to have high prevalence in 2005.

PSP over 2001/6. Comparisons were made for mussels only. The pattern was similar for PSP>0, PSP>40 and PSP>80 μg/100g, with a steady decline from 2002 to 2005, followed by an increase in 2006 (Figure 23).

Figure 21: Estimated DSP prevalence (%) over 6 years, for those species where models could be fitted to the toxin data.

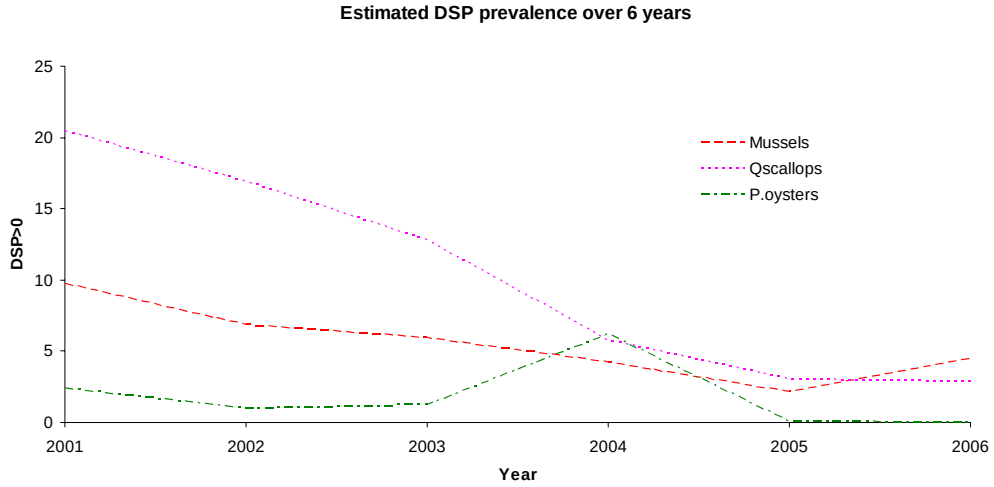


Figure 22: Estimated ASP prevalence (%) over 6 years, for those species where models could be fitted to the toxin data.

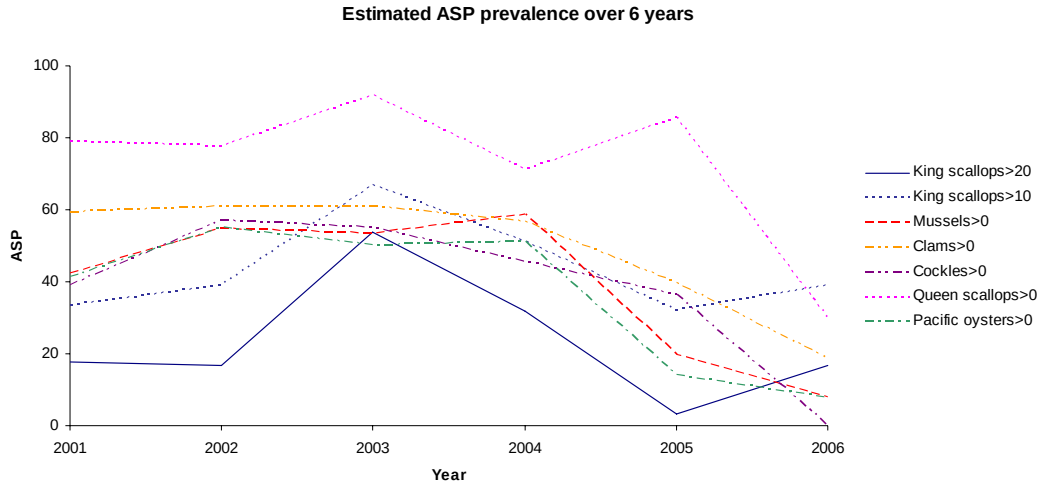
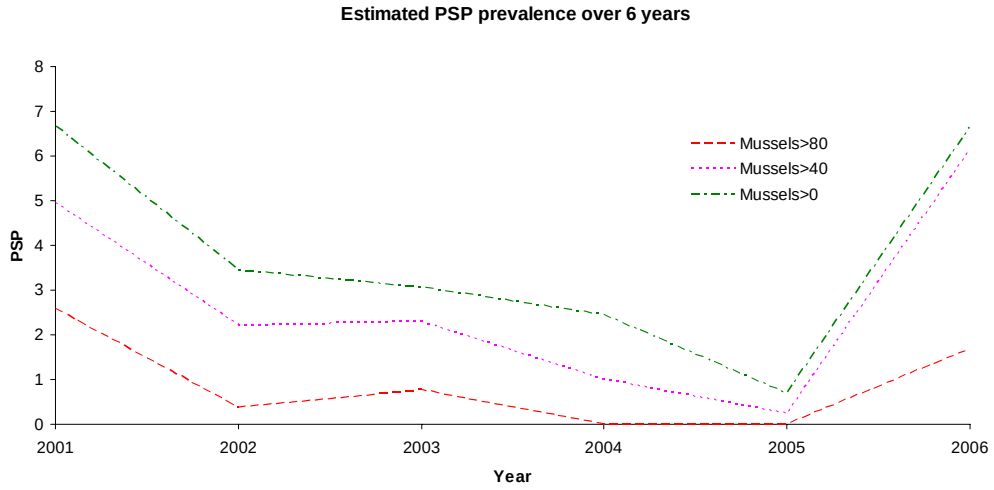


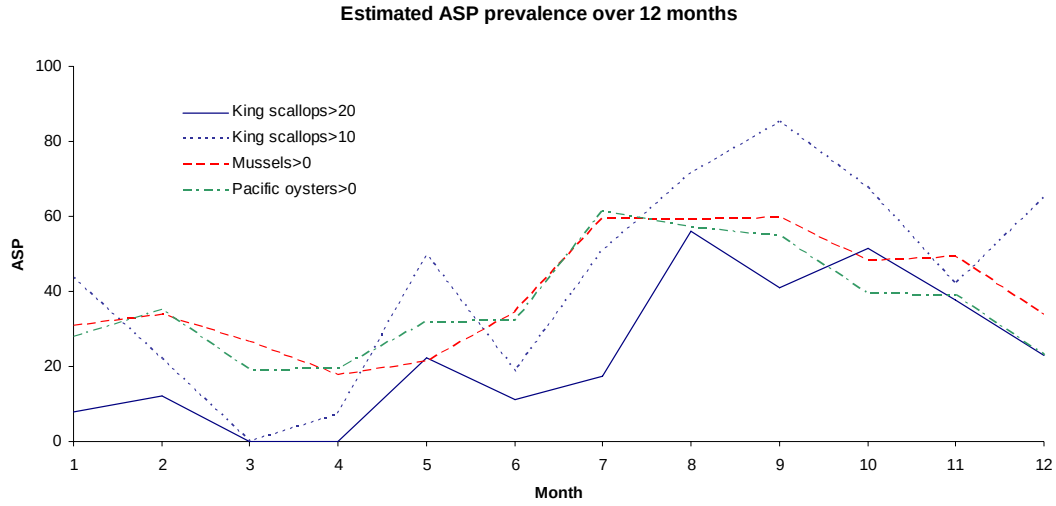
Figure 23: Estimated PSP prevalence (%) over 6 years, for mussels.



ASP during the 12 months of the year. For those species that had a Month effect included in the models, it can be seen that ASP was relatively more prevalent in autumn and less so in spring. There was also a similar prevalence in mussels and pacific oysters, which can be seen in Figure 24.

PSP over the 12 months of the year. Only the mussel data contained sufficient information to allow for modelling of Year effects. These results have already been presented in Figure 8. Prevalence was estimated to be high in early summer with a second peak in late summer, but low during the winter months.

Figure 24: Estimated ASP prevalence (%) during 12 months of the year, for those species where models that included a term for Month could be fitted to the toxin data.



3.7 Random variation between years

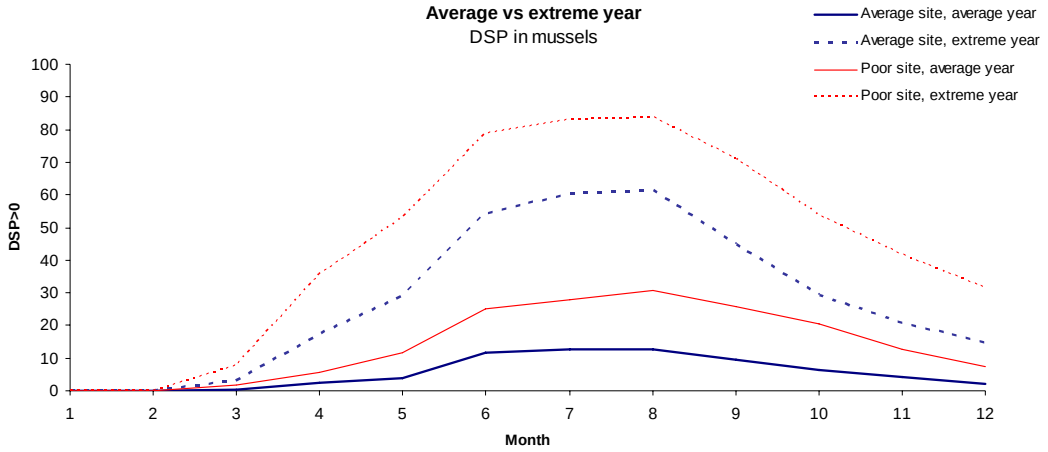
Worst case scenarios were also investigated. As the models allowed for random variation between years (see e.g. equation (3) in Section 2.2.3) we can look at what would happen in an extreme year. Let 'extreme year' be defined as a year in which prevalence of toxin is extremely high, and assume that such a year will, on average, only happen once every 20 years. Table 14 shows what would happen to the average toxicity for an average site at an average month, during such an extreme year. Note that the effect of an extreme year could only be assessed for those species/toxin combinations for which models could be developed. It can be seen that for DSP the prevalence would be between 4 (queen scallops) to 15 (pacific oysters) times higher during an extreme year. For PSP in mussels, the chance of field closure would be approximately 15 times higher. For ASP, the estimated effect of an extreme year is less pronounced, and the estimated prevalence would only be 2 to 3 times higher than for an average year.

The following sub-sections look at the 'extreme year' scenario in more detail.

3.7.1 DSP in mussels

The solid blue line in Figure 25 shows the prevalence of DSP in mussels for an average site (e.g. Shetland-W, Table 8) for an average year (2002, say). Note that this line corresponds to the 'avg' curve in Figure 12b. The dotted blue line indicates the estimated prevalence of DSP in an 'extreme year'. It shows that, on average, DSP prevalence would be around 10% during the summer months, but that during an extreme year the prevalence would increase to 60%. Furthermore, the graph also shows the average prevalence for a bad site (Ayr-LochStriven), which is more than twice as high as for an average site. In an 'extreme year', the prevalence is estimated to be 80% during the summer months for this site.

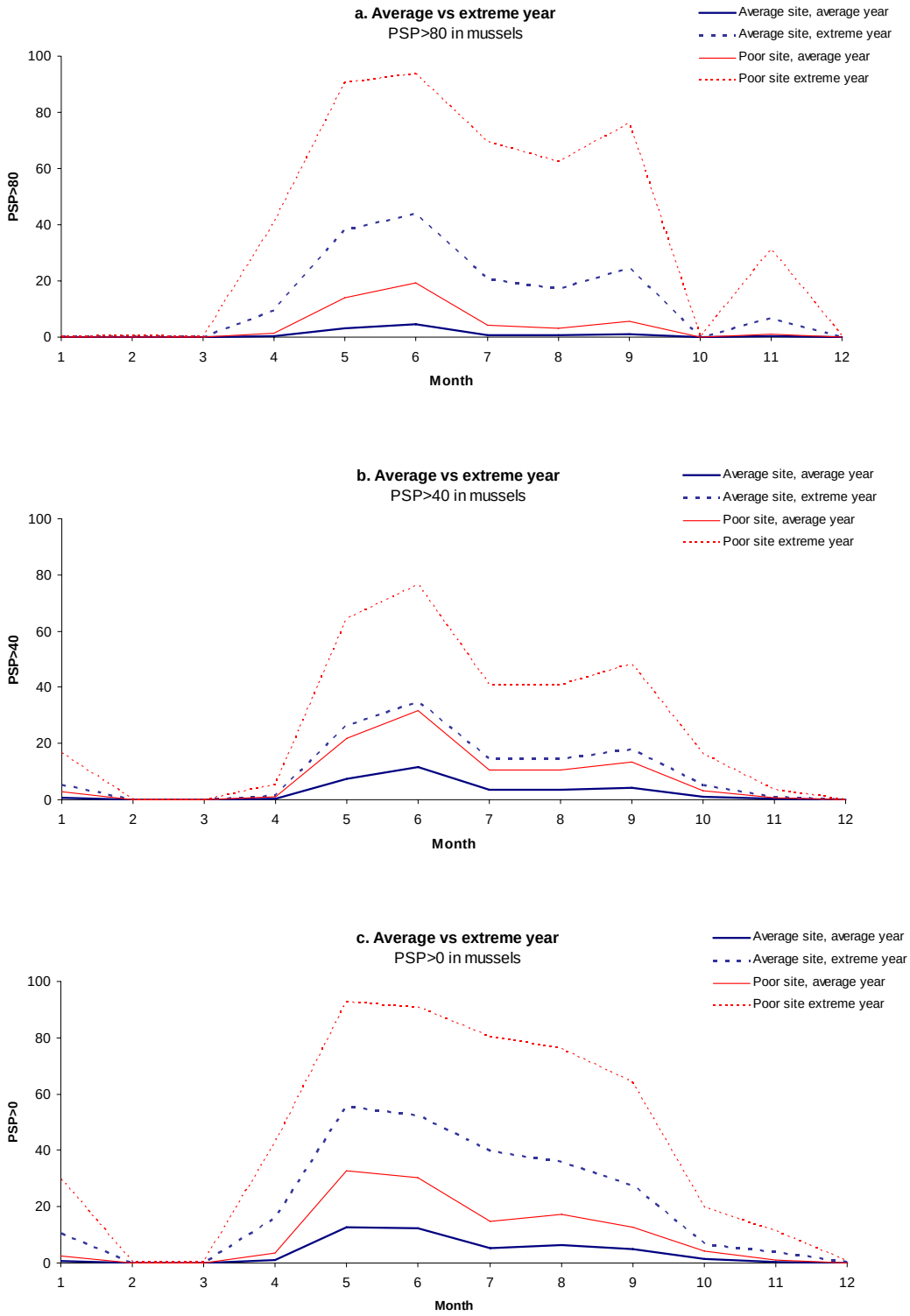
Figure 25: Prevalence (%) of DSP in mussels for an average year and an extreme year (only 1 in 20 years the prevalence is estimated to be as bad as this), for an average site and a poor site (Ayr-LochStriven).



3.7.2 PSP in mussels

For an average site (e.g. Ayr-LochStriven, Table 5), during an average year (2003, say), the estimated likelihood of field closure is small (blue line in Figure 26a). For an extreme year, however, it is estimated to increase to 40% in early summer. For a bad site (Shetland-NW) the likelihood of field closure is 40% in early summer for an average year, but is estimated to increase to nearly 100% during an extreme year. Similar patterns are observed for the likelihood of PSP >40 and PSP > 0 µg/100g in mussels (Figures 26b and c).

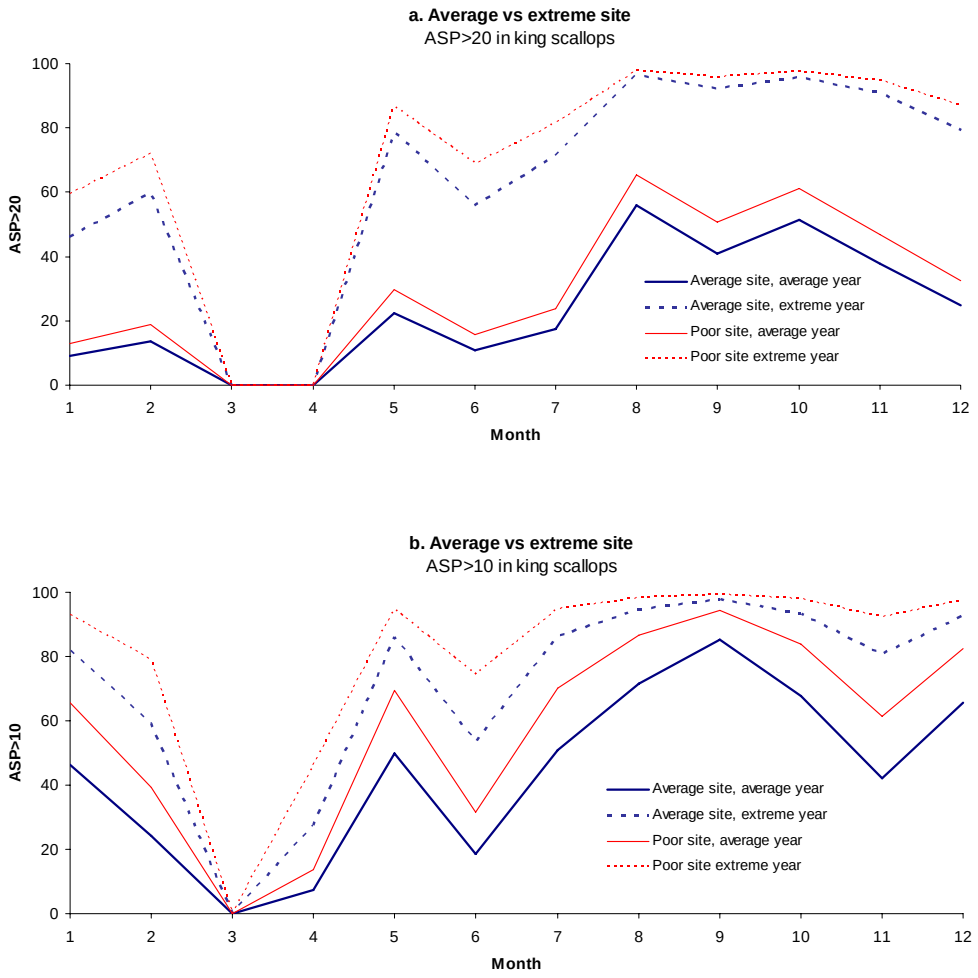
Figure 26: Prevalence (%) of PSP in mussels for an average year and an extreme year, for a site with an average toxin prevalence and a site with high toxin prevalence (Shetland-NW).



3.7.3 ASP in king scallop gonads

From Figure 27 it can be observed that, when looking at toxin prevalence during an average year, there is little difference between an average site and a poor site (NWC-Ullapool). Furthermore, it highlights that the likelihood of field closure can rise to nearly 100% for an 'extreme year'.

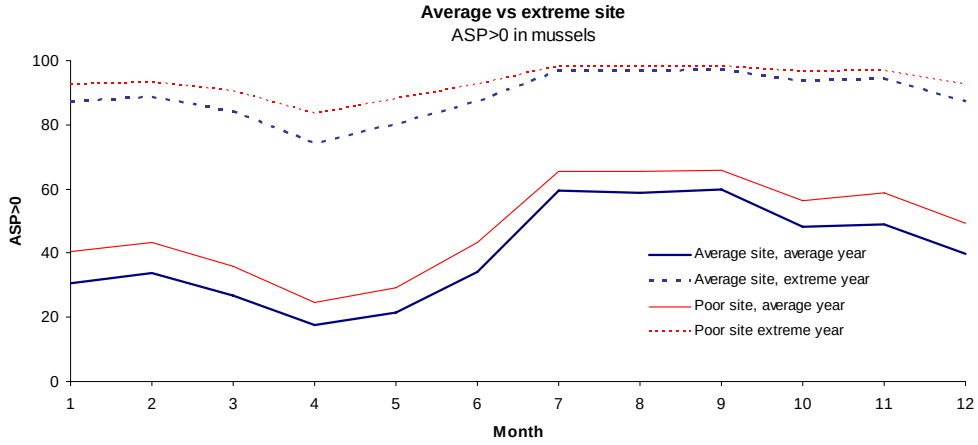
Figure 27: Prevalence (%) of ASP in king scallops for an average year and an extreme year, for a site with an average toxin prevalence and a site with high toxin prevalence (NWC-Ullapool).



3.7.4 ASP >0 in mussels

The prevalence of the presence of ASP in mussels is shown in Figure 28. Even though there were 25 mussel sites included in the analysis, the difference between an average site and a poor site was observed to be small. It was found that in an extreme year, the estimated prevalence could be close to 100% throughout the year.

Figure 28: Prevalence (%) of positive ASP in mussels for an average year and an extreme year, for a site with an average toxin prevalence and a site with high toxin prevalence (Ayr-LochStriven).



3.8 Shetland data

3.8.1 Model

To investigate variation within and between groups of pods, the Shetland mussel data were further analysed, using data from individual pods (as opposed to groups of pods). Pods with 30 samples or more, covering all 6 years, were included in this analysis. The included pods were 56, 57, 58, 61, 64, 65, 67, 68, 69, 70, 71 and 72. These covered 419 mussel samples for PSP, 509 for DSP and 517 for ASP. As the amount of data is smaller than for the full Scotland analysis, models were limited to those that included Month as a fixed effect, and Year and Pod as random effects. This model structure is similar to the Scotland-based model for e.g. PSP > 80 (which had Month as a fixed effect and Year and Site as random effects), where Site (a group of pods) has been replaced with Pod.

These models were fitted to the mussel test results for DSP, PSP (>0, >40 and >80 µg/100g) and ASP (> 0 µg/g). The fitted values gave good agreement with the data (not shown), and the estimated toxicity pattern for the Shetland sites during twelve months of the year was similar for both the Scotland-based model and the model based on Shetland data only.

3.8.2 Variation between pods within a grouping and between groupings

Table 15 gives a summary of the estimated variance components. These are presented on the log-odds scale and large values correspond to large variations between pods and small values to small variation. For example, it shows that ASP showed only little variation between groups and pods within groups, whereas for PSP>80 µg/100g this variation was large. This corresponds with earlier findings which showed that prevalence of ASP was similar across sites, while PSP exceeding field closure varied greatly between sites with some sites having no closures at all and other sites having relatively large probability of exceeding field closure.

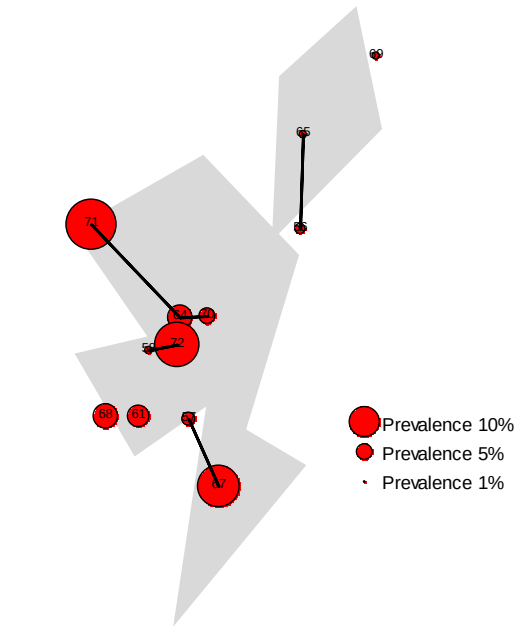
Table 15: Summary of sources of variation for Shetland data. Based on models that include Month as a fixed effect with Year, Group and Pod within Group as random effects. The variation is on the log-odds scale.

	variation between years	variation between groups[1]	variation between pods within a group[2]	total variation between pods[3]	variation between pods within a group as% of total variation between pods[4]
ASP>0	1.37	0.05	0.09	0.14	65
DSP	1.16	0.33	0.78	1.11	70
PSP>0	1.49	0.57	0.69	1.26	55
PSP>40	2.32	1.22	0.21	1.43	15
PSP>80	12.17	3.36	0.14	3.50	4

[1]Var(G); [2]Var(G.P); [3]Var(G)+Var(G.P); [4]Var(G.P)/[Var(G)+Var(G.P)] x 100%

To investigate whether pods within a grouping were more similar than between groups, it was necessary to compare the variation between pods within a group to the total variation between pods. This is shown in the rightmost column of Table 15. For ASP>0, DSP and PSP>0 the variation between pods within a group was relatively large, whereas the converse was true for PSP > 40 and PSP >80 (4 – 15% of the total variation between pods). The latter indicates that pods that were grouped together were much more similar (with respect to prevalence of PSP>40 or PSP>80) than pods that belong to different groups.

Figure 29: Estimated prevalence (%) of DSP in mussels in Shetland, for an average year and an average month. For individual pods. Pods that are connected by a line indicate groupings used for the Scotland-based analyses.

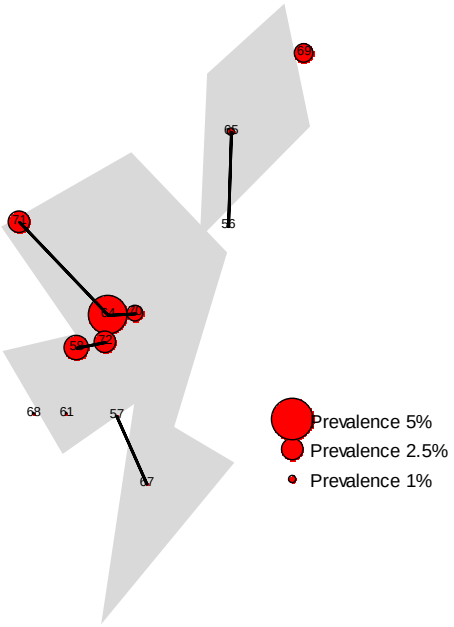


Figures 29, 30 and 31 show the average prevalence of DSP and PSP for the Shetland pods (based on model for Shetland data only). Compared to Figures 11, 9 and 14 the Scotland-based values are similar to those from Shetland only. The group-values (Figures (11, 9, and 14) are averages of the individual pod values for the Shetland model (Figures 29, 30 and 31).

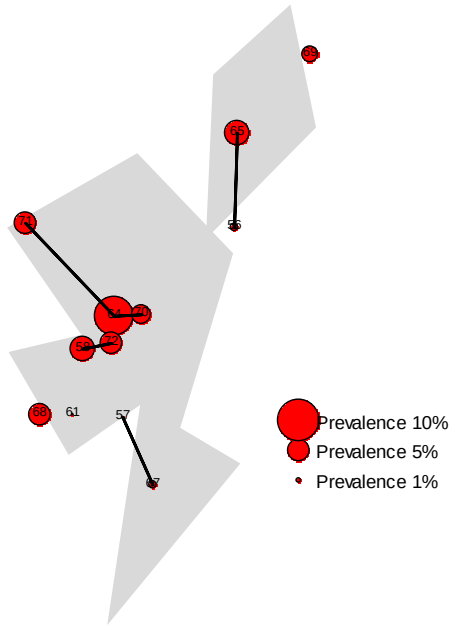
Generally, pods that were assigned to the same group showed similar behaviour. The only exception is for DSP in mussels for pods 58 (n = 90) and 72 (n=61), where pod 72 had a higher prevalence of DSP than pod 58. The difficulty is that, to be able to fit models, we need at least 1 or 2 samples per month per year (so for April 2001 to November 2006 that makes a minimum of 70 – 100 samples, say), and for the majority of pods this criterion was not met. This meant that pods that had limited numbers of samples had to be grouped with other pods in order to increase the numbers of samples. Although care was taken to group pods only if their toxin patterns resembled each other, this was not always easy to establish due to lack of data.

Figure 30: Estimated prevalence (%) of PSP in mussels in Shetland, for an average year and an average month (PSP > 80, PSP > 40 and PSP > 0 in Figures 81a, 81b and 81c, respectively) for individual pods. Pods that are connected by a line indicate groupings used for the Scotland-based analyses.

a. Average prevalence PSP>80 in mussels, Shetland



b. Average prevalence PSP>40 in mussels, Shetland



c. Average prevalence PSP>0 in mussels, Shetland

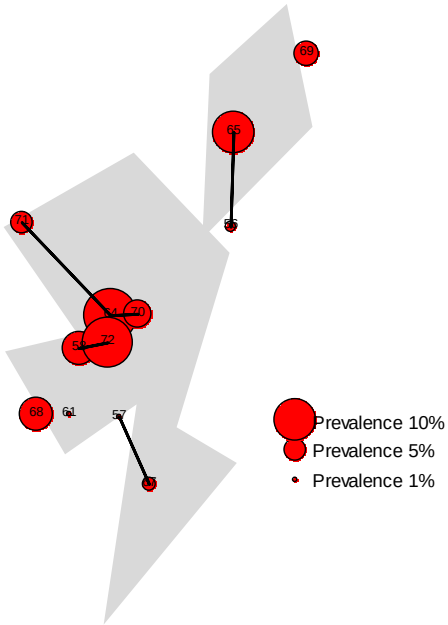
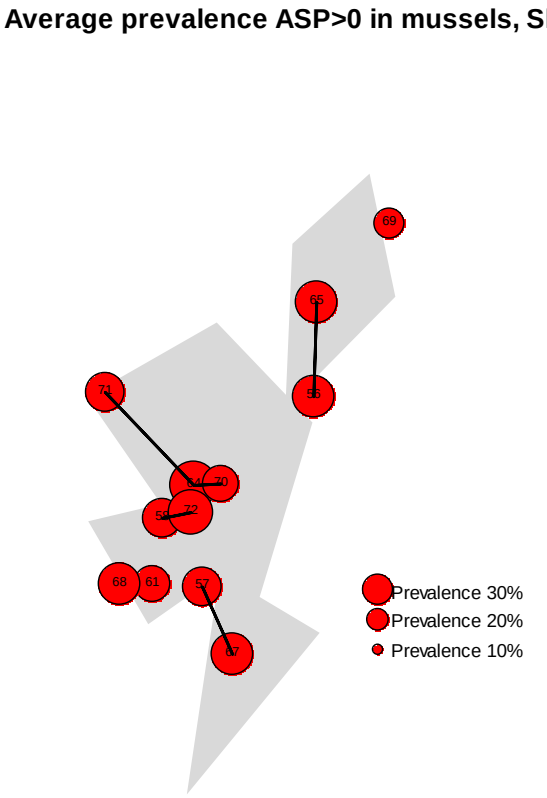


Figure 31: Estimated prevalence (%) of positive ASP in mussels in Shetland, for an average year and an average month for individual pods. Pods that are connected by a line indicate groupings used for the Scotland-based analyses.



4 RISK ASSESSMENT OF SAMPLING SCHEMES

The aim of the sampling strategy employed in the monitoring programme is to maximise confidence that a harvesting site is clear (i.e. toxin levels are below field closure). This is equivalent to minimising the risk that a site is unknowingly toxic. For the purposes of this study, this is referred to as the 'risk of non-detection'. To illustrate, if a site becomes toxic one week after a negative test result, this toxic event would go undetected under a monthly sampling scheme.

The risk of non-detection depends on the chance of the field being toxic; when this is higher the risk of non-detection will be higher also. In addition, sampling frequency also plays a role; the more frequently a field is being sampled, the less likely it will be that a toxic event goes undetected. In order to keep the risk of non-detection low, sampling schemes should be site and month specific, such that frequent sampling takes place when there is a high chance of the field being toxic, with less frequent sampling being sufficient when the chance of the field being toxic is low.

For simplicity, it is assumed that a clear test result is valid for one week. This means that if weekly sampling takes place the risk of non-detection is zero. The relationship between sampling frequency, field toxicity and the risk of non-detection is as follows (details in Materials and Methods):

- weekly sampling: risk of non-detection is zero,
- fortnightly sampling: risk of non-detection is 0.5 p,
- monthly sampling: risk of non-detection is 0.75 p,

where p is the chance that toxin levels exceed the field closure limit (as given in Tables 5, 8, 10).

Risk assessments were performed for DSP, PSP > 80 µg/100g, PSP > 0 µg/100g and ASP > 0 µg/g in mussels (as mussels are currently used as indicator species), and for ASP > 20µg/g in king scallop gonads (as ASP frequently exceeded field closure limit in this species).

4.1 Risk assessment of present monitoring scheme

Under the sampling scheme used at the time of this study (2006), the sampling frequencies were as follows

- PSP: weekly all year round
- DSP: weekly from April to November, fortnightly in December, monthly from January to March
- ASP: weekly from July to November, fortnightly from April to June, monthly from December to March

These frequencies are applicable to all sites and all shellfish species. The only exception is for ASP in king scallop gonads, which were tested weekly all year round.

PSP
Since the sampling regime being assessed at the time of this study involves weekly sampling for PSP from all sites, coupled with the assumption that a clear test result is valid for one week, the risk of non-detection is zero for PSP for all sites and all species.

DSP in mussels
The risk of non-detection is zero during April – November due to weekly sampling having taken place (Table 16). In December, when fortnightly sampling is applied, the risk is observed to increase to 4.4% for Ayr-LochStriven. During January-March the risk stays below 1%, despite sampling only once a month, except for Ayr-LochStriven which shows a risk up to 1.5% in March.

ASP in king scallop gonads exceeding field closure limit
As weekly monitoring for ASP in king scallops took place at the time of this study, the risk of non-detection is zero for ASP in king scallop gonads for all sites.

Positive ASP in mussels
When testing was less than once a week, the risk of non-detection of positive ASP samples in mussels ranged from 9 (April) to 25% (December-February). The risk of non-detection was similar across all sites (Table 17).

Table 16: Risk of non-detection (%), i.e. probability that a site is unknowingly toxic, for DSP in mussels using the sampling frequencies introduced in 2006. Risk of non-detection of 1% or more shown in bold.

			Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec
		Current frequency[1]	1	1	1	4	4	4	4	4	4	4	4	2
ID	Site	Avg[2]	0.00	0.00	0.30	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	**1.14**
G80	Eastcoast	0.15	0.01	0.01	0.45	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	**1.29**
G26	Dumfries	0.02	0.00	0.00	0.06	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.19
G8	Ayr-LochStriven	0.43	0.01	0.01	**1.40**	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	**3.69**
G16	WC-LochFyne	0.18	0.01	0.01	0.43	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	**1.73**
G10	WC-LochEtive	0.01	0.00	0.00	0.02	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.07
G9	WC-LochCreran	0.01	0.00	0.00	0.03	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.11
P5	Mull-LochSpelve	0.04	0.00	0.00	0.13	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.34
P7	Mull-LochScridain	0.10	0.00	0.00	0.23	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.93
G1	Mull-LochnaKeal	0.02	0.00	0.00	0.07	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.21
G31	WC-LochLeven	0.02	0.00	0.00	0.05	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.21
G28	WC-Lochaber	0.25	0.01	0.01	0.58	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	**2.41**
G41	Skye	0.24	0.01	0.01	0.67	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	**2.26**
G35	NWC-LochTorridon	0.16	0.00	0.00	0.26	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	**1.69**
G39	NWC-Ullapool	0.15	0.00	0.00	0.27	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	**1.55**
G48	NWC-other	0.26	0.01	0.01	0.53	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	**2.54**
G23	Lewis-LochRoag	0.08	0.00	0.00	0.17	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.83
G21	LewisHarrisUist	0.07	0.00	0.00	0.21	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.63
G54	Orkney	0.12	0.00	0.00	0.24	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	**1.15**
G57	Shetland-SE	0.10	0.00	0.00	0.22	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.98
P61	Shetland-SW-Gruting	0.11	0.00	0.00	0.28	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	**1.01**
P68	Shetland-SW-Vaila	0.08	0.00	0.00	0.22	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.77
G58	Shetland-W	0.12	0.00	0.00	0.25	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	**1.17**
G64	Shetland-NW	0.16	0.01	0.01	0.40	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	**1.56**
G56	Shetland-NE	0.07	0.00	0.00	0.14	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.68
P69	Shetland-N-Balta	0.05	0.00	0.00	0.16	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.44

[1]Current sampling frequency: 1=once per month, 2=fortnightly, 4=once per week.

[2]For each site the average risk was calculated, and for each month the average risk was calculated.

Table 17: Risk of non-detection (%), i.e. probability that a site unknowingly exceeds 0 µg/g, for ASP in mussels using the sampling frequencies introduced in 2006. Risk of non-detection of 10% or more shown in bold.

			Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec
		Current frequency[1]	1	1	1	2	2	2	4	4	4	4	4	1
ID	Site	Avg[2]	**23**	**25**	**20**	9	**11**	**17**	0	0	0	0	0	**25**
G80	Eastcoast	**11**	**22**	**25**	**19**	8	**10**	**17**	0	0	0	0	0	**25**
G26	Dumfries	**13**	**27**	**30**	**24**	**11**	**13**	**20**	0	0	0	0	0	**29**
G8	Ayr-LochStriven	**14**	**31**	**33**	**27**	**12**	**15**	**22**	0	0	0	0	0	**32**
G16	WC-LochFyne	7	**15**	**18**	**13**	6	7	**12**	0	0	0	0	0	**18**
G10	WC-LochEtive	**11**	**23**	**26**	**20**	9	**11**	**17**	0	0	0	0	0	**26**
G9	WC-LochCreran	**12**	**25**	**27**	**22**	10	**12**	**18**	0	0	0	0	0	**27**
P5	Mull-LochSpelve	**14**	**30**	**32**	**26**	**12**	**14**	**21**	0	0	0	0	0	**31**
P7	Mull-LochScridain	**12**	**26**	**29**	**23**	**10**	**12**	**19**	0	0	0	0	0	**28**
G1	Mull-LochnaKeal	**11**	**24**	**26**	**21**	9	**11**	**18**	0	0	0	0	0	**26**
G31	WC-LochLeven	7	**13**	**16**	**11**	5	6	**11**	0	0	0	0	0	**17**
G28	WC-Lochaber	**11**	**22**	**25**	**19**	8	**10**	**17**	0	0	0	0	0	**25**
G41	Skye	**12**	**25**	**27**	**21**	9	**11**	**18**	0	0	0	0	0	**27**
G35	NWC-LochTorridon	**13**	**28**	**30**	**24**	**11**	**13**	**20**	0	0	0	0	0	**29**
G39	NWC-Ullapool	**13**	**27**	**30**	**24**	**11**	**13**	**20**	0	0	0	0	0	**29**
G48	NWC-other	**11**	**23**	**25**	**20**	9	**10**	**17**	0	0	0	0	0	**25**
G23	Lewis-LochRoag	**14**	**29**	**32**	**26**	**12**	**14**	**21**	0	0	0	0	0	**31**
G21	LewisHarrisUist	**12**	**27**	**29**	**23**	**10**	**13**	**19**	0	0	0	0	0	**28**
G54	Orkney	10	**20**	**23**	**18**	8	9	**16**	0	0	0	0	0	**23**
G57	Shetland-SE	**10**	**22**	**24**	**19**	8	10	**16**	0	0	0	0	0	**24**
P61	Shetland-SW-Gruting	8	**16**	**19**	**14**	6	7	**13**	0	0	0	0	0	**19**
P68	Shetland-SW-Vaila	**11**	**23**	**25**	**20**	9	**11**	**17**	0	0	0	0	0	**25**
G58	Shetland-W	**10**	**21**	**24**	**18**	8	10	**16**	0	0	0	0	0	**24**
G64	Shetland-NW	**11**	**23**	**26**	**20**	9	**11**	**17**	0	0	0	0	0	**26**
G56	Shetland-NE	9	**19**	**22**	**17**	7	9	**15**	0	0	0	0	0	**22**
P69	Shetland-N-Balta	7	**13**	**16**	**11**	5	6	**11**	0	0	0	0	0	**16**

[1]Current sampling frequency: 1=once per month, 2=fortnightly, 4=once per week.
[2]For each site the average risk was calculated, and for each month the average risk was calculated.

4.2 Revised sampling schemes

From Tables 5, 6, 7 it was determined that PSP in mussels could be site-specific and season-bound. For example, the probability of testing positive for PSP in mussels was found to be almost zero throughout the year for WC-LochEtive, WC-LochCrerean and Mull-LochSpelve. The chance of testing positive for PSP in mussels was almost zero during February and March. These findings suggest that a blanket weekly monitoring frequency for all sites throughout the year may not be necessary.

For DSP in mussels (Table 8), it can be seen that DSP was much more prevalent at some sites (e.g. Ayr-LochStriven, WC-Lochaber) than others (WC-LochEtive, WC-LochLeven).

These findings indicate that re-allocation of sampling effort may be needed. The aim was to construct alternative sampling schemes such that the risk of non-detection does not exceed a pre-defined maximum value (denoted by R_{max}), while minimising the total number of samples required. Three possible sampling frequencies were considered, namely:

- once per month (monthly) when toxin levels are low,
- four times per month (weekly) when toxin levels are high,
- fortnightly for intermediate toxin levels,

where a month approximates four weeks. These alternative schemes were allowed to be site and time specific, so that each site was assigned its own monitoring scheme for which the sampling frequency could vary during the year.

For a given maximum acceptable risk of non-detection (denoted by R_{max}), alternative sampling schemes were constructed as follows.

- When toxin levels are high, with p exceeding p_{high}, weekly sampling is required.
- When toxin levels are low, with p less than p_{low}, monthly sampling will suffice.
- For intermediate toxin levels, with p exceeding p_{low} but less than p_{high}, fortnightly sampling is applied.

where p_{high} and p_{low} are cut-off levels that determine whether weekly sampling is necessary or monthly sampling will suffice. To minimise the number of samples needed, p_{high} and p_{low} were chosen as follows (details in Materials & Methods):

- let R_{max} denote the maximum acceptable risk of non-detection, which is set in advance,
- then $p_{high} = 2\ R_{max}$,
- and $p_{low} = 2/3\ p_{high}$.

To illustrate this approach, R_{max} was arbitrarily set at 1% and 5% for the purpose of this study, but it should be noted that the level of maximum acceptable risk of non-detection in Scotland should ultimately be the responsibility of the Food Standards Agency Scotland.

4.2.1 Risk assessment of alternative sampling schemes

Based on the approach outlined in the previous section, the following two alternative sampling schemes were constructed.

- Maximum acceptable risk of non-detection set at 5%, so that $p_{high} = 10\%$ and $p_{low} = 6.67\%$. Sampling frequency should be increased to once a week when $p \geq 10\%$, once a fortnight when $6.67\% < p < 10\%$ and once a month when $p \leq 6.67\%$.
- Maximum acceptable risk of non-detection set at 1%, so that $p_{high} = 2\%$ and $p_{low} = 1.33\%$. Sampling frequency should be increased to once a week when $p \geq 2\%$, once a fortnight when $1.33\% < p < 2\%$ and once a month when $p \leq 1.33\%$.

These schemes were implemented for PSP in mussels, DSP in mussels and ASP in king scallop gonads, based on the values of p (chance that toxin levels exceed field closure limit) given in Tables 5, 8 and 11. The sampling frequencies required for each site, which correspond to maximum acceptable risk of non-detection of 5% and 1%, are shown in Tables 18, 19 and 20.

PSP in mussels
In order to keep the risk of non-detection (i.e. the risk of missing PSP levels exceeding field closure) below 1%, monthly sampling would be sufficient during October – March. For 11 of the 25 sites, monthly sampling throughout the year would suffice (Table 18).

For interest, Table 21 shows the sampling frequencies required to keep the risk of non-detection of positive (i.e. > 0µg/100g) mussel samples below 5 or 1%. For 7 sites monthly sampling would suffice, while during Nov, Dec, Feb and Mar monthly sampling would suffice for all sites (but note that for Jan several Shetland sites, and Skye, would require more frequent sampling).

DSP in mussels
To keep the maximum risk of non-detection (i.e. the risk of missing DSP in mussels) below 1%, weekly sampling would be required for most of the year, except for January and February, where monthly sampling would suffice. These frequencies would have to be applied to all sites, with the exception of WC-LochEtive, for which it appears that monthly sampling throughout the year would be sufficient (Table 19).

ASP in king scallop gonads
Weekly sampling throughout the year would be required to keep the risk of not detecting ASP above those resulting in field closure (> 20µg/g) in king scallop gonads, with the exception of the month March, where monthly sampling would suffice (Table 20).

Table 18: Sampling frequencies (1 = once per month, 2 = every fortnight; 4 = every week) for sampling schemes with the maximum risk of non-detection (PSP exceeding field closure limit) set at 5 or 1%, for PSP in mussels. Sampling frequencies exceeding once per month are shown in bold.

		max risk 5%												max risk 1%											
		J	F	M	A	M	J	J	A	S	O	N	D	J	F	M	A	M	J	J	A	S	O	N	D
	Current frequency[1]	**4**	**4**	**4**	**4**	**4**	**4**	**4**	**4**	**4**	**4**	**4**	**4**	**4**	**4**	**4**	**4**	**4**	**4**	**4**	**4**	**4**	**4**	**4**	**4**
ID	Site																								
G80	Eastcoast	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	**2**	**4**	1	1	1	1	1	1
G26	Dumfries	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
G8	Ayr-LochStriven	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	**4**	**4**	1	1	1	1	1	1
G16	WC-LochFyne	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
G10	WC-LochEtive	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
G9	WC-LochCreran	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
P5	Mull-LochSpelve	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
P7	Mull-LochScridain	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	**4**	**4**	1	1	1	1	1	1
G1	Mull-LochnaKeal	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
G31	WC-LochLeven	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
G28	WC-Lochaber	1	1	1	1	1	**2**	1	1	1	1	1	1	1	1	1	1	**4**	**4**	**2**	1	**2**	1	1	1
G41	Skye	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	**4**	**4**	1	1	**2**	1	1	1
G35	NWC-LochTorridon	1	1	1	1	1	**2**	1	1	1	1	1	1	1	1	1	1	**4**	**4**	1	1	**2**	1	1	1
G39	NWC-Ullapool	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
G48	NWC-other	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	**4**	**4**	1	1	1	1	1	1
G23	Lewis-LochRoag	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	**2**	**4**	1	1	1	1	1	1
G21	LewisHarrisUist	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
G54	Orkney	1	1	1	1	**2**	**4**	1	1	1	1	1	1	1	1	1	1	**4**	**4**	**4**	**2**	**4**	1	1	1
G57	Shetland-SE	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
P61	Shetland-SW-Gruting	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
P68	Shetland-SW-Vaila	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	**2**	**4**	1	1	1	1	1	1
G58	Shetland-W	1	1	1	1	**4**	**4**	1	1	1	1	1	1	1	1	1	1	**4**	**4**	**4**	**4**	**4**	1	1	1
G64	Shetland-NW	1	1	1	1	**4**	**4**	1	1	1	1	1	1	1	1	1	**2**	**4**	**4**	**4**	**4**	**4**	1	1	1
G56	Shetland-NE	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	**2**	**4**	1	1	1	1	1	1
P69	Shetland-N-Balta	1	1	1	1	**4**	**4**	1	1	1	1	1	1	1	1	1	1	**4**	**4**	**4**	**4**	**4**	1	1	1

[1]Current sampling frequency: 1=once per month, 2=fortnightly, 4=once per week.

Table 19: Sampling frequencies (1 = once per month, 2 = every fortnight; 4 = every week) for sampling schemes with the maximum risk of non-detection (DSP positive) set at 5 or 1%, for DSP in mussels. Sampling frequencies exceeding once per month are shown in bold.

		max risk 5%												max risk 1%											
		J	F	M	A	M	J	J	A	S	O	N	D	J	F	M	A	M	J	J	A	S	O	N	D
	Current frequency[1]	1	1	1	**4**	**4**	**4**	**4**	**4**	**4**	**4**	**4**	**2**	1	1	1	**4**	**4**	**4**	**4**	**4**	**4**	**4**	**4**	**2**
ID	Site																								
G80	Eastcoast	1	1	1	1	**2**	**4**	**4**	**4**	**4**	**2**	**2**	1	1	1	1	**4**	**4**	**4**	**4**	**4**	**4**	**4**	**4**	**4**
G26	Dumfries	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	**4**	**4**	**4**	**4**	**2**	1	1
G8	Ayr-LochStriven	1	1	1	1	**4**	**4**	**4**	**4**	**4**	**4**	**4**	**2**	1	1	**2**	**4**	**4**	**4**	**4**	**4**	**4**	**4**	**4**	**4**
G16	WC-LochFyne	1	1	1	1	**2**	**4**	**4**	**4**	**4**	**2**	**2**	1	1	1	1	**4**	**4**	**4**	**4**	**4**	**4**	**4**	**4**	**4**
G10	WC-LochEtive	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
G9	WC-LochCreran	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	**4**	**2**	**2**	**2**	1	1	1
P5	Mull-LochSpelve	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	**4**	**4**	**4**	**4**	**2**	1	1
P7	Mull-LochScridain	1	1	1	1	1	**2**	**2**	**4**	**2**	1	1	1	1	1	1	1	**4**	**4**	**4**	**4**	**4**	**4**	**4**	**2**
G1	Mull-LochnaKeal	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	**4**	**4**	**4**	**2**	1	1	1
G31	WC-LochLeven	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	**4**	**4**	**4**	**4**	**2**	1	1
G28	WC-Lochaber	1	1	1	1	**2**	**4**	**4**	**4**	**4**	**4**	**2**	1	1	1	1	**4**	**4**	**4**	**4**	**4**	**4**	**4**	**4**	**4**
G41	Skye	1	1	1	1	**2**	**4**	**4**	**4**	**4**	**4**	**2**	1	1	1	1	**4**	**4**	**4**	**4**	**4**	**4**	**4**	**4**	**4**
G35	NWC-LochTorridon	1	1	1	1	1	**2**	**2**	**4**	**4**	**4**	1	1	1	1	1	**4**	**4**	**4**	**4**	**4**	**4**	**4**	**4**	**4**
G39	NWC-Ullapool	1	1	1	1	1	**2**	**4**	**4**	**4**	**2**	1	1	1	1	1	**4**	**4**	**4**	**4**	**4**	**4**	**4**	**4**	**4**
G48	NWC-other	1	1	1	1	1	**4**	**4**	**4**	**4**	**4**	**2**	1	1	1	1	**4**	**4**	**4**	**4**	**4**	**4**	**4**	**4**	**4**
G23	Lewis-LochRoag	1	1	1	1	1	**4**	**4**	**4**	**2**	1	1	1	1	1	1	1	**4**	**4**	**4**	**4**	**4**	**4**	**2**	**2**
G21	LewisHarrisUist	1	1	1	1	1	**4**	**4**	**4**	1	1	1	1	1	1	1	1	**4**	**4**	**4**	**4**	**4**	**4**	**4**	1
G54	Orkney	1	1	1	1	1	**4**	**4**	**4**	**4**	**2**	1	1	1	1	1	**4**	**4**	**4**	**4**	**4**	**4**	**4**	**4**	**4**
G57	Shetland-SE	1	1	1	1	1	**4**	**4**	**4**	**4**	1	1	1	1	1	1	**2**	**4**	**4**	**4**	**4**	**4**	**4**	**4**	**2**
P61	Shetland-SW-Gruting	1	1	1	1	1	**4**	**4**	**4**	**2**	1	1	1	1	1	1	**4**	**4**	**4**	**4**	**4**	**4**	**4**	**4**	**4**
P68	Shetland-SW-Vaila	1	1	1	1	**2**	**4**	**4**	**4**	**2**	1	1	1	1	1	1	**4**	**4**	**4**	**4**	**4**	**4**	**4**	**4**	**2**
G58	Shetland-W	1	1	1	1	1	**4**	**4**	**4**	**4**	**2**	1	1	1	1	1	**4**	**4**	**4**	**4**	**4**	**4**	**4**	**4**	**4**
G64	Shetland-NW	1	1	1	1	1	**4**	**4**	**4**	**4**	**2**	1	1	1	1	1	**4**	**4**	**4**	**4**	**4**	**4**	**4**	**4**	**4**
G56	Shetland-NE	1	1	1	1	1	**2**	**2**	**2**	**2**	1	1	1	1	1	1	1	**4**	**4**	**4**	**4**	**4**	**4**	**4**	**2**
P69	Shetland-N-Balta	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	**2**	**4**	**4**	**4**	**4**	**4**	**2**	1

[1]Current sampling frequency: 1=once per month, 2=fortnightly, 4=once per week.

Table 20: Sampling frequencies (1 = once per month, 2 = every fortnight; 4 = every week) for sampling schemes with the maximum risk of non-detection (ASP exceeding field closure limit) set at 5 or 1%, for ASP in king scallop gonads. Sampling frequencies exceeding once per month are shown in bold.

		max risk 5%												max risk 1%											
		J	F	M	A	M	J	J	A	S	O	N	D	J	F	M	A	M	J	J	A	S	O	N	D
	Current frequency[1]	1	1	1	**2**	**2**	**2**	**4**	**4**	**4**	**4**	**4**	1	1	1	1	**2**	**2**	**2**	**4**	**4**	**4**	**4**	**4**	1
ID	Site																								
G16	WC-LochFyne	**4**	**4**	1	**2**	**4**	**4**	**4**	**4**	**4**	**4**	**4**	**4**	**4**	**4**	1	**4**	**4**	**4**	**4**	**4**	**4**	**4**	**4**	**4**
G41	Skye	**4**	**4**	1	1	**4**	**4**	**4**	**4**	**4**	**4**	**4**	**4**	**4**	**4**	1	**4**	**4**	**4**	**4**	**4**	**4**	**4**	**4**	**4**
G39	NWC-Ullapool	**4**	**4**	1	**4**	**4**	**4**	**4**	**4**	**4**	**4**	**4**	**4**	**4**	**4**	1	**4**	**4**	**4**	**4**	**4**	**4**	**4**	**4**	**4**
G64	Shetland-NW	**4**	**4**	1	1	**4**	**2**	**4**	**4**	**4**	**4**	**4**	**4**	**4**	**4**	1	**4**	**4**	**4**	**4**	**4**	**4**	**4**	**4**	**4**

[1]Current sampling frequency: 1=once per month, 2=fortnightly, 4=once per week.

Table 21: Sampling frequencies (1 = once per month, 2 = every fortnight; 4 = every week) for sampling schemes with the maximum risk of non-detection of positive PSP samples (PSP > 0µg/100g)) set at 5 or 1%, for PSP in mussels. Sampling frequencies exceeding once per month are shown in bold.

		max risk 5%												max risk 1%											
		J	F	M	A	M	J	J	A	S	O	N	D	J	F	M	A	M	J	J	A	S	O	N	D
	Current frequency[1]	**4**	**4**	**4**	**4**	**4**	**4**	**4**	**4**	**4**	**4**	**4**	**4**	**4**	**4**	**4**	**4**	**4**	**4**	**4**	**4**	**4**	**4**	**4**	**4**
ID	Site																								
G80	Eastcoast	1	1	1	1	**4**	**4**	1	1	1	1	1	1	1	1	1	1	**4**	**4**	**4**	**4**	**4**	**2**	1	1
G26	Dumfries	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
G8	Ayr-LochStriven	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	**4**	**4**	**2**	**4**	**2**	1	1	1
G16	WC-LochFyne	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
G10	WC-LochEtive	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
G9	WC-LochCreran	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
P5	Mull-LochSpelve	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
P7	Mull-LochScridain	1	1	1	1	**4**	**4**	**2**	**4**	**2**	1	1	1	1	1	1	**2**	**4**	**4**	**4**	**4**	**4**	**4**	1	1
G1	Mull-LochnaKeal	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	**4**	**4**	**2**	**4**	**2**	1	1	1
G31	WC-LochLeven	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
G28	WC-Lochaber	1	1	1	1	**4**	**4**	**2**	**2**	**2**	1	1	1	1	1	1	**2**	**4**	**4**	**4**	**4**	**4**	**4**	1	1
G41	Skye	1	1	1	1	**4**	**4**	**4**	**4**	**2**	1	1	1	**2**	1	1	**4**	**4**	**4**	**4**	**4**	**4**	**4**	1	1
G35	NWC-LochTorridon	1	1	1	1	**4**	**4**	1	1	1	1	1	1	1	1	1	1	**4**	**4**	**4**	**4**	**4**	1	1	1
G39	NWC-Ullapool	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
G48	NWC-other	1	1	1	1	1	**2**	1	1	1	1	1	1	1	1	1	1	**4**	**4**	**4**	**4**	**4**	1	1	1
G23	Lewis-LochRoag	1	1	1	1	**2**	**2**	1	1	1	1	1	1	1	1	1	1	**4**	**4**	**4**	**4**	**4**	1	1	1
G21	LewisHarrisUist	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	**4**	**4**	**2**	**4**	**2**	1	1	1
G54	Orkney	1	1	1	1	**4**	**4**	**4**	**4**	**2**	1	1	1	**2**	1	1	**4**	**4**	**4**	**4**	**4**	**4**	**4**	1	1
G57	Shetland-SE	1	1	1	1	**4**	**4**	1	1	1	1	1	1	1	1	1	1	**4**	**4**	**4**	**4**	**4**	1	1	1
P61	Shetland-SW-Gruting	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	**4**	**4**	1	1	1	1	1	1
P68	Shetland-SW-Vaila	1	1	1	1	**4**	**4**	**4**	**4**	**4**	1	1	1	**2**	1	1	**4**	**4**	**4**	**4**	**4**	**4**	**4**	1	1
G58	Shetland-W	1	1	1	1	**4**	**4**	**4**	**4**	**4**	1	1	1	**4**	1	1	**4**	**4**	**4**	**4**	**4**	**4**	**4**	1	1
G64	Shetland-NW	1	1	1	1	**4**	**4**	**4**	**4**	**4**	1	1	1	**4**	1	1	**4**	**4**	**4**	**4**	**4**	**4**	**4**	1	1
G56	Shetland-NE	1	1	1	1	**4**	**4**	**4**	**4**	**4**	1	1	1	**2**	1	1	**4**	**4**	**4**	**4**	**4**	**4**	**4**	1	1
P69	Shetland-N-Balta	1	1	1	1	**4**	**4**	**4**	**4**	**4**	1	1	1	**2**	1	1	**4**	**4**	**4**	**4**	**4**	**4**	**4**	1	1

[1]Current sampling frequency: 1=once per month, 2=fortnightly, 4=once per week.

Table 22: Sampling frequencies (1 = once per month, 2 = every fortnight; 4 = every week) for sampling schemes with the maximum risk of non-detection of positive ASP samples (ASP > 0µg/g) set at 5 or 1%, for ASP in mussels. Sampling frequencies exceeding once per month are shown in bold.

		max risk 5%												max risk 1%											
		J	F	M	A	M	J	J	A	S	O	N	D	J	F	M	A	M	J	J	A	S	O	N	D
	Current frequency[1]	1	1	1	**2**	**2**	**2**	**4**	**4**	**4**	**4**	**4**	1	1	1	1	**2**	**2**	**2**	**4**	**4**	**4**	**4**	**4**	1
ID	Site																								
G80	Eastcoast	**4**	**4**	**4**	**4**	**4**	**4**	**4**	**4**	**4**	**4**	**4**	**4**	**4**	**4**	**4**	**4**	**4**	**4**	**4**	**4**	**4**	**4**	**4**	**4**
G26	Dumfries	**4**	**4**	**4**	**4**	**4**	**4**	**4**	**4**	**4**	**4**	**4**	**4**	**4**	**4**	**4**	**4**	**4**	**4**	**4**	**4**	**4**	**4**	**4**	**4**
G8	Ayr-LochStriven	**4**	**4**	**4**	**4**	**4**	**4**	**4**	**4**	**4**	**4**	**4**	**4**	**4**	**4**	**4**	**4**	**4**	**4**	**4**	**4**	**4**	**4**	**4**	**4**
G16	WC-LochFyne	**4**	**4**	**4**	**4**	**4**	**4**	**4**	**4**	**4**	**4**	**4**	**4**	**4**	**4**	**4**	**4**	**4**	**4**	**4**	**4**	**4**	**4**	**4**	**4**
G10	WC-LochEtive	**4**	**4**	**4**	**4**	**4**	**4**	**4**	**4**	**4**	**4**	**4**	**4**	**4**	**4**	**4**	**4**	**4**	**4**	**4**	**4**	**4**	**4**	**4**	**4**
G9	WC-LochCreran	**4**	**4**	**4**	**4**	**4**	**4**	**4**	**4**	**4**	**4**	**4**	**4**	**4**	**4**	**4**	**4**	**4**	**4**	**4**	**4**	**4**	**4**	**4**	**4**
P5	Mull-LochSpelve	**4**	**4**	**4**	**4**	**4**	**4**	**4**	**4**	**4**	**4**	**4**	**4**	**4**	**4**	**4**	**4**	**4**	**4**	**4**	**4**	**4**	**4**	**4**	**4**
P7	Mull-LochScridain	**4**	**4**	**4**	**4**	**4**	**4**	**4**	**4**	**4**	**4**	**4**	**4**	**4**	**4**	**4**	**4**	**4**	**4**	**4**	**4**	**4**	**4**	**4**	**4**
G1	Mull-LochnaKeal	**4**	**4**	**4**	**4**	**4**	**4**	**4**	**4**	**4**	**4**	**4**	**4**	**4**	**4**	**4**	**4**	**4**	**4**	**4**	**4**	**4**	**4**	**4**	**4**
G31	WC-LochLeven	**4**	**4**	**4**	**2**	**4**	**4**	**4**	**4**	**4**	**4**	**4**	**4**	**4**	**4**	**4**	**4**	**4**	**4**	**4**	**4**	**4**	**4**	**4**	**4**
G28	WC-Lochaber	**4**	**4**	**4**	**4**	**4**	**4**	**4**	**4**	**4**	**4**	**4**	**4**	**4**	**4**	**4**	**4**	**4**	**4**	**4**	**4**	**4**	**4**	**4**	**4**
G41	Skye	**4**	**4**	**4**	**4**	**4**	**4**	**4**	**4**	**4**	**4**	**4**	**4**	**4**	**4**	**4**	**4**	**4**	**4**	**4**	**4**	**4**	**4**	**4**	**4**
G35	NWC-LochTorridon	**4**	**4**	**4**	**4**	**4**	**4**	**4**	**4**	**4**	**4**	**4**	**4**	**4**	**4**	**4**	**4**	**4**	**4**	**4**	**4**	**4**	**4**	**4**	**4**
G39	NWC-Ullapool	**4**	**4**	**4**	**4**	**4**	**4**	**4**	**4**	**4**	**4**	**4**	**4**	**4**	**4**	**4**	**4**	**4**	**4**	**4**	**4**	**4**	**4**	**4**	**4**
G48	NWC-other	**4**	**4**	**4**	**4**	**4**	**4**	**4**	**4**	**4**	**4**	**4**	**4**	**4**	**4**	**4**	**4**	**4**	**4**	**4**	**4**	**4**	**4**	**4**	**4**
G23	Lewis-LochRoag	**4**	**4**	**4**	**4**	**4**	**4**	**4**	**4**	**4**	**4**	**4**	**4**	**4**	**4**	**4**	**4**	**4**	**4**	**4**	**4**	**4**	**4**	**4**	**4**
G21	LewisHarrisUist	**4**	**4**	**4**	**4**	**4**	**4**	**4**	**4**	**4**	**4**	**4**	**4**	**4**	**4**	**4**	**4**	**4**	**4**	**4**	**4**	**4**	**4**	**4**	**4**
G54	Orkney	**4**	**4**	**4**	**4**	**4**	**4**	**4**	**4**	**4**	**4**	**4**	**4**	**4**	**4**	**4**	**4**	**4**	**4**	**4**	**4**	**4**	**4**	**4**	**4**
G57	Shetland-SE	**4**	**4**	**4**	**4**	**4**	**4**	**4**	**4**	**4**	**4**	**4**	**4**	**4**	**4**	**4**	**4**	**4**	**4**	**4**	**4**	**4**	**4**	**4**	**4**
P61	Shetland-SW-Gruting	**4**	**4**	**4**	**4**	**4**	**4**	**4**	**4**	**4**	**4**	**4**	**4**	**4**	**4**	**4**	**4**	**4**	**4**	**4**	**4**	**4**	**4**	**4**	**4**
P68	Shetland-SW-Vaila	**4**	**4**	**4**	**4**	**4**	**4**	**4**	**4**	**4**	**4**	**4**	**4**	**4**	**4**	**4**	**4**	**4**	**4**	**4**	**4**	**4**	**4**	**4**	**4**
G58	Shetland-W	**4**	**4**	**4**	**4**	**4**	**4**	**4**	**4**	**4**	**4**	**4**	**4**	**4**	**4**	**4**	**4**	**4**	**4**	**4**	**4**	**4**	**4**	**4**	**4**
G64	Shetland-NW	**4**	**4**	**4**	**4**	**4**	**4**	**4**	**4**	**4**	**4**	**4**	**4**	**4**	**4**	**4**	**4**	**4**	**4**	**4**	**4**	**4**	**4**	**4**	**4**
G56	Shetland-NE	**4**	**4**	**4**	**4**	**4**	**4**	**4**	**4**	**4**	**4**	**4**	**4**	**4**	**4**	**4**	**4**	**4**	**4**	**4**	**4**	**4**	**4**	**4**	**4**
P69	Shetland-N-Balta	**4**	**4**	**4**	**2**	**4**	**4**	**4**	**4**	**4**	**4**	**4**	**4**	**4**	**4**	**4**	**4**	**4**	**4**	**4**	**4**	**4**	**4**	**4**	**4**

[1]Current sampling frequency: 1=once per month, 2=fortnightly, 4=once per week.

Positive ASP in mussels

Weekly sampling throughout the year at all sites would be required to keep the risk of not detecting positive ASP mussel samples below 5% (Table 22). However it should be remembered that positive samples are classed as those that have any level of toxin present. Due to the very limited number of samples (3 out of 3791) exceeding field closure levels, it was not possible to develop site- and month-specific models for the risk of not detecting levels of ASP toxins in mussels that exceed field closure levels. Based on simple models that ignore site and month effects (see Table 14) however, it follows that monthly sampling would suffice to keep the risk of non-detection below 1%.

5 DISCUSSION

Analysis of the 2004/6 toxin data and comparing results to the earlier findings from 2001/4 (Holtrop & Horgan, 2004) made it clear that toxin patterns had changed. For example, DSP had a much shorter peak season in 2004/6 compared to 2001/4, while PSP now showed a second peak in late summer, which was not seen in the earlier data. In order to better understand variation between years, and how it may affect predictions for future years, it was decided to analyse the 2001/6 data in a combined manner.

For DSP, PSP and ASP in mussels, and ASP in king scallop gonads and pacific oysters, models were formulated containing terms to allow for random variation between years and sites, as well as an effect for month of the year. The estimated prevalence shows good agreement with the data (e.g. Figures 7, 15, 17 and 20).

5.1 Issues arising from data and models

Assigning data to pods
The pod identification system was introduced in November 2006, which meant that previous samples had to be assigned to pods retrospectively. This was done by FSAS, and while every care was taken in doing so, lack of unique location identifiers in the older data means that some errors may have been introduced. Furthermore, new 'pods' were introduced (assigned numbers of 100 and above) for those sites not covered by the current pod identification system.

Combining pods
To allow for fitting models that contain effects for month, site and year, at least 1 to 2 samples per month per year per site are required. Unfortunately not all pods met these requirements and, as previously indicated, this meant that pods with insufficient numbers of samples had to be combined with neighbouring pods (a group of pods is referred to as a 'site'). Care was taken to combine pods only when their toxin patterns appeared similar. Due to the limited data per pod, it was not always possible to assess the justification of combining pods in detail. Further analysis of the Shetland mussel data revealed that for DSP, two of the pods that were combined in the Scotland-wide analysis were probably somewhat dissimilar in their DSP prevalence. This demonstrates that grouping of pods needs to be reassessed regularly. Note that when frequent data become available for each pod the grouping of pods will no longer be necessary.

Orkney
Orkney was an anomalous site in that data were only available for 2001 and 2004 (Figures A1 – A6) and could not be combined with other sites. To investigate how influential Orkney was in the analyses, the models were also fitted to mussel data excluding data from Orkney. It was found, however, that this did not alter the findings and therefore all results are presented that include Orkney.

Table 23: Rapid onset of PSP in mussels for Shetland pods in September 2006.

Site	ID	Pod	Date Collected	PSP µg/100g	#days previously
Shetland-NW	G64	64	17/07/06	0	
			07/08/06	45	21
			28/08/06	46	21
			05/09/06	287	8
			11/09/06	413	6
			18/09/06	226	7
			25/09/06	109	7
			02/10/06	50	7
			09/10/06	42	7
			16/10/06	38	7
			23/10/06	0	7
Shetland-NW	G64	70	08/08/06	0	
			14/08/06	36	6
			21/08/06	35	7
			28/08/06	0	7
			04/09/06	0	7
			12/09/06	138	8
			18/09/06	60	6
			25/09/06	48	7
			02/10/06	0	7
Shetland-W	G58	58	28/08/06	0	
			04/09/06	71	7
			11/09/06	321	7
			17/09/06	127	6
			20/09/06	127	3
			24/09/06	51	4
			27/09/06	57	3
			02/10/06	76	5
			03/10/06	47	1
			06/10/06	36	3
			09/10/06	45	3
			11/10/06	0	2
			15/10/06	36	4
			18/10/06	0	3

Rapid onset of PSP

Generally the onset of PSP is gradual in that it takes several weeks to move from zero to values exceeding the field closure limit. An exception was an outbreak in Shetland in September 2006, where toxin levels increased rapidly from zero to more than 100 µg/100g (e.g. Pod 70 in Table 23). Similar increases were found for Pods 64 and 58, also during the same time period. This highlights the importance of looking at toxin levels from several sites (or pods) simultaneously, so that if one site shows high levels then other sites in the proximity may be at risk of high toxin levels also.

Similarity of pods that are close together
Detailed analysis of the mussel data for Shetland showed that for PSP in mussels, especially for PSP > 40 and > 80 µg/100g, the pods within a group were more similar than between groups. For positive PSP, DSP and ASP samples, however, similarity of pods within a group was less pronounced. Differences between sites that are close geographically were also observed. For example Figure 9 shows how P61 and P68 (Shetland) had a large difference in prevalence, and the same is the case for P7, P5 and G1 (Mull). These findings suggest that the area covered by a 'pod-unit' cannot be enlarged without losing information on variability within such an area.

5.1.1 Absence of toxin for certain sites and certain months

Continual absence of PSP for several sites
From Table 7 it can be seen that for 7 sites the predicted probability of positive PSP results in mussels was less than 0.5%. When checking against the data (Table 4), we can see that all mussel samples from these 7 sites tested negative for PSP (i.e. 0 µg/100g). These sites were: Dumfries, WC-LochFyne, WC-LochEtive, WC-LochCreran, Mull-LochSpelve, WC-LochLeven and NWC-Ullapool. The numbers of samples tested for these sites were 90, 83, 112, 129, 79, 167 and 86, respectively. Of course it is important to ensure that these samples were not all taken during the winter months when reduced toxicity would be expected. Closer inspection of the data revealed that for each of these sites the samples cover all 6 years (except for 2004 when PSP testing was greatly reduced) and the samples were taken across all months of the year, so providing a good picture of the past 6 years (see also Figure A2 in Appendix A). In conclusion, both the model and the data indicate that for these 7 sites the incidence of PSP in mussels was negligible during 2001/6.

PSP absent in mussels for several months of the year
During February, March and December, the predicted probability of positive PSP test results in mussels was less than 0.5% (Table 7). When checking against the data, we find that no samples tested positive for PSP (out of 124, 131 and 80 samples, respectively). During January, although 1 out of 79 mussel samples tested positive, it was below 80µg/100g. These findings suggest that sampling frequency could be reduced during the winter months (December – March). Of course it is important to keep monitoring on a regular basis, and if the need arises, to increase monitoring frequency.

DSP absent in mussels for some months of the year
There is only one site that consistently tested negative (WC-LochEtive, 139 samples, Table 4) for DSP in mussels, and the predicted prevalence was less than 0.5% for this site (Table 8). For January and February, the predicted prevalence was less than 0.5% for all sites, and this was confirmed by the raw data, which showed that all sites tested negative for all five years (141 and 186 samples for January and February, respectively).

5.1.2 Comparison across species

For each toxin, models were fitted to each species and each toxin level separately. So, for PSP in mussels, separate models were fitted to PSP>0, PSP>40 and PSP>80 μg/100g. Despite this, agreement between the models was good (Figure s 8 and 23), with similar patterns across time, and with PSP prevalence estimated to be highest for PSP > 0 and lowest for PSP > 80 μg/100g. This is not surprising as it is more likely for a sample to exceed 0 than to exceed 80 μg/100g.

When comparing the DSP toxin patterns across species (Figure 21) it can be seen that for those species for which a Year effect could be estimated, the trend is similar, namely a decline in DSP from 2001 to 2006. For ASP (Figures 22 and 24) similar agreement between species was found.

Such similarities are due to all shellfish species being exposed to the same source of toxin, namely algae in the water. Although the rates of accumulation and clearance vary between shellfish species, the models used were not refined enough to detect such differences. This is because the change in toxicity was modelled on a month by month basis

The rates of accumulation and clearance may vary between shellfish species, which would imply that a peak in toxin levels may occur sooner for some species than for others. The models used in the present analyses did not detect such differences, this may indicate either that accumulation and clearance rates are not dissimilar between shellfish species, or it may be that the monthly time window used in the models was too large to detect such differences. Either way, these findings do not disagree with mussels forming an appropriate indicator species for monitoring toxin prevalence in shellfish.

Table 24: Sources of variation for various models fitted to toxin data. The variance components are on the log-odds scale.

		Sources of variation			
toxin level	Species	Year	Year by Month	Year by Site	Site
DSP>0	Mussels	0.38	1.56	1.58	0.85
PSP>0	Mussels	1.02	3.94		5.03
PSP>40	Mussels	1.80			5.75
PSP>80	Mussels	11.19			7.48
ASP>0	Mussels	2.16	1.49		0.17
ASP>10	King scallops	1.37			0.97
ASP>20	King scallops	3.45			0.32
ASP>0	Pacific oysters	1.69			0.03
ASP>0	Clams	1.86			
ASP>0	Cockles	0.14			
ASP>0	Queen scallops	2.74			
DSP>0	Pacific oysters	7.62			
DSP>0	Queen scallops	1.40			

5.1.3 Sources of random variation

Table 24 provides a summary of the various sources of random variation for each of the models fitted to the various toxin levels. The estimated variances are on the log-odds scale, and although their values do not have an easy direct interpretation, the magnitude of the value does relate directly to the magnitude of variation. This table reveals the following:

- For ASP in mussels, pacific oysters, and king scallops (especially ASP > 20μg/g) the variation between sites was estimated to be small. This was also reflected in the estimated prevalence of ASP (Figures 13, 16, 19)
- For PSP in mussels, the variation between sites was large. This was also reflected in the model estimates (Table 5, 6, 7), showing that PSP tends to be absent for some sites and prevalent for others.
- There was variation between years for all species/toxin combinations analysed. For DSP in pacific oysters and PSP>80 in mussels this is especially large, but to some extent this is due to the toxin being absent during certain years and present in others.
- The variation between years depends on the month of the year. For example, for PSP > 0 in mussels this variance component is quite large, and reflects that in earlier years PSP tended to be present in early summer and absent from September onwards, while in 2006 it was present in September.
- For DSP in mussels, the variation between years also depends on site. For example, WC-LochFyne (G16) had the highest prevalence of all sites in 2003, whereas in e.g. 2006 it was below average (Figure 10).

In our models we have assumed that we have no knowledge about the causes of such random variations. In reality, some of the variation between sites may be due to differences in geography, and variation between years may be due to different levels of toxins in algae. Inclusion of such aspects in the models would help to better understand and predict toxin prevalence but would require detailed knowledge of each of the sites and of algal blooms.

We focussed on one aspect of variation in more detail, namely random variation between years. This showed that, whilst ignoring other sources of random variation (including uncertainty associated with the model parameter estimates), the estimated prevalence could be increased by up to five-fold for an ‘extreme’ year.

Extreme year predictions

Some caution should be exercised when considering the ‘extreme year’ predictions. Figures 25-27 show the predicted prevalence of a toxin during an extreme year, i.e. a year during which toxin is very prevalent, where it was assumed that such a year only occurs once every 20 years. For these results, some of the months show an upper limit of nearly zero prevalence. For example, for DSP in mussels (Figure 25), the estimated prevalence is estimated to be nearly zero during January, even for an extreme year and assumes that future toxin patterns will be similar to those observed in 2001/6. From the model structure, given in equation (3), it follows that an extreme year will increase the odds in a multiplicative manner. If for January the odds are estimated to be close to zero, the effect of an extreme year will still result in nearly zero odds for January (as multiplication of zero by a number results in zero). In

reality, we do not know whether or not DSP will always be absent in January, and it seems unrealistic to assume that this would inevitably be the case.

Prediction of future toxin levels
For a future year, at a given site, the best prediction we can make about prevalence of e.g. DSP in mussels, will be based on the average prevalence from preceding years. For example, if we take an average site then prevalence would be 4% in May up to 13% in June and July (Table 8). When taking into account variation between years, however, we can also look at the expected toxin levels during an extreme year (defined as a year for which toxin levels are unusually high, such a year is assumed to happen only once every 20 years, on average). This is shown in Figure 25, which shows that for an extreme year prevalence could be up to 60% or more for an average site. The alternative way of looking at this that, for an average site, on average, prevalence will be around 13% during the summer months, and will stay below 60% for most years.

5.1.4 Other model-related issues

Models allow for removal of bias
Models containing terms for site, year and month allow for removal of bias in the data caused by infrequent sampling. For example, for DSP in mussels, 9.5% of the data tested positive, but some of this was caused by more frequent sampling during periods when DSP was more likely to be present. Based on the model predictions, for an average site, an average year and an average month, the estimated prevalence of DSP was only 5%.

Inclusion of terms for Site, Year and Month in models
The reasons why some models include effects for Site, Year, Year by Month and Year by Site interactions whereas other models do not, are two-fold.

- It depends on the amount of data available. For example, if there are many sites which do not have data for all 6 years, a Year by Site interaction can not be estimated.
- It depends on whether both absence and presence of the toxin occur for each site and each year, as data are only informative if there are both positive and negative samples. If a toxin is always absent, then, even if the number of samples is large for each site for each year, it will not be possible to model the effects of site, year and their interactions as each of these will be estimated to be (close to) zero.

Model assumptions
The statistical analyses are based on the following assumptions.

1. It was assumed that the toxin levels found in the tested sample are representative of the shellfish toxin levels at that particular site. For example, if a sample tends to be taken from a location such that it is less likely to contain high toxin levels, then this would result in actual toxin levels being underestimated.
2. It was assumed that the test result is correct, i.e. falls in the correct ‘zone’. For example, if the test result was 69 µg PSP /100g then it was assumed that the

true toxin level would have been between 40 and 80µg/100g. It is known that, at least for PSP, the variation in test results is large. For example, when the true toxicity is 80µg/100g the estimated toxicity can vary from 40 to 120 µg/100g (Holtrop et al., 2004, 2006). Likewise, toxin levels are known to vary within a field. These factors may result in a situation that a test result is below field closure, while in reality average toxin levels are above field closure (or vice versa). Such variation in test results has been ignored in the models used in this study.

3. It was assumed that repeated testing following field closure did not bias the data. When toxin levels in a sample exceed the statutory field closure limit, the field is closed until two consecutive samples, at least one week apart, are both below the limit. As shellfish farmers are likely to be keen for the field to be reopened, they may want to send off weekly samples following closure of the field. This has the potential for the data to be biased towards high toxin levels, as there might have been relatively more samples on occasions when toxin levels exceeded the field closure limit. If this were to be the case, however, the model results will err on the safe side as estimated prevalence of toxin would have been overestimated.
4. It was assumed that the random effects were normally distributed on the log-odds scale.

Violation of assumptions 1 and 3 could have consequences for the estimated chance of the field being toxic. No information on assumption 1 is available but inspection of the data for the validity of assumption 3 revealed that there was no significant increase in sampling effort following a positive sample. The data were also inspected for temporal autocorrelation, which might be thought of as positive or negative samples would tend to be followed by similar samples after a short time interval. After allowing for site and month effects, however, there was no clear indication of such patterns in the data for any of the toxins. Assumption 4 is hard to test formally but informal inspection of the estimated values and residual plots indicated that this assumption was reasonable.

More complex models

The toxicity data were regarded as binary data (i.e. only two possible outcomes, namely toxin levels above or below field closure limit), resulting in relatively simple models of toxicity. Future extensions could be to model toxin levels (PSP>0, PSP>40 and PSP>80) simultaneously by means of multinomial models with random effects. Other possibilities are to develop models that describe actual toxicity levels (as opposed to the current field open or closed approach), smooth changes of toxicity with time, modelling of relationships between toxin patterns of neighbouring sites, and inclusion of temporal autocorrelation in the models.

To aid the decision-making processes involved in field closures and safe harvesting of shellfish, it might be useful to create a website where recent test results are available for each pod along with the (estimated) test results from previous years from the same period. The models developed so far could then be used to forecast expected toxin patterns for the next two weeks, say, based on the most recent test results and corresponding model results from previous years.

Despite the limitations of the data, which necessitated grouping of bed locations and aggregation of data to monthly values, we are confident that the analyses presented in this report give a good indication of when and where high toxin levels did occur during 2001-2006.

5.2 Issues arising from risk assessment

The risk assessment was concerned with asking: what is the chance of failing to detect a toxic event for the present and alternative monitoring schemes? It was assumed that the monitoring scheme is solely concerned with monitoring of toxin levels. Field closure and retesting following closure (so that field can be reopened when two successive results, at least 7 days apart, are below the statutory limit) were not considered in the risk assessment. As a consequence, it was assumed that monitoring is continued at its prescribed frequency even when a field is closed.

It was assumed that a test result is valid for one week. As a consequence, weekly sampling implies that the risk of non-detection is zero. In reality this may not hold, but as in practical terms it is not feasible to increase the monitoring frequency to more than once every week this assumption seems unavoidable.

5.2.1 Risk of non-detection under the current (2006) monitoring scheme

The risk of non-detection was zero for PSP in mussels, as weekly monitoring is taking place. It seems, however, that monitoring could be reduced for certain sites and during October – April (see Table 18), when monthly sampling would still result in a risk of non-detection of 1% or less.

For DSP in mussels the current sampling frequency is weekly during April – November. This is in agreement with a risk of non-detection of 1% or less (see Table 19). During December, when fortnightly sampling takes place, the maximum risk of non-detection is nearly 4% for Ayr-LochStriven (Table 16), and if this were deemed too high a risk by FSAS then weekly sampling would be required for this site. During January – March the current frequency is once a month and the maximum risk of non-detection is 1.4% (Ayr-LochStriven). Overall, the sampling frequency for DSP seems appropriate except perhaps for December.

The current sampling frequency for ASP in king scallops is weekly and, as the prevalence of toxin levels exceeding field closure limits is high throughout the year, the current sampling frequency seems appropriate.

Flexible sampling frequencies

Analysis of the 2001/6 data has indicated that the prevalence of toxin can change from year to year, and the prevalence pattern during the 12 months of the year can change also. It is therefore important that sampling frequencies should be flexible in that they should be easily adaptable to more frequent sampling when toxin patterns rapidly change. It may also be possible to alternate sampling frequencies between pods that

are close together (and that have shown similar behaviour in the past), e.g. monthly sampling at pod A and monthly sampling at pod B, where the latter is off-set by a fortnight with respect to pod A. It may also be possible to combine testing methods, where the screening method could be used weekly and the regulatory test monthly. Furthermore, it may be possible to link algal monitoring test results with the shellfish monitoring programme.

As one aspect of the shellfish monitoring programme is to build information on changes in toxin levels throughout the year, shellfish sampling should take place at least once per month.

5.3 Future data

For future data it would be useful to annotate each sample, indicating whether it forms part of the regular monitoring regime or whether it is analysed at request of the shellfish farmer. It would also be useful to record what the status of the pod is with respect to 'above alert' level or field closure. These records will make it easier to assess the monitoring scheme as such.

To increase our understanding of duration of toxin outbreaks, and the speed at which an outbreak builds up and disappears, it would be useful to implement weekly monitoring at a limited number of locations. This would also provide information on autocorrelation patterns (i.e. dependency of present test result on last weeks' test result).

In addition, when further data become available, the following issues should be considered.

- Is the grouping of pod locations, as outlined in Table 1, still appropriate? In most cases, groupings were based on limited data, so when more data become available this should be re-checked. Also, toxin patterns may change for some locations but not for others, which may require a different grouping of sites.

- Have toxin patterns changed? For example, sites that were previously non-toxic may have become toxic. Furthermore, the onset and duration of toxic events may change.

If changes as described above are observed then the models that describe the probability of a sample being toxic should be revised. As more data will become available with time it may be feasible to develop more realistic statistical models that describe actual toxin levels (as opposed to below/above field closure limit in the present model) that change smoothly with time (as opposed to the monthly stepwise changes in the present model). Furthermore, based on the new modelling results, monitoring schemes should be reassessed for the risk of not detecting a toxic event.

The present data (April 2001 – March 2006) only allowed for modelling of year, site and month specific toxin levels for DSP and PSP in mussels, and ASP in scallop gonads and pacific oysters. When more data become available such detailed models may be possible also for other species/toxin combinations.

6 RECOMMENDATIONS

Due to the limited data available it should be borne in mind that a full picture of variation in toxicity between years has yet to be established.

- ASP levels in whole scallops almost always exceeded the field closure limit during 2001-2006. Therefore, it is recommended that whole scallops continue to be 'shucked' before being placed on the market for human consumption.

- Accurate monitoring of changes in toxin levels across Scotland throughout the year requires sampling to be carried out at least monthly for every site.

- The current (2006) sampling scheme for PSP, which comprises weekly sampling at all sites, could be relaxed for several sites and for certain months of the year, either by means of reduced sampling frequency or by means of testing based on cheaper screening methods.

- For DSP the current (2006) sampling scheme would require some minor modifications during certain months of the year.

- When more data become available it may be possible to focus more on forecasting of toxin levels, using data from the ongoing year to predict toxin levels for later that same year.

- Sampling schemes should be flexible and may require modification in future, as toxin patterns may change and information on distribution of toxin levels over years is limited.

- It is therefore recommended that the risk assessment presented in this report is updated either on a yearly basis, or after a further three years of monitoring data has been collected.

- It will be necessary for the Food Standards Agency to set acceptable levels for the risk of non-detection on which to base suitable sampling schemes for future monitoring of all three toxin groups.

7 ACKNOWLEDGEMENTS

The Food Standards Agency Scotland is thanked for funding this research.

8 REFERENCES

Holtrop, G, Petrie, J., McElhiney, J., Dennison, N. (2006) Can general anaesthesia be used for the Paralytic Shellfish Poison bioassay? Toxicon, 47, 336-347

Holtrop, G., & Horgan, G.W. (2004) Risk assessment of the FSA Scotland monitoring programme for biotoxins in shellfish harvested from classified inshore areas in Scotland: evaluation of the current scheme and development of improved alternatives based on historical data. Report to Food Standards Agency Scotland, Project code S01026. December 2004.

Holtrop, G., Petrie, J., & Dennison, N. (2004) Study to investigate the effect of general anaesthesia on the paralytic shellfish poison bioassay. Report to Food Standards Agency Scotland, Project code S02012. April 2004.

Lee, Y., & Nelder, J.A. (1996) Hierarchical generalized linear models. Journal of the Royal Statistical Society, series B 58: 619-678.

Lee, Y., & Nelder, J.A. (2001) Hierarchical generalized linear models: a synthesis of generalised linear models, random-effect models and structured dispersions. Biometrika 88: 987-1006.

McCullagh, P., & Nelder, J.A. (1989) Generalised Linear Models, 2nd edition, Chapman and Hall, London.

McKenzie, J.D., & Bavington, C. (2002) Measurement of domoic acid in King Scallops processed in Scotland. Report to Food Standards Agency Scotland, Project code S02011. June 2002.

APPENDIX A: RAW DATA

Figure A1: Proportion of mussel samples testing positive for DSP for each month for each site. Rows of sites start from bottom left with Eastcoast, each row up follows the coastline of Scotland approximately clockwise.

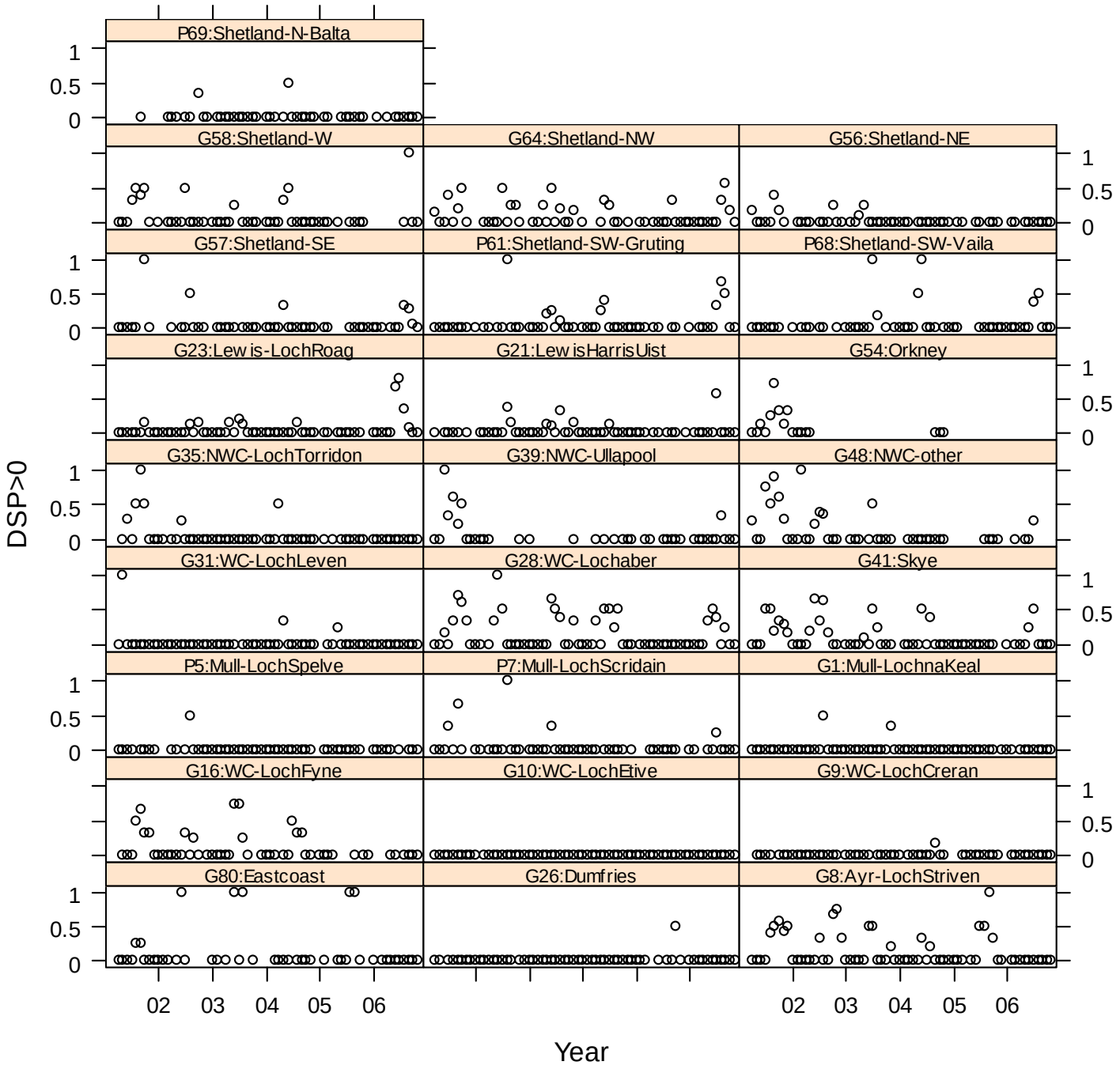


Figure A2: Proportion of mussel samples for which PSP > 0 µg/100g for each month for each site. Rows of sites start from bottom left with Eastcoast, each row up follows the coastline of Scotland approximately clockwise.

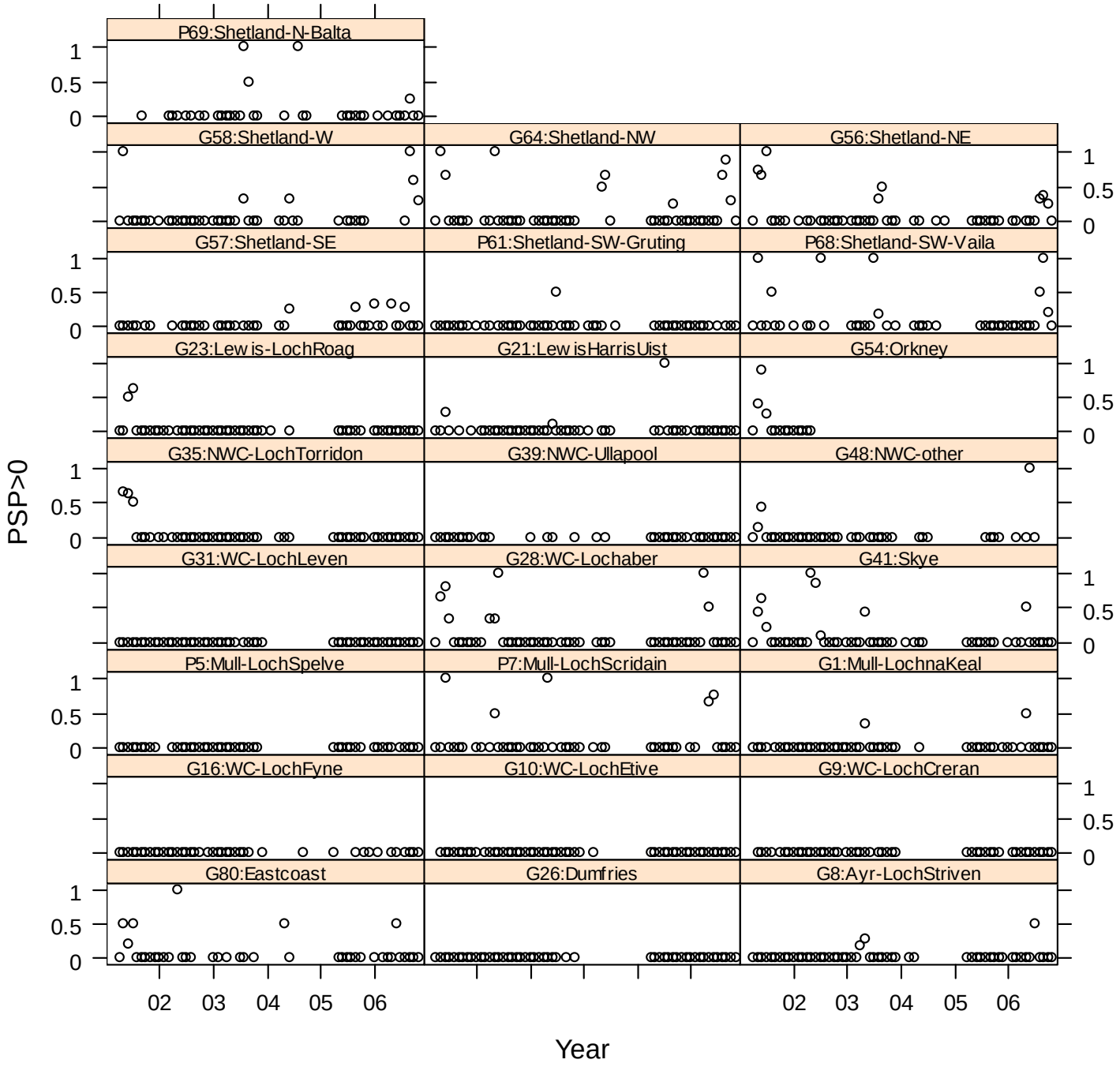


Figure A3: Proportion of mussel samples for which PSP > 40 µg/100g for each month for each site. Rows of sites start from bottom left with Eastcoast, each row up follows the coastline of Scotland approximately clockwise.

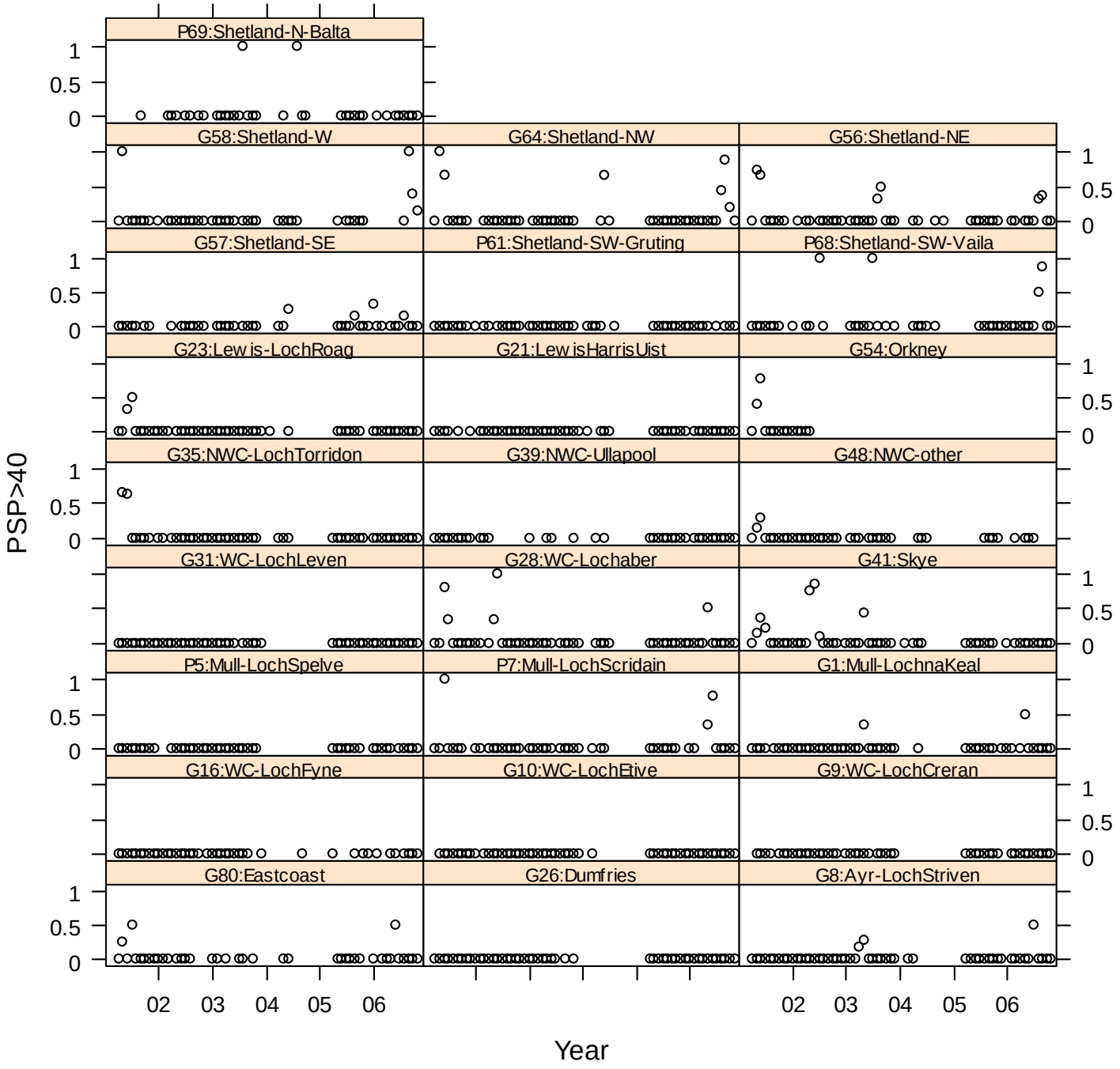


Figure A4: Proportion of mussel samples for which PSP > 80 µg/100g for each month for each site. Rows of sites start from bottom left with Eastcoast, each row up follows the coastline of Scotland approximately clockwise.

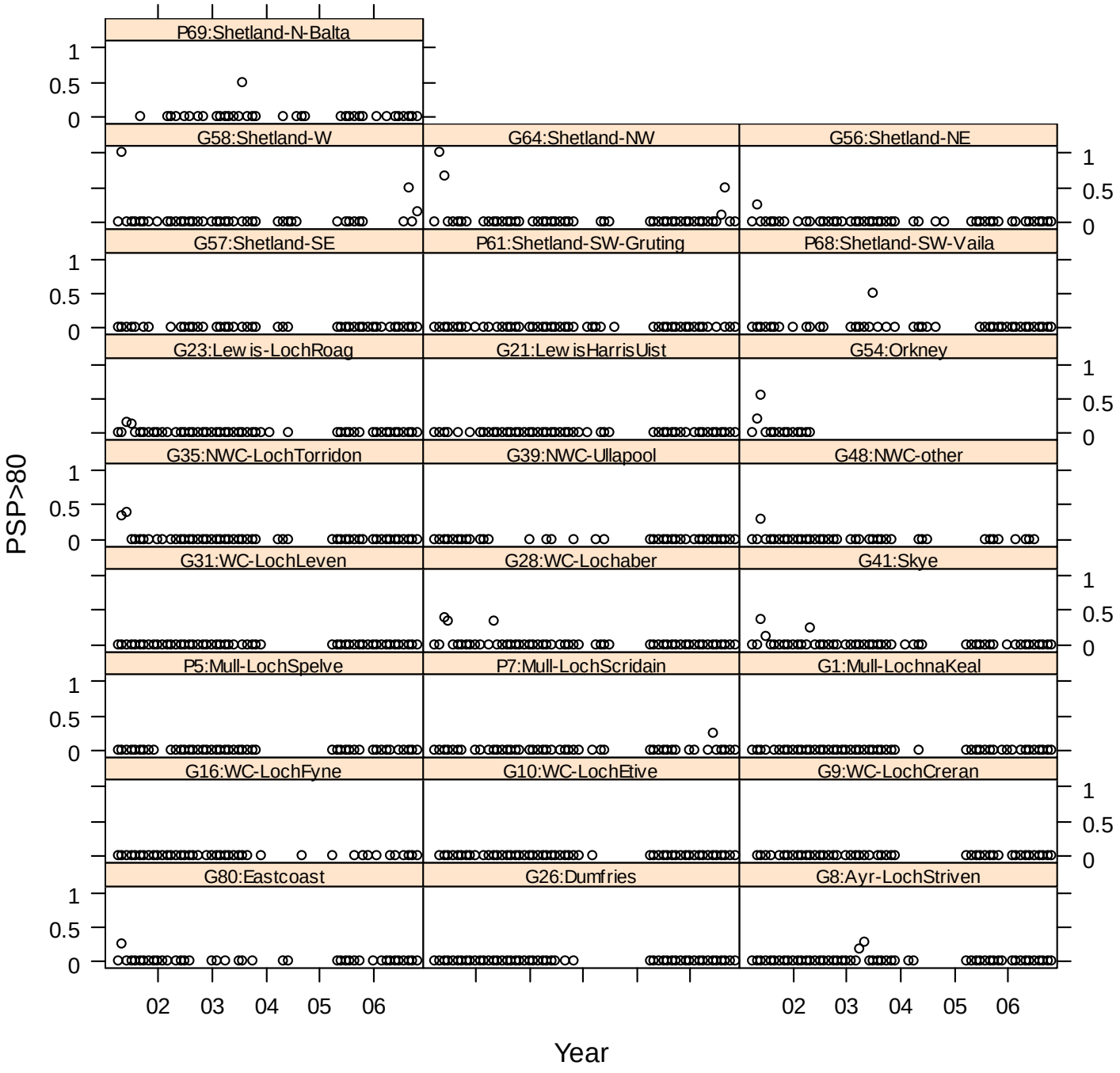


Figure A5: Proportion of mussel samples for which ASP > 0 µg/g for each month for each site. Rows of sites start from bottom left with Eastcoast, each row up follows the coastline of Scotland approximately clockwise.

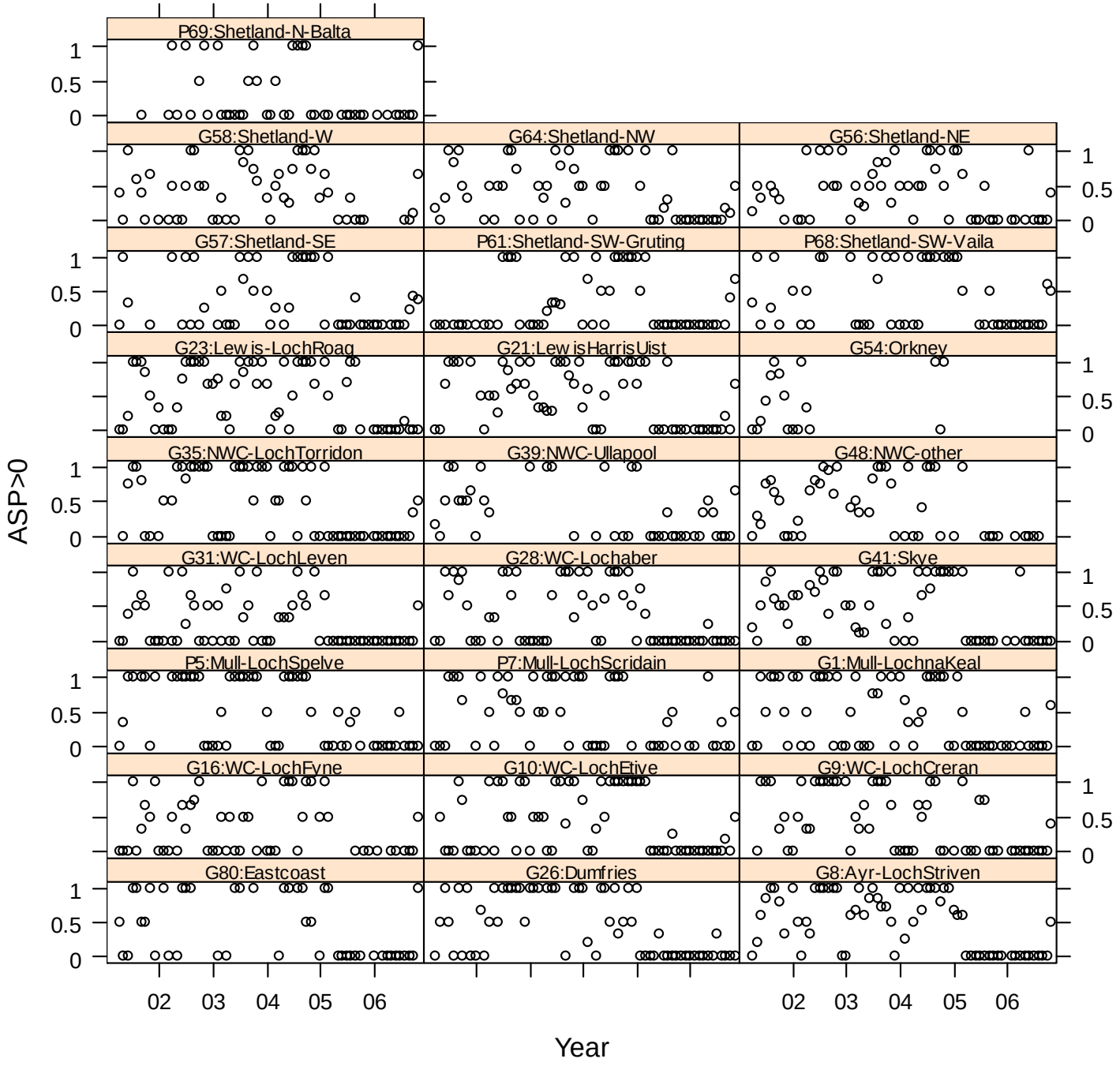


Figure A6: Proportion of mussel samples for which ASP > 10 µg/g for each month for each site. Rows of sites start from bottom left with Eastcoast, each row up follows the coastline of Scotland approximately clockwise.

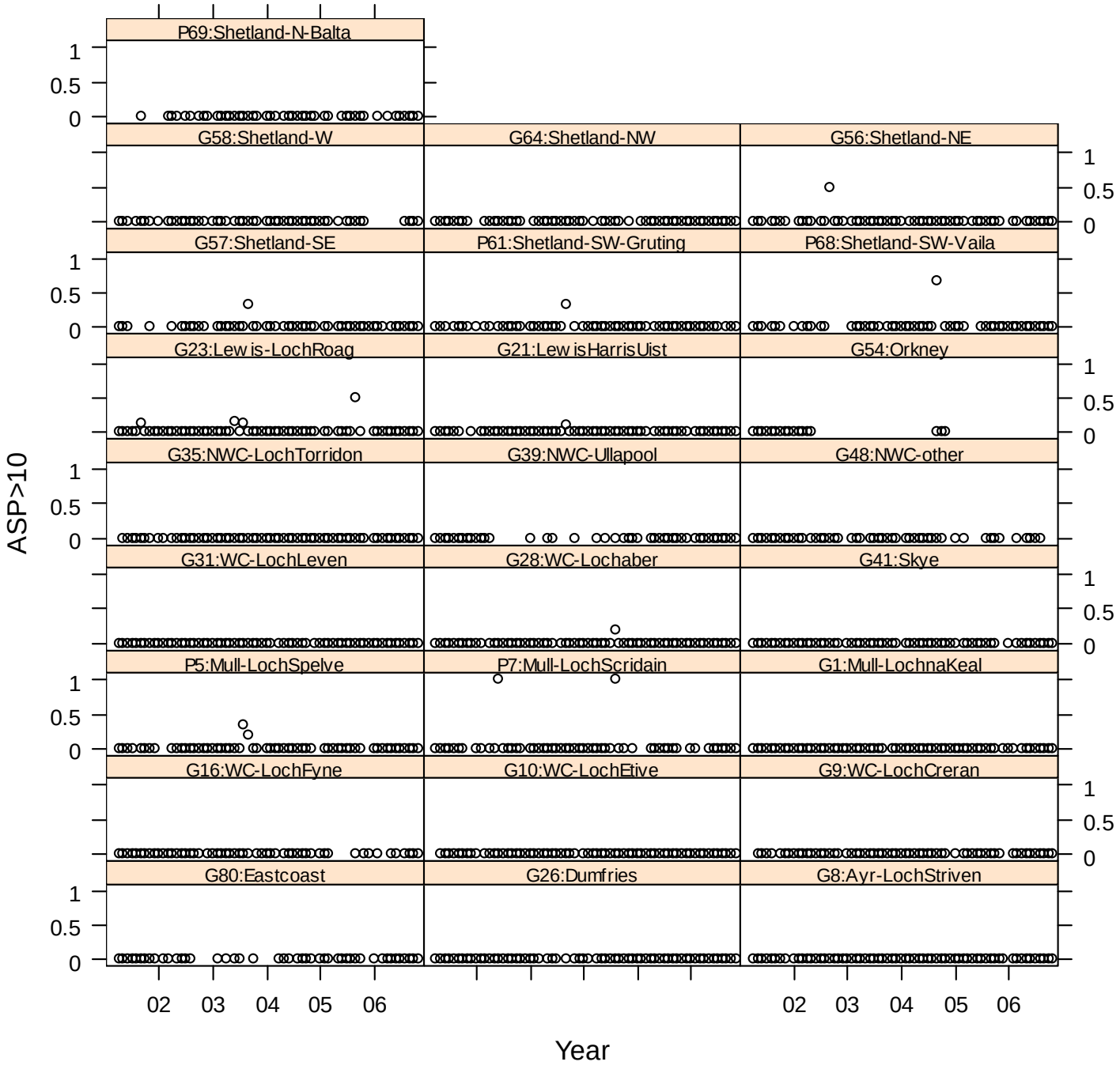


Figure A7: Proportion of king scallop gonad samples for which ASP > 20 µg/g for each month for each site.

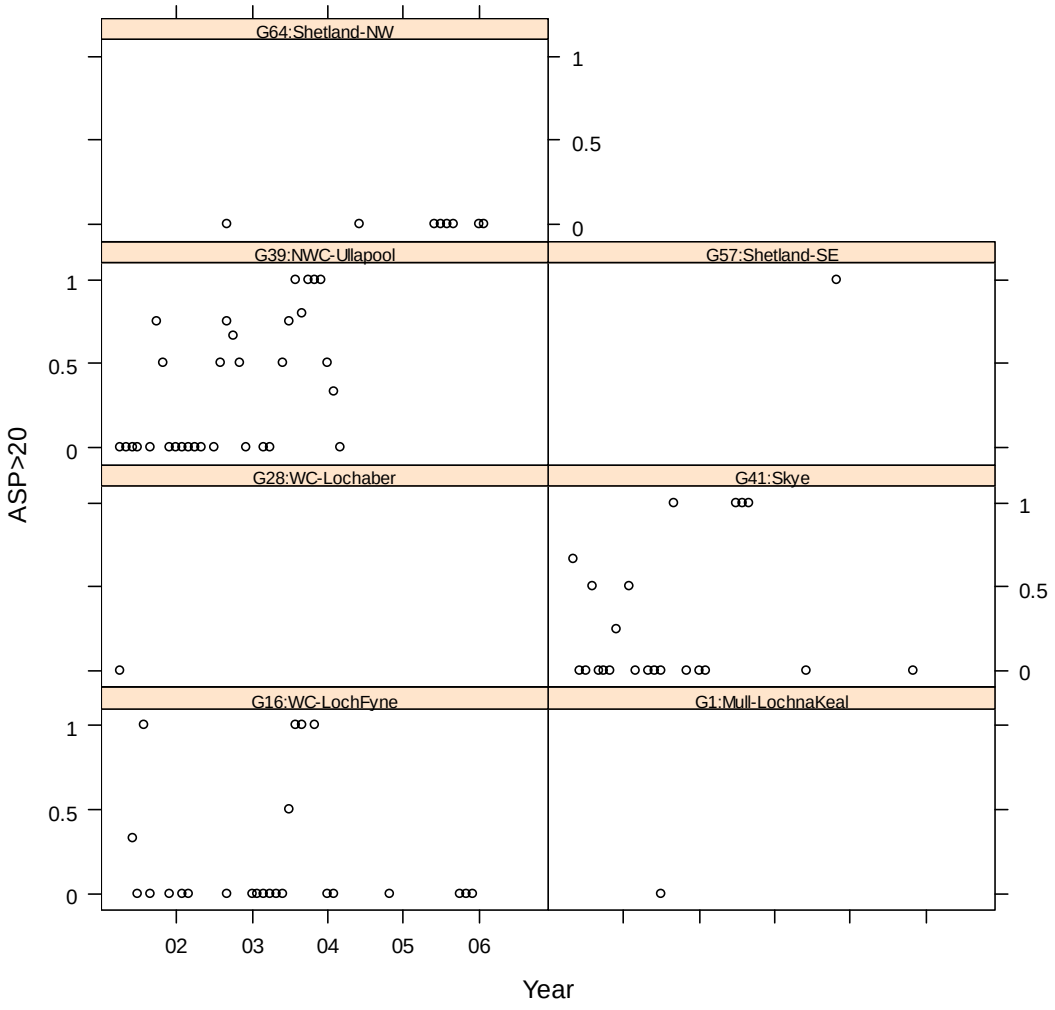


Figure A8: Proportion of king scallop gonad samples for which ASP > 10 µg/g for each month for each site.

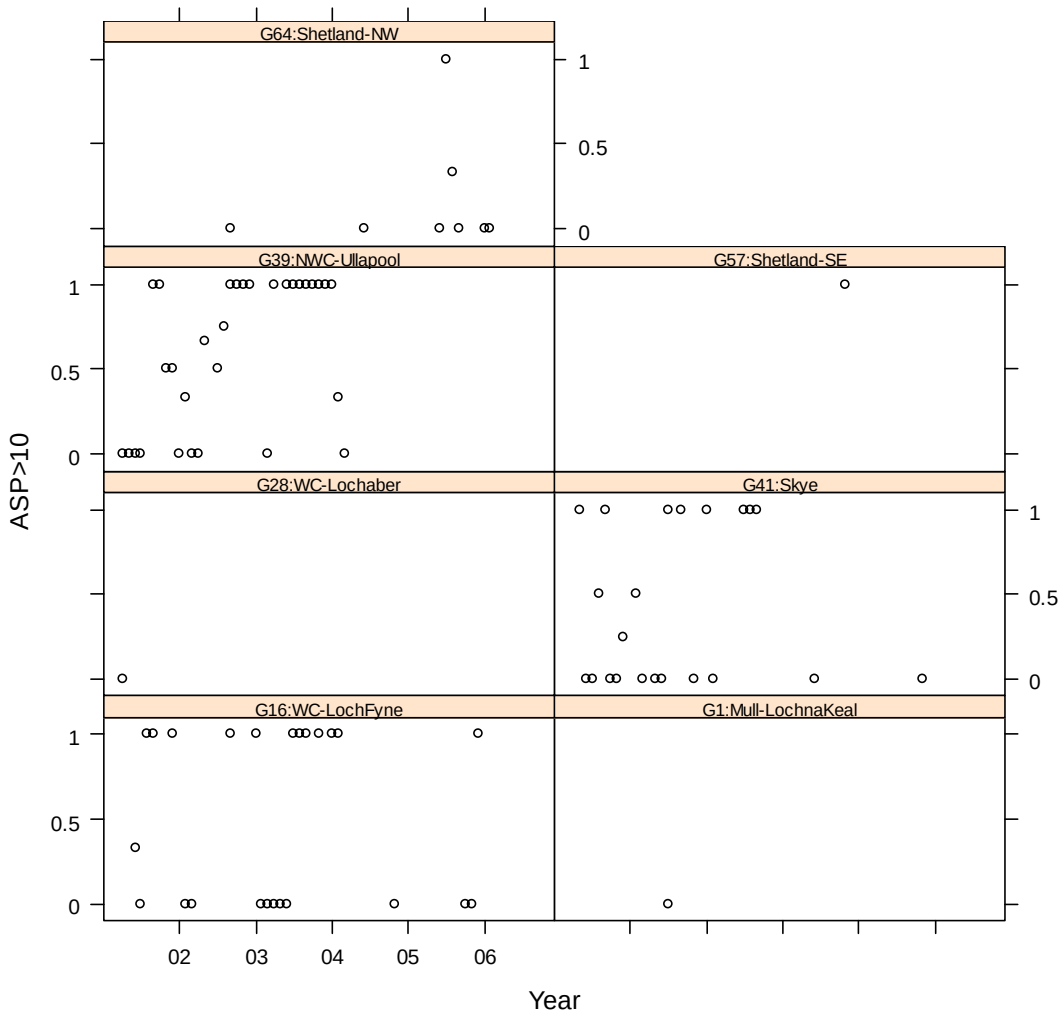


Figure A9: Proportion of pacific oysters samples for which ASP > 0 µg/g for each month for each site.

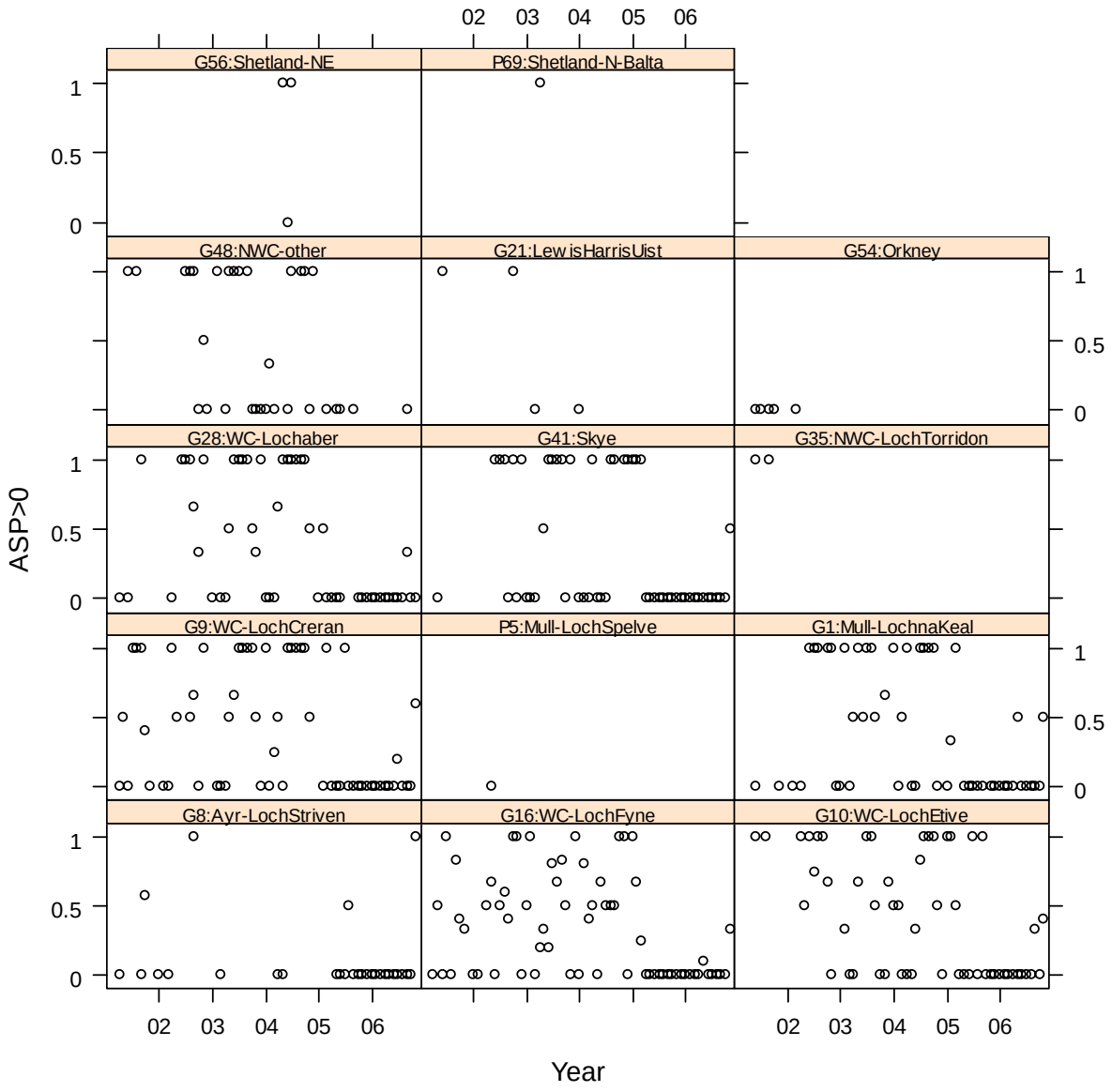


Figure A10: Proportion of clam samples for which ASP > 0 µg/g for each month for each site.

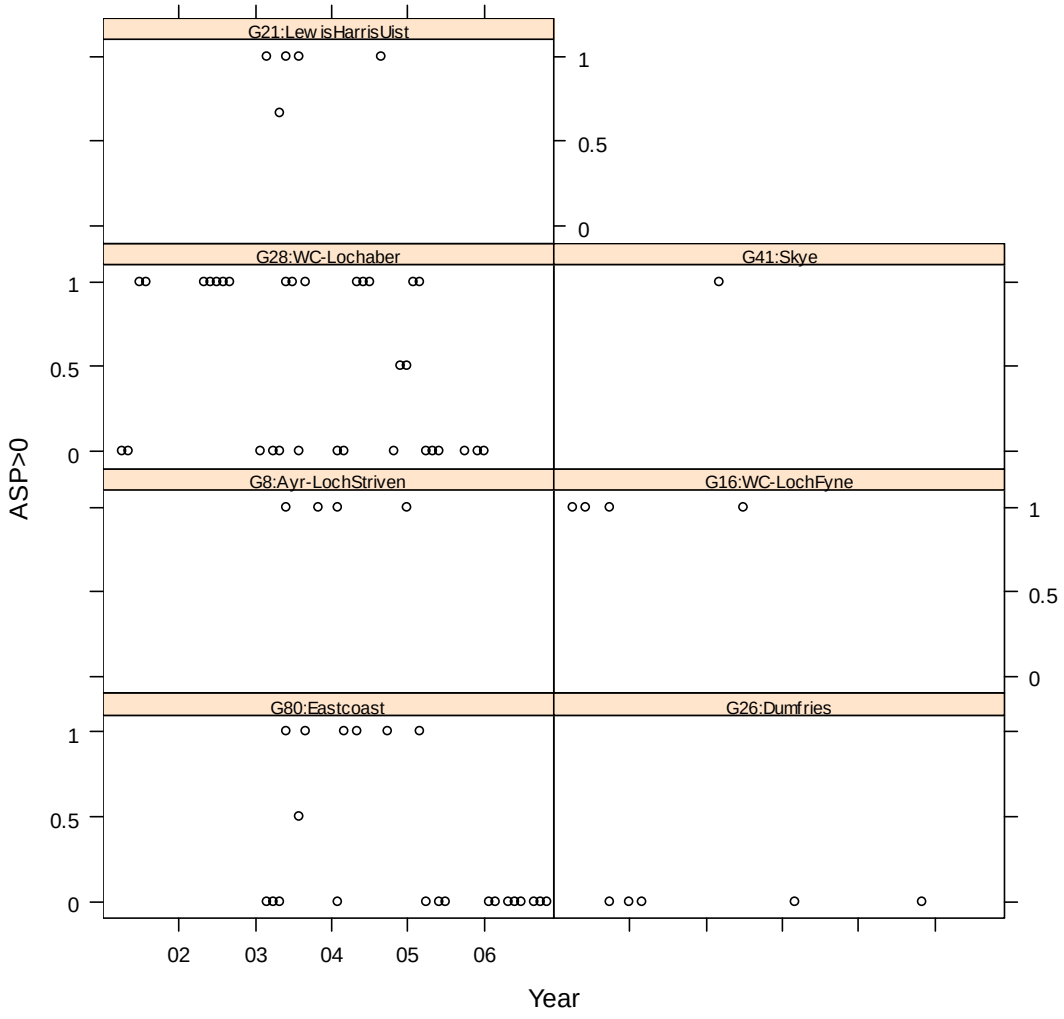


Figure A11: Proportion of cockle samples for which ASP > 0 µg/g for each month for each site.

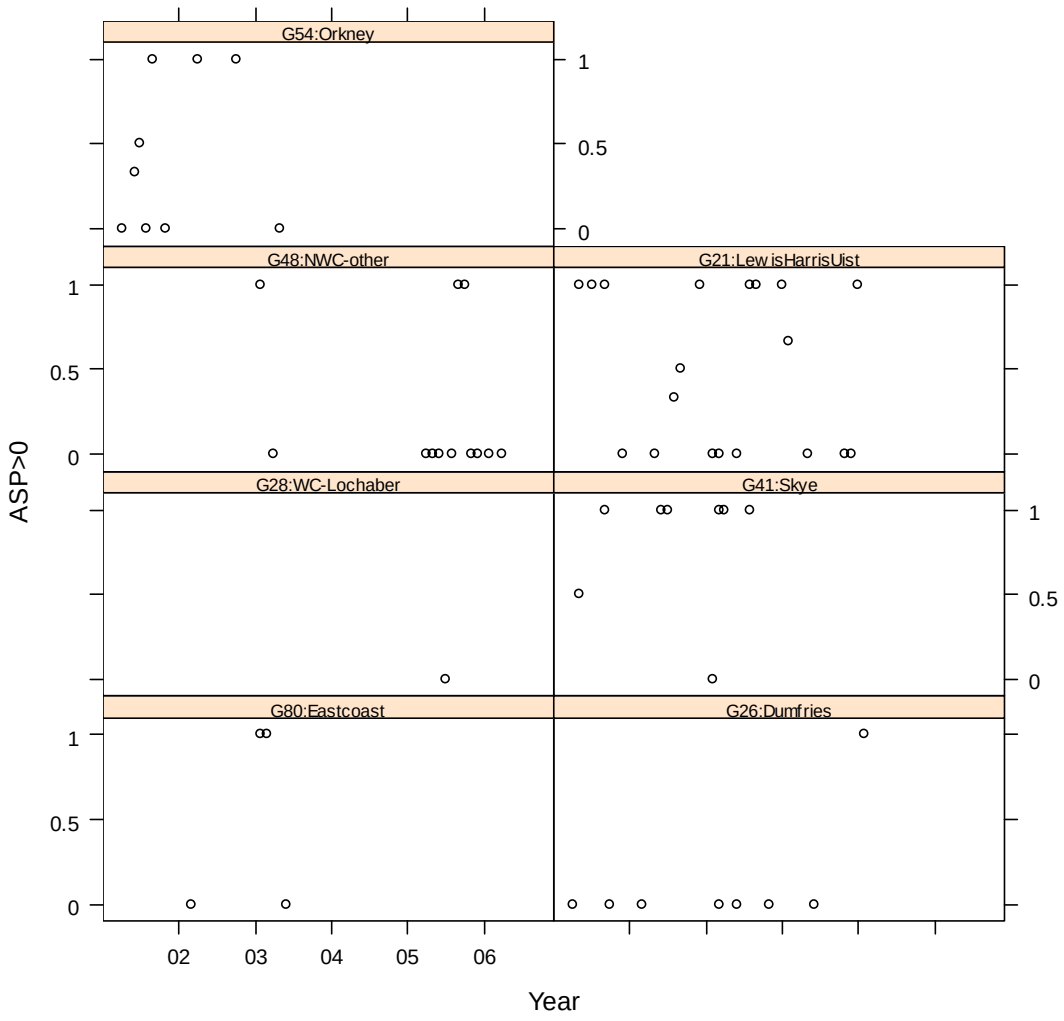


Figure A12: Proportion of queen scallop samples for which ASP > 0 µg/g for each month for each site.

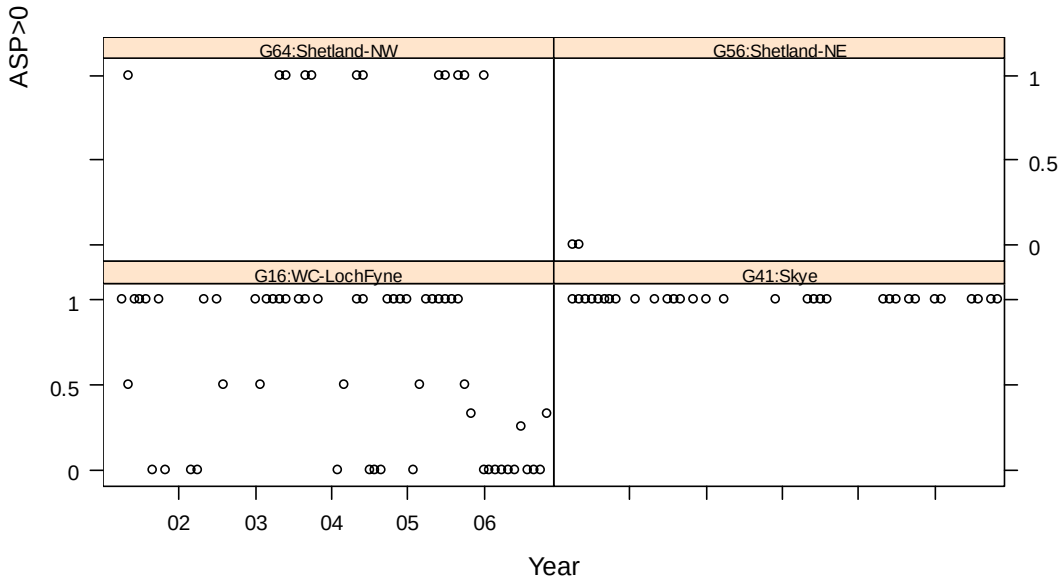


Figure A13: Proportion of pacific oysters samples for which DSP was positive for each month for each site.

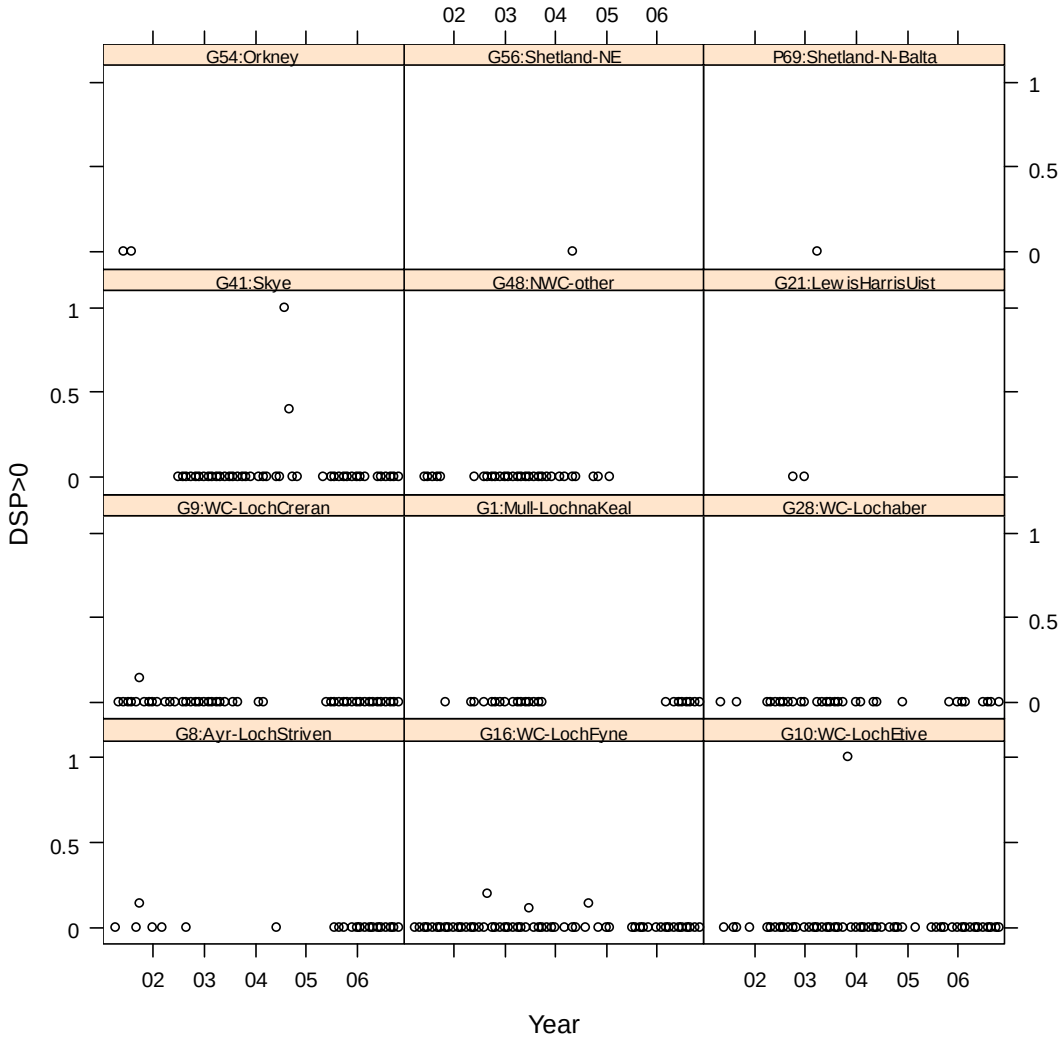
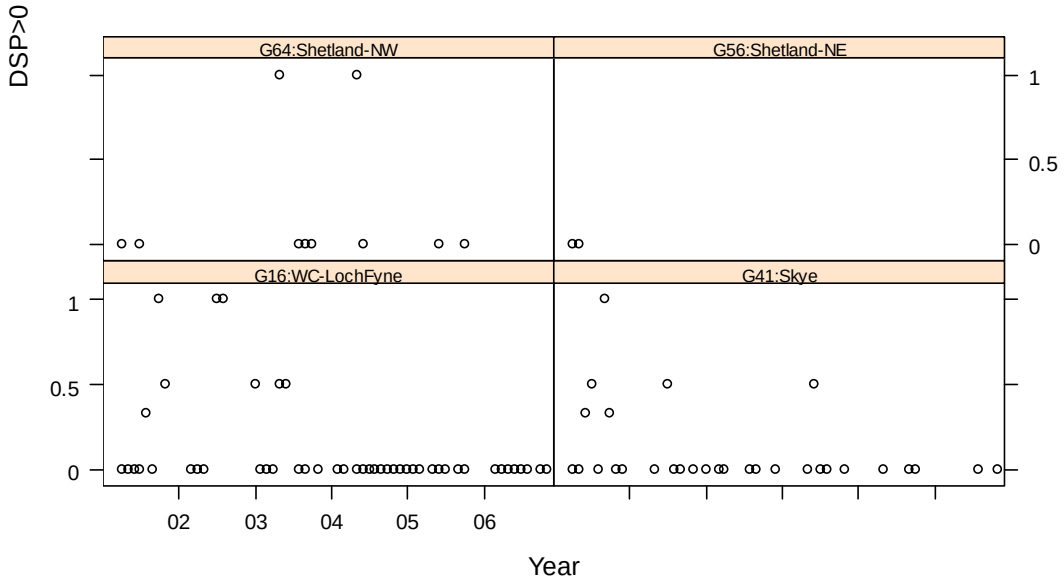


Figure A14: Proportion of queen scallop samples for which DSP was positive for each month for each site.



APPENDIX B: MODELS FOR ASP > 5 UG/G IN MUSSELS

B.1 Introduction

Amnesic Shellfish Poison (ASP) levels in mussels are mainly less than 10 µg/g and often > 0µg/g. Results in the present report have shown that neither of these cut-offs are very informative for detecting patterns of ASP in mussels over time and location. Therefore, this Appendix looks at ASP in mussels exceeding 5µg/g, using data from April 2001 to November 2006. The work presented in this Appendix was conducted after the original report had been written, and any findings and conclusions are in addition to those presented in the main report.

B.2 Results

Data
Figure B1 shows the proportion of mussel samples for which ASP > 5µg/g. Of the 25 sites[3], 8 sites had no samples exceeding 5µg/g. These where Shetland-N-Balta, Orkney, NWC-LochTorridon, NWC-Ullapool, WC-LochLeven, Eastcoast, Dumfries and Ayr-LochStriven.

Of the 3791 mussel samples tested, 54 exceeded 5µg/g (Table B1). The percentage of mussel samples exceeding 5 µg/g peaked in 2003 and 2004 (2.2 and 2.1%, respectively) and was low in 2001 (0.8%) and 2006 (0.1%, see Table B1).

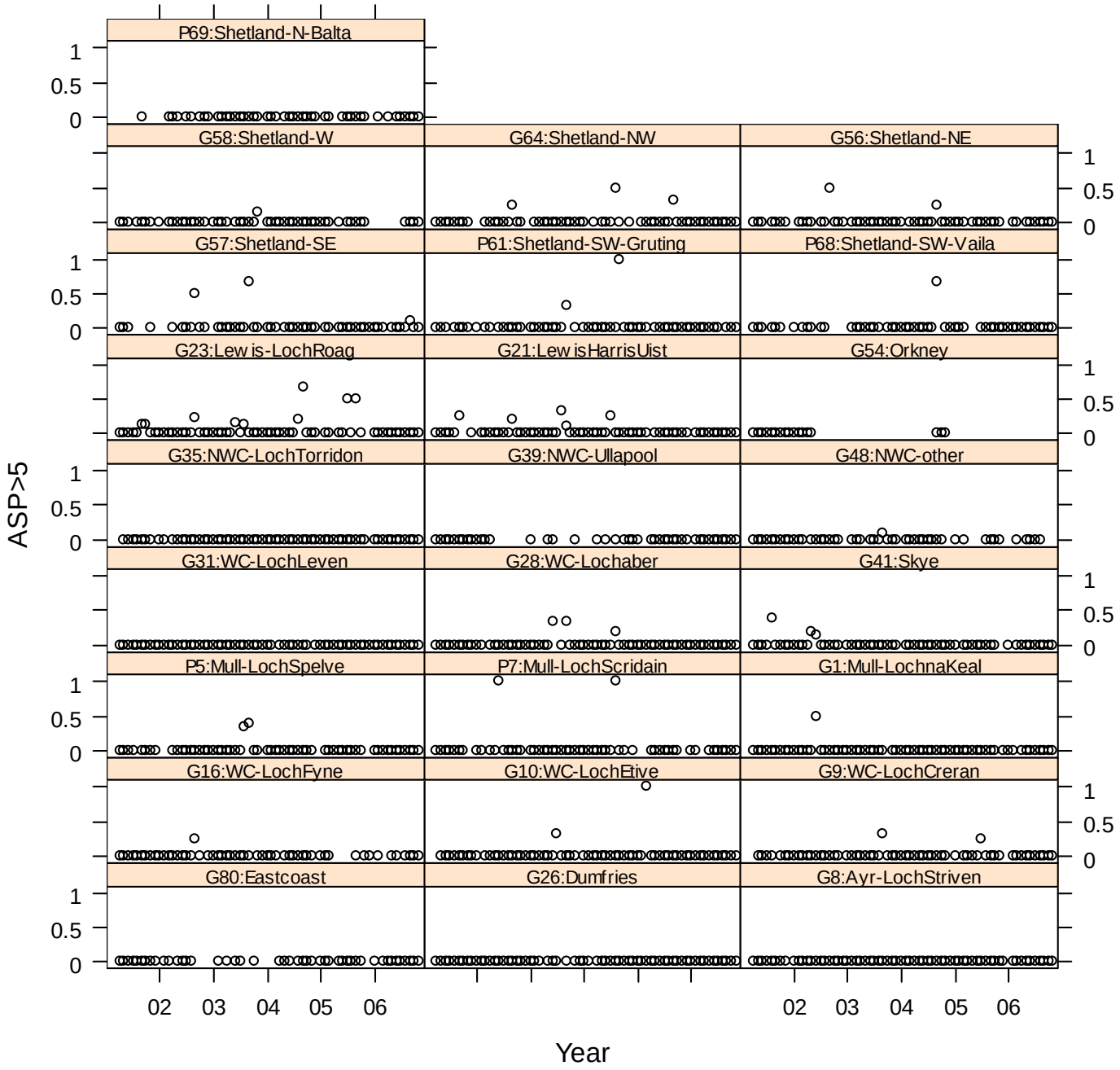
Table B1: Number of mussel samples per ASP toxin level for each year. Values resulting in field closure are shown in bold.

	ASP category[1]					
	0	0-5	5-10	10-20	**20+**	Total
2001	323	299	4		**1**	627
2002	211	360	9	1	**1**	582
2003	314	398	9	6	**1**	728
2004	229	333	8	4		574
2005	438	94	8	1		541
2006	661	77	1			739
Total	2176	1561	39	12	**3**	3791

[1]Categories are 0 µg/g; >0 and < 5 (denoted by 0-5); ≥ 5 and < 10 (denoted by 5-10); ≥10 and < 20 (denoted by (10-20) and ≥ 20 (denoted by 20+, is also field closure limit).

[3] Pods were grouped into sites, as described in Table 1.

Figure B1: Proportion of mussel samples for which ASP > 5µg/g for each month for each site. Rows of sites start from bottom left with Eastcoast, each row up follows the coastline of Scotland approximately clockwise.



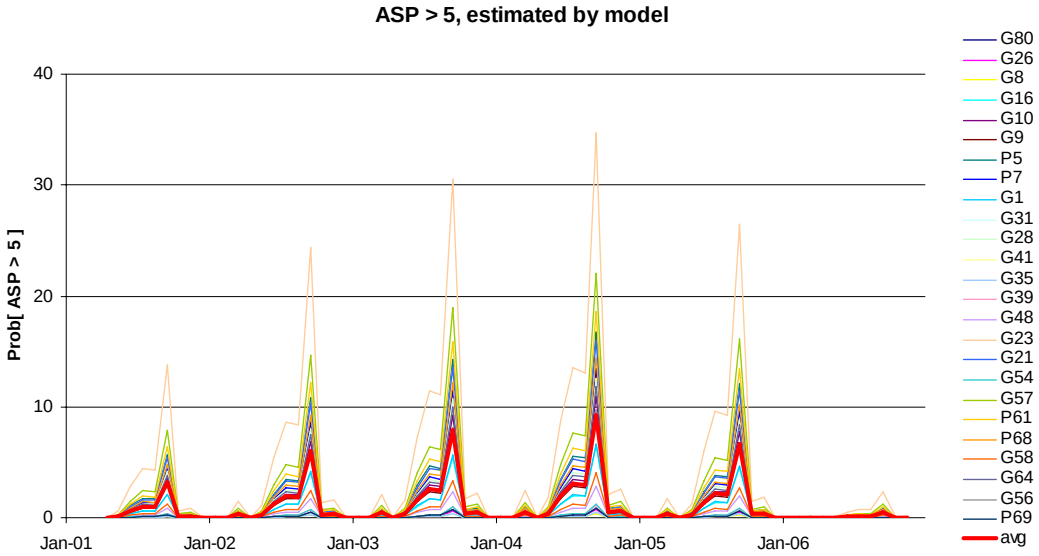
Model

The following model[4] described the data best. It allowed for

- Random variation between sites, i.e. some sites are more likely to have high ASP levels than others
- Random variation between years, i.e. some years tend to have high prevalence of ASP while other years tend to have low prevalence.
- Furthermore, the model contains a term to allow for modelling of toxicity for each month of the year. This is relevant for assessing and developing monitoring schemes, where the frequency of sampling may vary during the year.

Figure B2 shows the estimated probability of ASP in mussels exceeding 5µg/g over time for each site. The estimated toxin pattern confirms the observation made earlier that 2001 and 2006 had relatively low prevalence. For some sites, the prevalence is up to twofold higher than the average prevalence.

Figure B2: Estimated probability (%) of ASP > 5µg/g in mussels for each site



[4] A hierarchical generalised linear model was used to describe the data. Data were assigned zero if the test result was less than 5µg/g, and one otherwise. A binomial distribution with logistic link was assumed. Model selection indicated that a model with Month regarded as a fixed effect, and Site and Year regarded as random effects gave the best fit. Full details are given in Section 2.2.

Figure B3: Estimated probability (%) of ASP in mussels > 5µg/g, for each of six years.

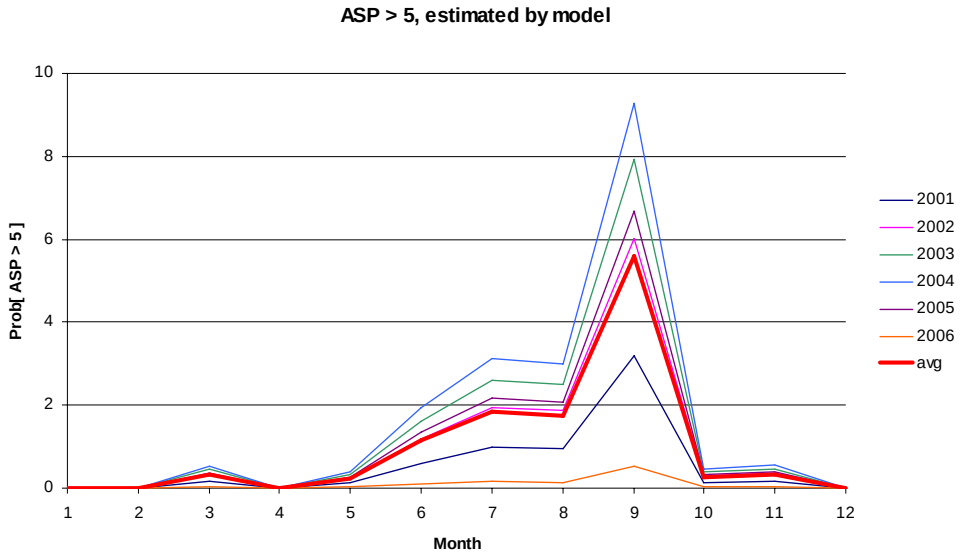


Figure B3 shows the toxin patterns during the year, for each of six years. The highest prevalence was observed in 2004, followed by 2003, while 2001 and 2006 were below average.

The estimated prevalence per site is shown in more detail in Table B2 and Figure B4. Lewis-LochRoag showed the highest prevalence, 4% on average but up to 22% in September, followed by Shetland-SE (2.3% on average), Shetland-SW-Gruting (1.9% on average), Mull-LochSpelve (1.7% on average), and Lewis-HarrisUist (1.6%). The remaining sites had a prevalence of 1.5% or less. Prevalence tended to be low during the winter months and early spring (Oct – May, less than 0.3%, on average) and then peaked in September with 5.6% on average (Table B2, Figure B3).

Random variation between years

Let an extreme year be defined as a year in which prevalence of ASP is extremely high, and assume that such a year will, on average, only happen once every 20 years. Figure B5 shows that the prevalence of ASP > 5µg/g for an average year and an average site (solid blue line) does not exceed 6% (in September), whereas for an extreme year this prevalence would increase to 20%. For a poor site, such as Lewis-LochRoag, the prevalence in September would be 22%, on average, while for an extreme year this would triple to 60%.

Figure B4: Estimated prevalence (%) of ASP > 5µg/g in mussels, for an average year and an average month. Although the outline of Shetland is shown enlarged, to keep findings compatible the sizes of the symbols are the same as for mainland Scotland.

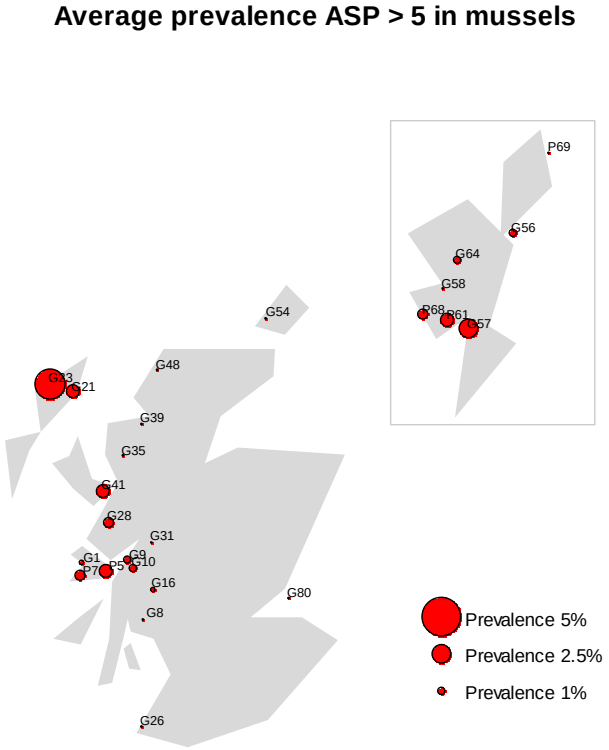


Figure B5: Prevalence (%) of ASP > 5µg/g in mussels for an average year and an extreme year, for a site with average toxin prevalence and a site with high toxin prevalence (Lewis-LochRoag).

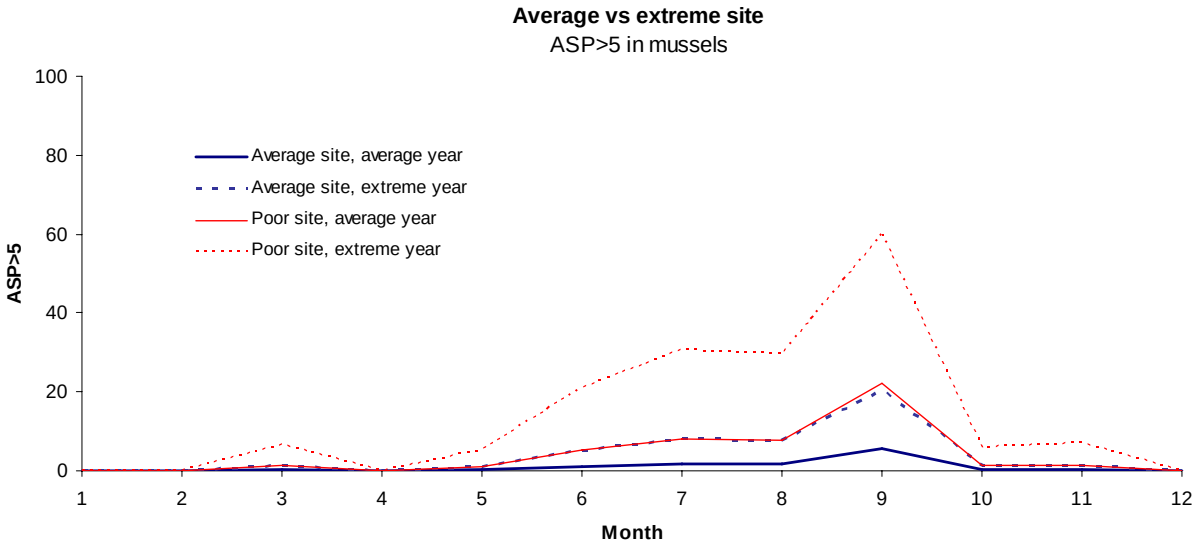


Table B2: Estimated[1] probability that ASP levels in mussels exceed 5μg/g, for each site per month, averaged over 6 years. The value 0% represents a small positive number having a value of less than 0.5%. Probabilities of 1% and higher are shown in bold.

ID	Site	Avg[2]	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec
			0.0	0.0	0.3	0.0	0.2	**1.1**	**1.8**	**1.8**	**5.6**	0.3	0.3	0.0
G80	Eastcoast	0.1	0.0	0.0	0.0	0.0	0.0	0.1	0.1	0.1	0.5	0.0	0.0	0.0
G26	Dumfries	0.1	0.0	0.0	0.0	0.0	0.0	0.1	0.1	0.1	0.4	0.0	0.0	0.0
G8	Ayr-LochStriven	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.1	0.1	0.2	0.0	0.0	0.0
G16	WC-LochFyne	0.6	0.0	0.0	0.2	0.0	0.1	0.7	**1.2**	**1.1**	**3.8**	0.2	0.2	0.0
G10	WC-LochEtive	**1.1**	0.0	0.0	0.3	0.0	0.3	**1.3**	**2.0**	**1.9**	**6.5**	0.3	0.4	0.0
G9	WC-LochCreran	0.9	0.0	0.0	0.3	0.0	0.2	**1.0**	**1.7**	**1.6**	**5.3**	0.2	0.3	0.0
P5	Mull-LochSpelve	**1.7**	0.0	0.0	0.6	0.0	0.4	**2.0**	**3.3**	**3.1**	**10.1**	0.5	0.6	0.0
P7	Mull-LochScridain	**1.4**	0.0	0.0	0.4	0.0	0.3	**1.6**	**2.6**	**2.5**	**8.0**	0.4	0.5	0.0
G1	Mull-LochnaKeal	0.6	0.0	0.0	0.2	0.0	0.1	0.7	**1.2**	**1.2**	**3.9**	0.2	0.2	0.0
G31	WC-LochLeven	0.0	0.0	0.0	0.0	0.0	0.0	0.1	0.1	0.1	0.3	0.0	0.0	0.0
G28	WC-Lochaber	**1.3**	0.0	0.0	0.4	0.0	0.3	**1.5**	**2.4**	**2.3**	**7.5**	0.3	0.4	0.0
G41	Skye	**1.6**	0.0	0.0	0.5	0.0	0.4	**1.9**	**3.1**	**3.0**	**9.6**	0.4	0.6	0.0
G35	NWC-LochTorridon	0.0	0.0	0.0	0.0	0.0	0.0	0.1	0.1	0.1	0.3	0.0	0.0	0.0
G39	NWC-Ullapool	0.1	0.0	0.0	0.0	0.0	0.0	0.1	0.2	0.2	0.7	0.0	0.0	0.0
G48	NWC-other	0.3	0.0	0.0	0.1	0.0	0.1	0.3	0.5	0.5	**1.7**	0.1	0.1	0.0
G23	Lewis-LochRoag	**4.0**	0.0	0.0	**1.5**	0.0	**1.1**	**5.1**	**8.1**	**7.8**	**22.0**	**1.2**	**1.5**	0.0
G21	LewisHarrisUist	**1.6**	0.0	0.0	0.5	0.0	0.4	**1.9**	**3.1**	**3.0**	**9.6**	0.4	0.6	0.0
G54	Orkney	0.1	0.0	0.0	0.0	0.0	0.0	0.1	0.2	0.2	0.7	0.0	0.0	0.0
G57	Shetland-SE	**2.3**	0.0	0.0	0.8	0.0	0.6	**2.8**	**4.5**	**4.3**	**13.5**	0.7	0.8	0.0
P61	Shetland-SW-Gruting	**1.9**	0.0	0.0	0.6	0.0	0.5	**2.3**	**3.7**	**3.5**	**11.3**	0.5	0.7	0.0
P68	Shetland-SW-Vaila	**1.4**	0.0	0.0	0.5	0.0	0.3	**1.7**	**2.7**	**2.6**	**8.6**	0.4	0.5	0.0
G58	Shetland-W	0.4	0.0	0.0	0.1	0.0	0.1	0.4	0.7	0.7	**2.3**	0.1	0.1	0.0
G64	Shetland-NW	**1.2**	0.0	0.0	0.4	0.0	0.3	**1.4**	**2.2**	**2.1**	**7.0**	0.3	0.4	0.0
G56	Shetland-NE	1.0	0.0	0.0	0.3	0.0	0.2	**1.1**	**1.8**	**1.7**	**5.7**	0.3	0.3	0.0
P69	Shetland-N-Balta	0.1	0.0	0.0	0.0	0.0	0.0	0.1	0.1	0.1	0.5	0.0	0.0	0.0

[1]From HGLM with Site and Year as random effects and Month as fixed effect.

[2]For each site the average probability over 12 months over 6 years was calculated, and for each month the probability over all sites over 6 years was calculated.

B.3 Risk assessment

Under the sampling scheme used at the time of this study (2006), the sampling frequencies for ASP in mussels were as follows: weekly from July to November, fortnightly from April to June, monthly from December to March.

Let the risk of non-detection be defined as the probability of not detecting a toxic event (in this case ASP in mussels > 5µg/g). Furthermore, it was assumed that a test result is valid for one week, so that with weekly sampling the risk of non-detection is zero. Full details are given in Section 2.3. In brief, if p is the estimated probability of toxin level exceeding 5µg/g (as reported in Table B2), then the risk of non-detection is zero for weekly sampling, 0.5 p for fortnightly sampling, and 0.75 p for monthly sampling.

Risk assessment of present (2006) monitoring scheme
As weekly sampling takes place during July – November, the risk of non-detection is zero for these months (Table B3). Even though sampling takes place only once a month during December – March, the maximum risk of non-detection is only 1.1% (Lewis-LochRoag). During June, the risk of non-detection increases to up to 2.4%. For approximately half the sites the risk of non-detection is less than 0.5% throughout the year.

Alternative monitoring schemes
Table B4 shows two alternative schemes, one where the risk of non-detection is 5% or less, and a more severe scheme for which the risk of non-detection is set at 1% or less. For 8 sites, monthly sampling throughout the year would suffice, while for the remaining sites weekly sampling would be required in summer to keep the risk of non-detection below 1%.

Table B3: Risk of non-detection (%), i.e. probability that a site unknowingly exceeds 5 µg/g, for ASP in mussels using the sampling frequencies introduced in 2006. Risk of non-detection of 1% or more shown in bold.

			Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec
		Current frequency[1]	1	1	1	2	2	2	4	4	4	4	4	1
ID	Site	Avg[2]	0.0	0.0	0.2	0.0	0.1	0.6	0.0	0.0	0.0	0.0	0.0	0.0
G80	Eastcoast	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
G26	Dumfries	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
G8	Ayr-LochStriven	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
G16	WC-LochFyne	0.0	0.0	0.0	0.1	0.0	0.1	0.4	0.0	0.0	0.0	0.0	0.0	0.0
G10	WC-LochEtive	0.1	0.0	0.0	0.3	0.0	0.1	0.6	0.0	0.0	0.0	0.0	0.0	0.0
G9	WC-LochCreran	0.1	0.0	0.0	0.2	0.0	0.1	0.5	0.0	0.0	0.0	0.0	0.0	0.0
P5	Mull-LochSpelve	0.1	0.0	0.0	0.4	0.0	0.2	**1.0**	0.0	0.0	0.0	0.0	0.0	0.0
P7	Mull-LochScridain	0.1	0.0	0.0	0.3	0.0	0.2	0.8	0.0	0.0	0.0	0.0	0.0	0.0
G1	Mull-LochnaKeal	0.0	0.0	0.0	0.1	0.0	0.1	0.4	0.0	0.0	0.0	0.0	0.0	0.0
G31	WC-LochLeven	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
G28	WC-Lochaber	0.1	0.0	0.0	0.3	0.0	0.1	0.7	0.0	0.0	0.0	0.0	0.0	0.0
G41	Skye	0.1	0.0	0.0	0.4	0.0	0.2	1.0	0.0	0.0	0.0	0.0	0.0	0.0
G35	NWC-LochTorridon	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
G39	NWC-Ullapool	0.0	0.0	0.0	0.0	0.0	0.0	0.1	0.0	0.0	0.0	0.0	0.0	0.0
G48	NWC-other	0.0	0.0	0.0	0.1	0.0	0.0	0.2	0.0	0.0	0.0	0.0	0.0	0.0
G23	Lewis-LochRoag	0.4	0.0	0.0	**1.1**	0.0	0.5	**2.6**	0.0	0.0	0.0	0.0	0.0	0.0
G21	LewisHarrisUist	0.1	0.0	0.0	0.4	0.0	0.2	1.0	0.0	0.0	0.0	0.0	0.0	0.0
G54	Orkney	0.0	0.0	0.0	0.0	0.0	0.0	0.1	0.0	0.0	0.0	0.0	0.0	0.0
G57	Shetland-SE	0.2	0.0	0.0	0.6	0.0	0.3	**1.4**	0.0	0.0	0.0	0.0	0.0	0.0
P61	Shetland-SW-Gruting	0.2	0.0	0.0	0.5	0.0	0.2	**1.1**	0.0	0.0	0.0	0.0	0.0	0.0
P68	Shetland-SW-Vaila	0.1	0.0	0.0	0.3	0.0	0.2	0.8	0.0	0.0	0.0	0.0	0.0	0.0
G58	Shetland-W	0.0	0.0	0.0	0.1	0.0	0.0	0.2	0.0	0.0	0.0	0.0	0.0	0.0
G64	Shetland-NW	0.1	0.0	0.0	0.3	0.0	0.1	0.7	0.0	0.0	0.0	0.0	0.0	0.0
G56	Shetland-NE	0.1	0.0	0.0	0.2	0.0	0.1	0.5	0.0	0.0	0.0	0.0	0.0	0.0
P69	Shetland-N-Balta	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0

[1]Current sampling frequency: 1=once per month, 2=fortnightly, 4=once per week.

[2]For each site the average risk was calculated, and for each month the average risk was calculated.

Table B4: Sampling frequencies (1 = once per month, 2 = every fortnight; 4 = every week) for sampling schemes with the maximum risk of non-detection (ASP exceeding 5µg/g) set at 5 or 1%, for ASP in mussels. Sampling frequencies exceeding once per month are shown in bold.

		max risk 5%												max risk 1%											
		J	F	M	A	M	J	J	A	S	O	N	D	J	F	M	A	M	J	J	A	S	O	N	D
	Current frequency[1]	**1**	**1**	**1**	**2**	**2**	**2**	**4**	**4**	**4**	**4**	**4**	**1**	**1**	**1**	**1**	**2**	**2**	**2**	**4**	**4**	**4**	**4**	**4**	**1**
ID	Site																								
G80	Eastcoast	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
G26	Dumfries	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
G8	Ayr-LochStriven	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
G16	WC-LochFyne	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	**4**	1	1	1
G10	WC-LochEtive	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	**4**	**2**	**4**	1	1	1
G9	WC-LochCreran	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	**2**	**2**	**4**	1	1	1
P5	Mull-LochSpelve	1	1	1	1	1	1	1	1	**4**	1	1	1	1	1	1	1	1	**4**	**4**	**4**	**4**	1	1	1
P7	Mull-LochScridain	1	1	1	1	1	1	1	1	**2**	1	1	1	1	1	1	1	1	**2**	**4**	**4**	**4**	1	1	1
G1	Mull-LochnaKeal	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	**4**	1	1	1
G31	WC-LochLeven	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
G28	WC-Lochaber	1	1	1	1	1	1	1	1	**2**	1	1	1	1	1	1	1	1	**2**	**4**	**4**	**4**	1	1	1
G41	Skye	1	1	1	1	1	1	1	1	**2**	1	1	1	1	1	1	1	1	**2**	**4**	**4**	**4**	1	1	1
G35	NWC-LochTorridon	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
G39	NWC-Ullapool	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
G48	NWC-other	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	**2**	1	1	1
G23	Lewis-LochRoag	1	1	1	1	1	1	**2**	**2**	**4**	1	1	1	1	1	**2**	1	1	**4**	**4**	**4**	**4**	1	**2**	1
G21	LewisHarrisUist	1	1	1	1	1	1	1	1	**2**	1	1	1	1	1	1	1	1	**2**	**4**	**4**	**4**	1	1	1
G54	Orkney	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
G57	Shetland-SE	1	1	1	1	1	1	1	1	**4**	1	1	1	1	1	1	1	1	**4**	**4**	**4**	**4**	1	1	1
P61	Shetland-SW-Gruting	1	1	1	1	1	1	1	1	**4**	1	1	1	1	1	1	1	1	**4**	**4**	**4**	**4**	1	1	1
P68	Shetland-SW-Vaila	1	1	1	1	1	1	1	1	**2**	1	1	1	1	1	1	1	1	**2**	**4**	**4**	**4**	1	1	1
G58	Shetland-W	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	**4**	1	1	1
G64	Shetland-NW	1	1	1	1	1	1	1	1	**2**	1	1	1	1	1	1	1	1	**2**	**4**	**4**	**4**	1	1	1
G56	Shetland-NE	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	**2**	**2**	**4**	1	1	1
P69	Shetland-N-Balta	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1

[1]Current sampling frequency: 1=once per month, 2=fortnightly, 4=once per week

B.4 Discussion

ASP is currently tested with High Performance Liquid Chromatography, which is extremely sensitive in detecting low levels of ASP. Furthermore, it appears that residual levels of ASP were found in mussels throughout the period of investigation (2001-2006). As a consequence, 41% of the mussel samples tested had levels between the limit of detection (approximately 1 µg/g) and 5 µg/g, with only 1.4% of all samples exceeding 5µg/g. As a result, models developed for ASP > 0 µg/g in mussels turned out to have only limited value, especially as the prevalence of positive samples was evenly distributed over all sites throughout the year (Table 10, Figure A5).

The analyses presented here focus on ASP > 5µg/g and this appears to be more informative. About a third of the sites tested negative throughout (Figure B1), and prevalence was estimated to be relatively high only during June through to September (Table B2).